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ASSESSMENT OF OVULATION OF COMMON CARP (*CYPRINUS CARPIO*) FEMALES SELECTED FOR INDUCED SPAWNING ON THE BASIS OF EXTERNAL MORPHOLOGICAL CHARACTERISTICS

T. SZABÓ[1*], R. SZABÓ[2], B. URBÁNYI[1] and L. HORVÁTH[1]

[1]Department of Applied Genetics and Breeding, Biotechnology Laboratory, Gödöllő University of Agricultural Sciences, Institute of Animal Husbandry, H-2103 Gödöllő, Páter K. u. 1, Hungary; [2]National Federation of Hungarian Fish Producers, Dinnyés Fish Farm, Dinnyés, Hungary

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Secondary sexual characteristics such as softening and rounding of the abdomen as well as reddening and protrusion of the anal papilla and vent can be of help to breeders in selecting common carp (*Cyprinus carpio*) females prepared for propagation. To assess the reliability of this method, long-term data obtained on induced spawning of common carp at a large-scale fish hatchery were evaluated. The average spawning ratio of 2,620 females receiving hormonal injections was 79.8%. The average pseudogonadosomatic index (PGSI) calculated from data on the egg production of 2,086 females was 16.3 ± 5.87% (mean ± SD) for the same period. There was a correlation between fish weight and the time of induction determined by the breeder on the basis of external morphological characteristics. The similarity of the responses of females, including both spawning ratio and PGSI, among the different weight categories proved the reliability of this method for identification.

Key words: Common carp, induced breeding, ovulation, maturity

The common carp (*Cyprinus carpio*) as a domesticated fish species is cultured in most parts of Europe, all over Asia, and on a small scale in some countries of Africa and Latin America (Balon, 1995). In intensive common carp farming, effective seed production demands special techniques of artificial propagation. Induced spawning enables farmed broodstocks to ovulate under conditions of intensive culture and allows the production of eggs to be adjusted as required to suit procedures on the farm. The technique that uses pituitary extracts to induce ovulation is known as hypophysation. Besides the advantage of regulating the time of spawning, hypophysation enables the adoption of other

[*]E-mail: tszabo@fau.gau.hu; Fax: +36 (28) 410 804

methods of artificial propagation, including hand-stripping, fertilisation, incubation, hatching, and larval rearing.

Although hatchery production of common carp has been practised for more than three decades, studies on the results of induced breeding based on long-term data are non-existent. This is because routine hatchery work and precise data collection are hard to conduct simultaneously. Also, long-time storage of data collected under field conditions would be difficult to carry out.

In the spawning season, there may be considerable differences in the reproductive development of individual females, even within the same population (Rothbard and Yaron, 1995). Hypophysation is only effective in inducing spawning in fish that have reached the final stages of maturation. This means that the germinal vesicle (GV) must have begun or preferably have completed its migration towards the periphery. Reductions in the quality or survival of eggs occur if treatments are made too early in development (Bromage, 1992). Hypophysation inducing the final oocyte maturation without ovulation can even result in the death of the female, because of the mass atresia of the oocytes (Horváth et al., 1992). Even if the fish were to survive, propagation should be postponed until the next year. Therefore, the identification of the females that will be able to respond to pituitary injection is of basic importance to the artificial propagation of fish.

Oocyte development can be monitored by taking a small sample from the ovary by inserting a plastic catheter via the genital pore into the gonad. The ovarian sample (of about 30–40 oocytes) should be cleared in a solution of ethanol:formalin (40% formaldehyde):glacial acetic acid (6:3:1; by volume) (Rothbard and Yaron, 1995). This solution enables the position of the germinal vesicle to be seen easily under a dissecting microscope at approximately 20 × magnification. In the experiments of Yaron and Levavi-Zermonsky (1986), carp females responded to hypophysation only if initially more than 65% of their oocytes had already started GV migration. The procedure of oocyte examination in ovarian biopsies of female carp destined for spawning has already been applied in several hatcheries of Israel as a routine. By employing this method, only suitable females are selected for spawning induction, while others are returned to the pond until their oocytes show further progress, namely, initial migration of GV (Yaron and Levavi-Zermonsky, 1986; Rothbard and Yaron, 1995).

Although biopsy is reliable for the identification of responsive females, the technique can be stressful to fish or cause damage to internal organs. It is also time consuming when large numbers of females are to be spawned. In common carp, secondary sexual characteristics such as softening and rounding of the abdomen as well as reddening and protrusion of the anal papilla and vent can be of help to breeders in selecting females prepared for propagation (Fig. 1) (Horváth and Tamás, 1984). The procedure is relatively quick and can be done by the pond; however, the success of selection largely depends on the breeder's experience.

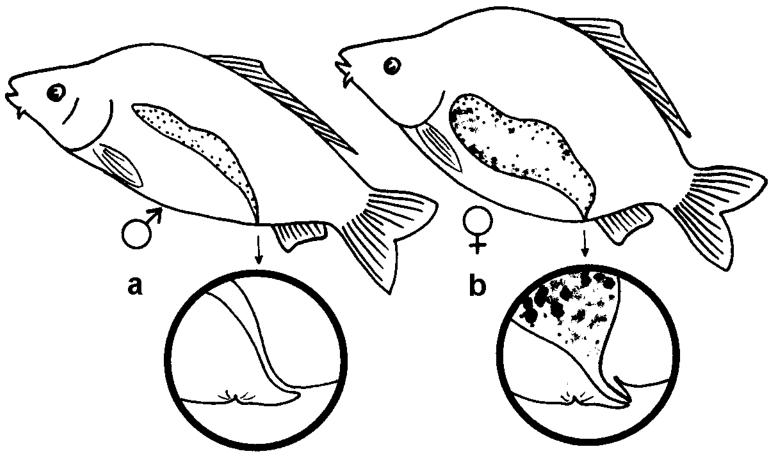

Fig. 1. Secondary sexual characteristics in common carp males (a) and females (b) during the spawning season

The Dinnyés Fish Hatchery and Farm, the production unit of the National Federation of Hungarian Fish Producers, was established in 1958. The farm was the first in the world to be equipped with an egg-incubating and a fry-rearing facility for carp breeding using the Woynarovich method (Woynarovich, 1962) which has received worldwide acceptance (Huet, 1986). At present the facility can simultaneously produce and rear 150 million fish fry in a breeding season. The farm produces fry originating from genetically tested common carp stock, both mirror and scaled varieties. For propagation of common carp, the standard technology of induced spawning has been applied (Woynarovich and Horváth, 1980).

In this study, we analyzed the following data on induced spawning of common carp, obtained between 1980 and 1997 at the Dinnyés Fish Hatchery and Farm: (1) response of females to hormonal treatment and relative quantity of stripped eggs expressed as the spawning ratio and the pseudogonadosomatic index (PGSI), respectively (their calculation will be shown later); (2) fish weight relative to the time of induction determined by the breeder on the basis of secondary sexual characteristics. In order to form an opinion on the reliability of the identification of responsive females by examining secondary sexual characteristics, we compared the spawning ratios and the mean PGSI values among different weight categories. Breeders expect similar responsiveness of females showing signs of maturity irrespective of their body weights.

Materials and methods

Between 1980 and 1997, data for the following aspects were collected at the Dinnyés Fish Hatchery and Farm: number of common carp females selected for propagation, weight of the females, time of induction, response of females to hormonal treatment, and the relative quantity of stripped eggs. The last two reproductive parameters were expressed by the spawning ratio (no. of responsive females/no. injected) and PGSI, respectively. PGSI was calculated as follows: (weight of stripped egg mass/body weight before stripping) × 100 (Freund et al., 1995). The spawning ratio was analyzed by the chi-square test ($p < 0.05$). PGSI data were subjected to one-way analysis of variance (ANOVA, $p < 0.05$).

In examinations where fish weight was concerned, except for the determination of PGSI – which is an individual reproductive parameter – weight categories were set up as follows: (< 3 kg, 4 kg, … 13 > kg). When comparing the spawning ratios and mean PGSI values among categories, the last two or three categories were put together. The females selected for breeding, irrespective of the year of propagation, were classified into the appropriate category on the basis of their weight.

The time of induction was appointed as follows: in each year, the reproductive season, lasting approximately four to six weeks, was divided into three periods of the same length of time. The time of induction of each female propagated in the season independently of the year of propagation was considered as an event in the first, the second or the third period.

In large-scale breeding work at the Dinnyés Fish Hatchery and Farm, ovarian biopsy would not be a very practical method for determining the stage of maturity of females. Breeders have therefore to depend largely on practical experience and field observations. Females ready for spawning are identified by external morphological characteristics such as the well-rounded and soft abdomen and the swollen genital opening. Less mature fish are returned to the holding pond for reexamination at a later date.

Results and discussion

Response of females to hormonal treatment and relative quantity of stripped eggs

Between 1980 and 1997, 2,091 out of the 2,620 carp females selected for propagation and treated with pituitary homogenate responded to treatment. The average spawning ratio for the period of 18 spawning seasons is 79.8% (Table 1). From data of egg production of 2,086 females, the average PGSI was calculated as 16.3 ± 5.87% (mean ± SD) for the same period (Table 2). The validity of the above two indices is supported by the facts that (1) the standard technology of induced spawning of common carp was applied, and (2) the indices were calculated from a large number of data.

Fish weight relative to the time of induction determined on the basis of secondary sexual characteristics

Figure 2 indicates a correlation between fish weight and the time of induction determined by the breeder on the basis of secondary sexual characteristics. Most of the females weighing 7 kg or more show signs of maturity, that is, a well-rounded and soft abdomen and swollen genital opening, early in the reproductive season. Therefore, 53.5–68.1% of these females were selected for propagation in the first period of the spawning season, while a minor proportion of them (11.3–21.9%) was induced to spawn in the third period. In contrast, the majority of the females with a body weight less than 7 kg were propagated in the third period (24.6–58.4%), and only 22.4–45.9% were selected for spawning induction in the first period. In the second period of the spawning season, proportions of different weight categories are similar to those in the whole population destined for spawning induction.

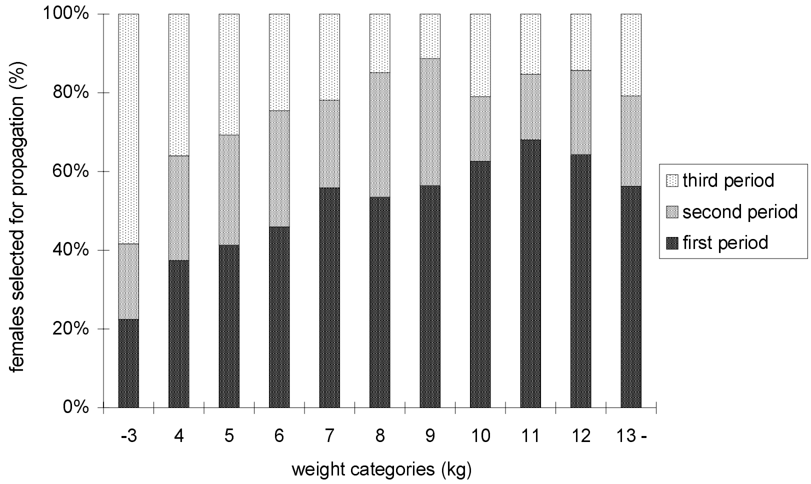

Fig. 2. Composition of females in different weight categories based upon the time of induction. Females were selected for propagation between 1980 and 1997

By comparing the spawning ratios and the mean PGSI values in different weight categories, we tried to form an opinion on the reliability of the identification of responsive females on the basis of secondary sexual characteristics. Spawning ratios were similar among different weight categories in any period of the season or in the spawning season as a whole (chi-square test; $p < 0.05$) (Table 1 and Fig. 3).

Table 1

Number of females selected for induced spawning between 1980 and 1997, and the spawning ratios in different weight categories in the first, second and third period of the spawning season, and in the whole season disregarding the time of induction

Weight categories (kg)	Reproductive season							
	First period		Second period		Third period		Total	
	No. of females	Spawning ratio (%)	No. of females	Spawning ratio (%)	No. of females	Spawning ratio (%)	No. of females	Spawning ratio (%)
< 3	48	89.6	41	80.5	125	75.2	214	79.4
4	113	86.7	80	82.5	109	76.2	302	81.8
5	191	78.0	129	90.7	142	76.3	462	81.2
6	194	83.0	124	79.0	104	78.9	422	80.8
7	221	81.9	88	88.6	87	65.5	396	79.8
8	161	78.3	95	80.0	45	64.4	301	76.7
9	110	81.8	63	84.1	22	72.7	195	81.5
10	95	74.7	25	80.0	32	59.4	152	72.4
11	49	85.7	12	58.3	11	81.8	72	80.6
> 12	63	84.1	23	82.6	18	66.7	104	80.8
Total	1245	81.4	680	83.4	695	73.4	2620	79.8

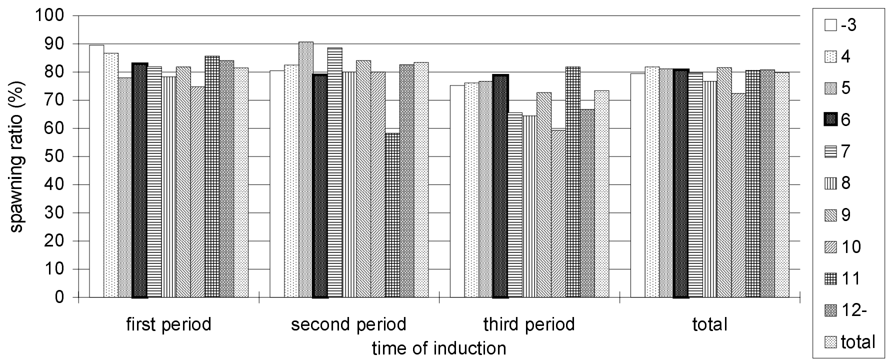

Fig. 3. Spawning ratio in relation to fish weight in the first, second and third period of the spawning season and in the whole season disregarding the time of induction. The chi-square test indicated that there was no significant effect of fish weight on spawning ratio in any period or in the spawning season as a whole

There was no significant difference among mean PGSI values in the first and second period of the season, or in the spawning season as a whole (ANOVA; $p < 0.05$). In the third period, the mean PGSI for the 8 kg weight category was significantly higher than those for the lower categories (ANOVA; $F = 2.04$; $p = 0.041$) (Table 2 and Fig. 4). However, ANOVA indicated this significant difference by a relatively low number of females in the 8 kg weight category (29 individuals). The deviation could also be hard to explain from a physiological point of view. In the other comparisons, the 8 kg weight category represented itself with relatively more individuals, and the mean PGSI for the group was similar to those for the others.

Table 2

Number of females responding to hormonal treatment between 1980 and 1997, and the PGSI (mean ± SD) in different weight categories in the first, second and third period of the spawning season, and in the whole season disregarding the time of induction

Weight categories (kg)	Reproductive season: First period		Second period		Third period		Total	
	No. of females	PGSI (%) mean ± SD	No. of females	PGSI (%) mean ± SD	No. of females	PGSI (%) mean ± SD	No. of females	PGSI (%) mean ± SD
< 3	43	18.7±5.96	33	17.6±4.94	94	14.2±6.52	170	16.0±6.39
4	98	15.9±5.75	67	15.9±5.18	83	16.0±6.72	248	16.0±5.93
5	147	16.5±4.97	117	16.3±5.11	109	15.4±6.06	373	16.0±5.36
6	159	16.4±6.29	98	16.4±6.18	76	15.3±5.84	333	16.2±6.16
7	185	16.0±5.84	78	16.3±6.48	57	15.7±6.28	320	16.0±6.06
8	126	16.7±5.74	75	16.6±5.52	29	18.7±5.77	230	17.0±5.68
9	89	16.8±6.26	53	15.7±5.46	16	17.3±4.99	158	16.5±5.87
10	67	18.2±5.76	20	15.7±4.88	23	17.2±5.17	110	17.5±5.52
> 11	99	16.7±5.91	25	14.0±5.82	20	16.8±4.77	144	16.3±5.81
Total:	1013	16.6±5.84	566	16.2±5.61	507	15.7±6.18	2086	16.3±5.87

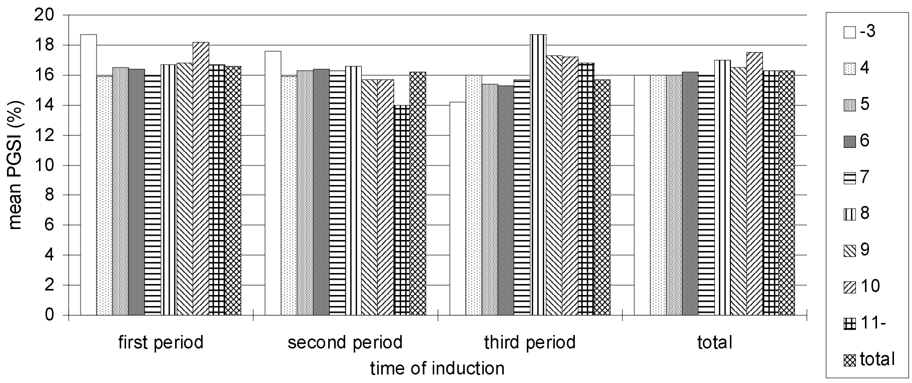

Fig. 4. PGSI in relation to fish weight in the first, second and third period of the spawning season and in the whole season disregarding the time of induction. ANOVA indicated that there was no significant effect of fish weight on the relative quantities of stripped eggs in the first and second period or in the spawning season as a whole. In the third period, the mean PGSI for the 8 kg weight category was significantly higher than those for the lower categories ($F = 2.04$; $p = 0.041$)

With the similarity of responses of the females with different body weights, the above results of statistical tests prove that identification of mature females by the breeders on the basis of external examinations is a reliable method.

The results presented above will provide guidelines for carp breeders in the selection of optimal brood fish for spawning induction. It will also allow breeders to calculate their hatchery's larval production in advance. These results are helpful in calculating the total weight of broodstock that must be kept in order to produce a specific number of eggs.

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