Characteristics of the reproduction effectiveness of four Hungarian breeding lines of carp *Cyprinus carpio* (L.)

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Abstract The effectiveness of reproduction of four Hungarian breeding lines 7, 8, W, and 0 of carp *Cyprinus carpio* was investigated. The highest percentage of ovulating females after Ovopel stimulation of ovulation ($1/5 + 1$ pellet kg^{-1} BW of females) was found in line 8 (100 %), in line 0, it was 86.21 %, and in lines 7 and W, the respective values were 73.68 and 76.47 %. It was noted that the breeding line significantly ($P \leq 0.05$) determined the weight of the eggs (expressed in grams and percentage of female BW), the fertilization percentage, the percentage of living embryos after 24- and 36-h incubation, the total number of eggs, and the number of live embryos after 36-h incubation. The highest weight of eggs was obtained from females of line 7 (1,083.41 g) and line 8 (981.54 g), while the lowest weight was noted in the case of females of line W (804.94 g). The highest number of eggs was found in line 7 (722,300) and in line 8 (654,400). For these lines, the highest number of live embryos (36 h) was also found (624,400 and 622,200, respectively). Within each line investigated, the regression equations were derived in order to predict the weight of eggs (g), percentage of live embryos, and the number of live embryos after 36-h incubation.

Keywords *Cyprinus carpio* · Hungarian breeding lines · Reproduction effectiveness

Introduction

The carp spawner herd owned by the Institute of Ichthyobiology and Aquaculture in Gołysz (Polish Academy of Sciences) comprise 17 breeding lines and constitute a live “bank of genes” unique on the European scale. This genetically diverse population of carp was formed by breeding and genetic studies, which took several decades. Among the

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breeding lines of carp in Gołysz, there are lines of different origins: Hungarian, Polish, French, German, Lithuanian, Ukrainian, and Israeli (Białowas et al. 2008). This very genetically valuable material forms the basis for the national programs of preserving the genetic reserves of fish and for genetic improvements to the carp population in Poland. It is also an exceptional material for genetic (Rakus et al. 2003; Kachamakova et al. 2006), immunological (Jurecka et al. 2009) and breeding (Brzuska 2006a, b; Brzuska and Adamek 1997; Brzuska and Białowas 2002) studies.

Genetic characterization of carp from the Hungarian lines was presented by Imnazarow and Białowas (1995). They possess numerous desired characteristics, which make them precious material for cross-breeding between different lines. It is worth mentioning that the spawners from the Hungarian W line were used in the first studies on the effects of heterosis in carps after cross-breeding between lines (Białowas 2000).

Interest in the effects of controlled breeding fish from these lines (both during the breeding season and beyond it) was expressed in the implementation of numerous studies, in which both natural and synthetic preparations were used as ovulation stimulators (Brzuska 1990, 1991, 2000, 2004, 2005, 2006b, 2010, 2011; Białowas 2008; Cejko 2007; Jamiński 2005; Brzuska and Adamek 1989; Brzuska and Ryszka 1990). Based on the results of these studies, it was impossible to answer the question whether the differences expressed occur in the effects of breeding the fish from Hungarian lines with the use of Ovopel (D-Ala⁶, Pro⁹NEt-mGnRH + metoclopramide; Horváth et al. 1997), which is currently the most commonly used product for stimulating ovulation.

The aim of the presented studies was to compare the results of breeding in Ovopel-stimulated carps belonging to the four Hungarian breeding lines and predict the effectiveness of breeding within each of the lines.

Materials and methods

The data used for analyses were obtained during controlled spawning conducted in the following years: 1998–1999, 2001, 2004–2005, 2009–2011 in the Institute of Ichthyobiology and Aquaculture in Gołysz (Polish Academy of Sciences). Carp from four Hungarian carp breeding lines (line 7, line 8, line 0, and line W; Białowas et al. 2008) were used as study material. The average body weight in females from line 7 amounted to 7.80 ± 2.23 , line 8— 8.16 ± 1.17 , line W— 8.06 ± 3.17 kg, and from line 0 to 7.45 ± 1.35 kg. These four lines were not present in all 8 years of breeding. Line W was present in six breeding seasons, lines 0 and 7 in three seasons and line 8 only in one season. All spawnings that were used to obtain data were conducted in mid-May, and the data were obtained only from one spawning in each year. During every spawning that was conducted, the fish were stimulated simultaneously.

Before the breeding season, the spawners were held in different ponds (1.5 m deep, 1.5 ha). After capturing, females with clearly visible characteristics of maturity were selected (Szabó et al. 2000). After being transported to the hatchery, the fish were placed in 3-m³ pools, 6–8 fish per pool. During the adaptation period, which usually lasted 24 h, the water temperature was increased to 20 ± 1 °C. Afterward, the females were subjected to ovulation stimulation with Ovopel, which was administered intraperitoneally (1/5 + 1 pellet kg⁻¹ BW; Horváth et al. 1997). The males were also stimulated with Ovopel (1 pellet kg⁻¹ BW). The conditions of the natural photoperiod were maintained during the entire time the spawners spent in the hatchery. After breeding, the fish were transported to the ponds as quickly as possible.

The data collected determined the reproduction results of 78 spawning females (out of 96 females treated with Ovopel) from 3.70 to 12.00 kg, aged 4, 7–10, and 12–14 years.

The eggs obtained from each female were weighed and then fertilized with pooled milt from 3 to 4 males. After elimination of stickiness (Woynarovich and Woynarovich 1980), the eggs of each fish were incubated in a separate 7-L Weiss glass in water of 21 ± 1 °C. The following parameters were calculated for each of the females: percentage of egg fertilization after 12-h incubation, the percentage of living embryos after 24- and 36-h incubation, the total number of eggs and the number of living embryos after 36-h incubation. The arithmetic mean calculated for the investigated features is presented in Table 1.

The percentage of ovulating females within particular breeding lines was calculated.

In order to determine the effect of the breeding line on the features being studied, i.e., egg weight expressed in grams, egg weight expressed as the percentage of female body weight, percentage of egg fertilization (12 h), percentage of living embryos (24 and 36 h), number of eggs and number of living embryos after 36-h incubation analysis of variance using the least squares method was conducted (Harvey 1987). The females examined were characterized by different body weights and ages. For that reason, the linear model included cubic regression for the age and weight of females in order to increase the precision of estimating the effect of the line on the investigated traits. In order to minimize the variability regarding the influence of the year, in which spawning took place, the multiplier corrections were estimated, and each observation was corrected with their use (Miller et al. 1966).

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

$$Y_{ij} = \alpha + l_i + bW_{ij} + dM_{ij} + e_{ij}$$

where Y_{ij} is the observation revised for the year; α is the theoretical general mean with the assumption that $W_{ij} = 0$ and $M_{ij} = 0$; l_i is the effect of line ($i = 1 \dots 4$); b is partial regression on the age of the female; W_{ij} is the age of the female within line i ; d is partial regression on female body weight; M_{ij} is female body weight within line i ; and e_{ij} is the random error associated with observation j .

The solution of the simultaneous equations formed on the basis of this linear model allowed the least squares means (LSM) listed in Table 1 to be estimated. The significance of the effect of the main classification factor, i.e., breeding line, on the investigated traits, was verified by an F -test and the significance of differences between the means within the analyzed traits for the investigated lines—with Duncan's multiple range test with Kramer's modification (Harvey 1987).

Multiple regression analysis was used in order to predict the weight of eggs in grams, the percentage of living embryos after 36-h incubation, and number of living embryos (36 h). This analysis was conducted within every line investigated. For the prediction of egg weight (g), age and body weight were the independent variables in the equation. For the prediction of the percentage and the number of living embryos after 36-h incubation, the initial equation included the following independent variables: female age, female body weight, weight of the eggs in grams and in the percentage of female body weight. In order to determine the way in which introducing successive independent variables determines the precision of predicting the percentage of living embryos after 36-h incubation, the equations that followed included percentage of fertilized eggs and percentage of living embryos after 24-h incubation.

Table 1 Arithmetical means ($\bar{x} \pm \text{SD}$) and least square means (LSM) for investigated reproduction traits of four Hungarian breeding lines and results of Duncan's test

Breeding line	<i>n</i>	Weight of eggs (g)		Weight of eggs (% of female body weight)		Percentage of fertilized eggs after 12-h incubation	
		$\bar{x} \pm \text{SD}$	LSM	$\bar{x} \pm \text{SD}$	LSM	$\bar{x} \pm \text{SD}$	LSM
Line 7	21	982.67 $\pm$ 398.04	1,083.41c	13.6 $\pm$ 6.08	14.70C	90.00 $\pm$ 9.56	91.11c
Line 8	9	966.11 $\pm$ 308.40	981.54cb	11.93 $\pm$ 3.81	12.43b	95.89 $\pm$ 3.59	96.87a
Line W	26	858.31 $\pm$ 551.24	804.94a	9.95 $\pm$ 4.24	9.83A	93.58 $\pm$ 4.73	93.82b
Line 0	22	833.55 $\pm$ 385.94	878.49ab	11.26 $\pm$ 5.07	11.44Ab	94.73 $\pm$ 7.60	94.40ab
Breeding line	<i>n</i>	Percentage of living embryos		Number of eggs (thousand)		Number of living embryos after 36-h incubation (thousand)	
		after 24-h incubation		after 36-h incubation			
		$\bar{x} \pm \text{SD}$	LSM	$\bar{x} \pm \text{SD}$	LSM	$\bar{x} \pm \text{SD}$	LSM
Line 7	21	84.85 $\pm$ 14.40	87.65a	82.62 $\pm$ 13.29	85.29ab	655.1 $\pm$ 265.3	722.3aB
Line 8	9	93.78 $\pm$ 3.81	96.60bc	91.00 $\pm$ 3.91	94.27C	644.00 $\pm$ 205.60	654.4a
Line W	26	91.46 $\pm$ 5.62	93.77b	88.61 $\pm$ 8.64	88.52b	572.20 $\pm$ 367.40	536.6A
Line 0	22	89.59 $\pm$ 11.95	88.78a	86.00 $\pm$ 14.73	84.69A	555.60 $\pm$ 257.20	585.7a
						475.50 $\pm$ 223.40	491.3b

Line means marked with the same letters do not differ significantly from one another. Mean values marked with different letters are significantly different: capital letters at $P \leq 0.01$ and small letters at $P \leq 0.05$

Results

Latent period and percentage of ovulating females after hormonal stimulation

In all females investigated, ovulation occurred 11–14 h after administering a resolving dose of Ovopel. The highest percentage of ovulating females was found in line 8 (100 % of spent fish). After Ovopel stimulation, eggs were obtained from 86.21 % of females of line 0 and from lines 7 and W 73.68 and 76.47 %, respectively.

The effect of the breeding line on the weight and quality of the eggs obtained, their number, and the number of living embryos after 36-h incubation

A significant ($P \leq 0.05$) effect of the breeding line on all the investigated traits, determining the efficiency of controlled reproduction, was demonstrated. The LSM evaluated for the weight of eggs in grams show that the highest weight of eggs was obtained from fish of line 7 and line 8 (1,083.41 and 981.54, respectively; Table 1). The lowest value of the LSM of 804.94 g was found in strain W (Table 1). The mean for the weight of eggs (g) estimated for line W differed significantly from the respective means calculated for line 7 and line 8 (Table 1). The LSM estimated for the weight of eggs expressed as % of female BW also had the lowest value of 9.83 % for line W, and this mean was significantly different to the respective means for line 7 and line 8 (Table 1). The highest LSM value of this trait differed significantly from the means noted in the remaining three lines was found in line 7 (14.70 %; Table 1).

The LSM for the percentage of fertilization of eggs obtained from females from the four investigated lines show that this trait was characterized by the highest value in line 8 females and the lowest value in those from line 7 (96.87 and 91.11 %, respectively; Table 1). The difference between these means was statistically significant (Table 1). The mean estimate for line 7 was also significantly lower than that for lines W and line 0 (93.82 and 94.40 %, respectively; Table 1).

The quality of eggs from line 8 was much higher than that found in the other lines investigated with respect to both the percentage of living embryos after 24- and 36-h incubation. The LSM for the percentage of living embryos (36 h) estimated for line 8 amounted to 94.27 %, and it was significantly different than the means for other examined lines (Table 1).

The least squares means estimated for the number of obtained eggs indicate that the LSM for this trait was highest for line 7 (722.3 thousand), and it differed significantly from the respective values calculated for line 0 and line W (585.7 and 536.6 thousand; Table 1).

When analyzing the LSM for the number of living embryos (36 h), it can be noted that for females from line 7 and line 8, similar means were obtained (623.4 and 622.2 thousand, respectively). Similar means for that trait (491.3 and 489.2 thousand, respectively) were also observed for lines 0 and W. The significance of differences between the means of investigated lines is presented in Table 1.

Regression

Multiple regression results are presented in Table 2. With R^2 as the determination index predicting the compliance between the linear model and empirical data, i.e., information on the proportion of variability of the variable accounted for by the model, the highest

Table 2 Multiple regression results

Line	Regression equations	R^2
7	$Y_1 = -521.76 + 201.048x_1 - 24.393x_2$	0.14
	$Y_2 = -49.98 + 12.34x_1 + 3.32x_2 - 0.001x_3 + 0.30x_4$	0.37
	$Y_2 = -74.15 + 4.65x_1 + 3.11x_2 - 0.008x_3 + 1.15x_4 + 0.95x_5$	0.74
	$Y_2 = -5.04 + 0.97x_1 + 0.34x_2 - 0.001x_3 + 0.07x_4 + 0.03x_5 + 0.87x_6$	0.98
	$Y_3 = -786,000 + 86,598x_1 + 7,663x_2 + 653x_3 - 7,330x_4$	0.91
8	$Y_1 = -3,756.08 + 506.07x_1 + 78.47x_2$	0.13
	$Y_2 = 146.62 - 6.11x_1 - 1.38x_2 - 0.006x_3 + 0.96x_4$	0.61
	$Y_2 = -71.69 - 1.71x_1 + 8.39x_2 - 0.07x_3 + 6.01x_4 + 1.08x_5$	0.92
	$Y_2 = -12.81 - 4.25x_1 + 5.26x_2 - 0.05x_3 + 4.34x_4 + 0.02x_5 + 0.98x_6$	0.98
	$Y_3 = 362,199 - 409,096x_1 - 619x_2 + 472x_3 + 14,264x_4$	0.99
0	$Y_1 = 2,813.24 - 263.34x_1 + 71.64x_2$	0.17
	$Y_2 = 27.05 + 0.87x_1 + 7.03x_2 - 0.06x_3 + 4.53x_4$	0.08
	$Y_2 = -180.65 + 8.36x_1 + 2.10x_2 - 0.04x_3 + 2.57x_4 + 1.86x_5$	0.83
	$Y_2 = -60.27 + 2.23x_1 + 2.27x_2 - 0.006x_3 + 0.58x_4 + 0.27x_5 + 1.07x_6$	0.97
	$Y_3 = -383,335 + 19,393x_1 + 29,573x_2 + 246x_3 + 22,056x_4$	0.85
W	$Y_1 = -346.94 + 38.67x_1 + 103.59x_2$	0.67
	$Y_2 = 87.74 - 0.005x_1 - 0.66x_2 + 0.005x_3 + 0.15x_4$	0.08
	$Y_2 = -100.65 - 0.04x_1 + 0.95x_2 + 0.003x_3 - 0.95x_4 + 2.01x_5$	0.91
	$Y_2 = -88.11 + 0.01x_1 + 0.68x_2 + 0.004x_3 - 0.97x_4 + 1.44x_5 + 0.45x_6$	0.91
	$Y_3 = 12,572 + 2,425x_1 - 7,587x_2 + 658x_3 - 2,568x_4$	0.99

Dependent variables: Y_1 —weight of eggs (grams); Y_2 —percentage of living embryos after 36-h incubation; and Y_3 —number of living embryos after 36-h incubation

Independent variables: x_1 —age of female; x_2 —weight of female; x_3 —weight of eggs (g); x_4 —weight of eggs (% of female body weight); x_5 —percentage of fertilized eggs (12 h); and x_6 —percentage of living embryos after 24-h incubation

R^2 —determination index

accuracy of the prediction of egg weight in grams was obtained for line W ($R^2 = 0.67$) and the lowest for line 8 ($R^2 = 0.13$) (Table 2).

Given the assessment results for prediction accuracy with respect to the number of living embryos after 36-h incubation, it can be concluded that the prediction made on the basis of the age of female (x_1), female body weight (x_2), weight of eggs in grams (x_3), and weight of eggs expressed as the percentage of female body weight (x_4) did not produce satisfactory results for any of the lines analyzed. However, it needs to be emphasized that for line 8, the value of R^2 was the highest (0.61; Table 2), whereas the highest convergence coefficient ($\phi = 1 - R^2$; describing the proportion of the dependent variable determined by factors which had not been included in the model) was obtained for lines 0 and W. When the fertilization percentage (x_5) was included in the equation, satisfactory results of prediction were also observed for lines 8 and W (R^2 0.92 and 0.91, respectively; Table 2). Having included another trait in the equation, i.e., the percentage of living embryos after 24-h incubation (x_6), satisfactory results were obtained for all the lines.

Given the assessment results for prediction accuracy with respect to the number of living embryos (36 h), using an equation that included the following traits: age of female (x_1), female body weight (x_2), weight of eggs in grams (x_3), and weight of eggs expressed as the percentage of female BW (x_4), it can be concluded that the results obtained are

satisfactory for all four breeding lines (Table 2). However, it needs to be emphasized that the lowest value of the determination index was recorded for line 0 ($R^2 = 0.85$; Table 2).

Discussion

The results of the research indicate that among the Hungarian lines analyzed in the study, the highest reproduction effectiveness was displayed by line 8. Spawn was obtained from all the fish and the mean weight of eggs was high, reaching 981 g (which was over 12 % of female BW). The highest mean values for all three traits characterizing the quality of eggs were observed in females from line 8. Furthermore, the number of living embryos (36 h) was high and exceeded 622 thousand. Satisfactory prediction of both the percentage and the number of living embryos after 36-h incubation was noted within this line.

Brzuska (1991) reports a relatively low percentage (<60 %) of females from line 8 which laid eggs after CPH treatment (eggs of good quality and an average weight of 981.5 g). According to Białowas et al. (2008), the proportion of spawning females from line 8 was 60–70 %, and relative fecundity (thousand eggs/kg⁻¹ BW) was 95–115. The use of [D-Tle⁶, ProNHet⁹] GnRH (Lecirelin) resulted in an increased percentage of spawning females from line 8, whose eggs presented a higher weight compared to the hypophysed fish (Brzuska 2004).

Satisfactory breeding results were also obtained for line 7. Even though this group of fish displayed the lowest percentage of ovulating females, the mean weight of the eggs obtained was the highest both in grams and in percentage of female BW. The highest number of eggs was obtained (over 722 thousand), and the number of living embryos (36 h) was similar in lines 7 and 8. The prediction of the percentage and number of living embryos (36 h) was satisfactory for this line.

Brzuska (1991) recorded a high percentage of line 7 females that ovulated after hypophysation (the highest from among the 13 lines of carp that were analyzed), and the high weight of the eggs of inferior quality. Jamiński (2005) also observed a high percentage of females from line 7 ovulating after treatment with Ovopel (90 %). The author indicated that the spawning effectiveness coefficient (Se; Kucharczyk et al. 1997) for line 7 was considerably lower compared to the French line F and the Lithuanian lines B and V. Cejko (2007) indicated that within line 7, the percentage of ovulating females was higher after hypophysation than after treatment with Ovopel, whereas the egg weight expressed in grams and as a percentage of female body weight was higher after stimulation with Ovopel (the quality of eggs obtained after treatment with each of the stimulators did not vary). Białowas et al. (2008) reported a very low percentage of ovulating female (35–45 %) in line 7 and a very low relative fecundity for this line, namely 80–90 (thousand eggs/kg⁻¹ female body weight). After stimulation with Dagin [D-Arg⁶, Pro⁹NET-sGnRH + metoclopramide], a low percentage of fish reproduced and the eggs obtained were of low weight (and the worst quality) compared to the weight of eggs produced by the hypophysed females and those treated with Ovopel (Brzuska 2006b).

In the present study, eggs were produced by a relatively low percentage of females from the Hungarian line W (~76 %). The mean weight of the eggs expressed both in grams and as a percentage of female BW was the lowest of the four investigated lines. The number of eggs for the fish from this line and the number of living embryos (36 h) were also the lowest. The prediction of the number of living embryos (36 h) was satisfactory.

In her research, Brzuska (1991) indicated that after stimulation with CPH, ovulation was observed in slightly more than 60 % of line W females. Furthermore, the mean weight of

their eggs (expressed in grams and in the percentage of female BW) did not reach the mean value calculated for the 13 lines of carp analyzed. For line W, the number of living embryos after 24-h incubation was relatively low. Research by Cejko (2007) demonstrated that line W is one of those breeding lines of carp that presented a relatively low percentage of ovulating fish, both after hypophysation and after treatment with Ovopel. This has been confirmed by Brzuska and Białowas (2002) and by Brzuska (2005). In a report by Cejko (2007), line W was included in the group of lines that produced the lowest weight of eggs in grams, irrespective of the stimulator used. However, the quality of eggs from the females of line W was satisfactory after both 24- and 36-h incubation. The results of the research on the reproduction effectiveness of the Polish line 6, the French line F, and the Hungarian line W conducted by Białowas (2008) indicated that after Ovopel treatment, the percentage of ovulating females was the lowest for line W. This line also displayed the lowest survival rate of embryos (24 h), the lowest spawning effectiveness coefficient (Se), and the lowest value of the reproduction index (Ss). Białowas et al. (2008) also report a very low relative fecundity for this line, namely 60–65.

The present research has indicated that within line 0, ovulation occurred in over 86 % of females. The mean weight of the eggs obtained was not high (>878 g, 11.44 % of the female BW) compared to the value calculated for line 8 and line 7. Line 0 displayed the lowest percentage of living embryos (36 h) and a lower number of eggs and living embryos (36 h) compared to lines 8 and 7. Within line 0, prediction of both the percentage and the number of living embryos (36 h) was satisfactory (36 h). However, it needs to be mentioned that when predicting the number of living embryos (36 h), the determination index (R^2) for this line was the lowest compared to its respective values for the other three lines.

Research by Brzuska (1991) indicates that out of the 13 lines analyzed, line 0 was one where the percentage of ovulating females treated with CPH exceeded 75 %. It is also important that the fish from line 0 produced eggs of the highest mean weight (expressed in grams and in percentage of female BW). The quality of eggs was satisfactory as well. Brzuska (2010) reports a high percentage of ovulating females from this breeding line and a high weight of good-quality eggs after stimulation with either CPH or Ovopel. Białowas et al. (2008) also report a high percentage of ovulating fish from this line (85–95 %) and a relative fecundity of 100–120. The results of research by Cejko (2007), on the other hand, show that after treatment with Ovopel, the percentage of spawning females from line 0 was the lowest (only 50 %) compared to the other 9 breeding lines of carp from Gołysz. Having accounted for the interaction between the breeding line and the stimulator, the value of the least squares means for egg weight expressed in grams indicated that line 0 females produced eggs of a very low weight (but satisfactory quality after 36-h incubation) regardless of whether the fish were treated with CPH or Ovopel.

To sum up the research, it was concluded that Ovopel stimulation of fish from the Hungarian lines 7 and 8 can produce better reproduction results than in fish from lines 0 and W. The results of the study (conducted for the first time on four Hungarian lines simultaneously) are important for the fish practice because they allow for prediction of the most likely breeding results that can be obtained after administering Ovopel to fish from the lines in question to stimulate ovulation.

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