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EUROPEAN CATFISH (*SILURUS GLANIS* L.) REPRODUCTION OUTSIDE OF THE SPAWNING SEASON

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ABSTRACT. The aim of this work was to develop methods of artificial European catfish reproduction outside of its natural spawning season using thermal and hormonal stimulation. Ovulation was induced with combined hormonal stimulation using a priming dose of Ovopel (0.3 granules in one dose or 0.2 and 0.3 granules in two doses per kg of female body weight) and a resolving dose of carp pituitary extract (CPE) (3 mg per kg of female body weight). The latency period between the doses was 24 hours. The males were stimulated with one dose of Ovopel (1 granule per kg of body weight). The results obtained indicated that the application of the appropriate thermal and hormonal stimulation, without light stimulation, can induce artificial spawning in the European catfish between January and August. It was revealed that the time between the injection of the resolving dose and egg collection decreased over this period. Thus, ovulation could be stimulated faster than in winter and spring. The number of eggs obtained in pre-season spawning was greater when the thermal treatment was longer. The results obtained indicate that the application of Ovopel in two stimulation doses instead of one was advantageous for the effectiveness of European catfish pre-season spawning, the number of eggs obtained as well as its survival. The results obtained during artificial European catfish reproduction conducted before, during, and after its natural spawning period were comparable.

Key words: EUROPEAN CATFISH (*SILURUS GLANIS*), OUT-OF-SEASON SPAWNING, REPRODUCTION

INTRODUCTION

The European catfish, *Silurus glanis* L., is a thermophilic species, which spawns naturally in Poland in June when water temperatures exceed 18°C (Horoszewicz 1971). Females achieve spawning maturity when the accumulated water temperature exceeds 1000°D (Wisniewolski 1989). This species is cultivated traditionally in ponds (Horoszewicz 1971, Horvath 1977, Stevic et al. 1997) or under controlled conditions (Kouril and Hamackova 1982, Epler and Bieniarz 1989a, Linhart et al. 1997, Klodzinska and Okoniewski 1998) but only during its natural spawning period. Sometimes due to cold summers natural spawning in this period is not effective or these fish do not spawn at all (Wisniewolski 1989).

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A fundamental role in the controlled reproduction of European catfish is played by hormonal stimulation, mainly with carp pituitary extract (CPE), which is administered to mature specimens caught during the spawning season. The hypophysation of females is usually done with two doses of CPE (Linhart et al. 1997, Klodzinska and Okoniewski 1998, Brzuska and Adamek 1999). However, better reproduction effects were obtained when the females were treated with the analogue hormones combined with the dopamine inhibitors LH-RH with pimozid (Epler and Bieniarz 1989a, Brzuska and Adamek 1999) or Ovopel (Horvath et al. 1997), *i.e.*, the male analogue of GnRH with metoclopramide (Klodzinska and Okoniewski 1998, Brzuska 2001). Microscope observations of oocytes collected from European catfish females in fall (October) indicated that they are very similar morphologically to those collected at the beginning of the spawning season (Ulikowski 2002). Preliminary studies indicated that under controlled conditions European catfish spawners can mature sexually earlier than in the natural spawning season (Ulikowski et al. 1998). The best results were achieved when combined hormonal stimulation with Ovopel and CPE was applied (Ulikowski 2003).

The aim of his work was to develop methods for artificial European catfish reproduction before and after its natural spawning season using thermal and hormonal stimulation.

MATERIALS AND METHODS

The study material consisted of European catfish specimens three to six years old with an average body weight of 3.6 ± 2.2 kg. They had been cultivated under intensive pond culture conditions on artificial feed. During the summer the fish were held in ponds with an area of 0.2 ha and were fed once daily with trout granulate (46% protein, 14% fat) at a daily ration of 1.0-1.5% of the stock biomass. The fish were moved in October to smaller ponds (10×10×2 m) where they overwintered. Males and females were held separately at 8-10 specimens per pond. The fish were not fed during overwintering. The further treatment of the fish depended on the planned fish reproduction period. In all the experiments, the facility housing the recirculating system ponds was dark for 24 hours and no light stimulation was applied. The studies were carried out in four thermal and hormonal stimulation variants in three subsequent cultivation seasons (2000-2002).

THERMAL STIMULATION

In order to prepare spawners for earlier reproduction (groups A and B), fish were collected from ponds in the winter between December and March and moved to separate tanks (1.2 × 1.0 × 1.0 m) in the recirculating system. One party of fish was comprised of 2-4 females and 2 males. The water temperature at the moment the fish were collected was 3-5°C. Over the course of the following two to three days the water temperature in the recirculating system was increased to 16-18°C (Fig. 1). It was then maintained at a constant temperature of 18 ± 0.5°C. The fish were fed once daily *ad libitum* with trout granulate. The period during which the fish were held in group A varied from 2 to 14 weeks, while in group B they were always held for 10 weeks (Fig. 1). At the end of the thermal treatment stimulation period, 20-30 oocytes were collected from anesthetized females using a catheter (3 mm in diameter). These samples were fixed in Serra liquid (ethanol:formaldehyde:acetic acid, 6:3:1, v/v). The location of the nucleus was determined under a magnifying glass. If the majority of oocytes had migrating nuclei and the oocytes were of a similar size, it was assumed that fish were ready for hormonal stimulation (Ulikowski 2002). If these conditions had not yet

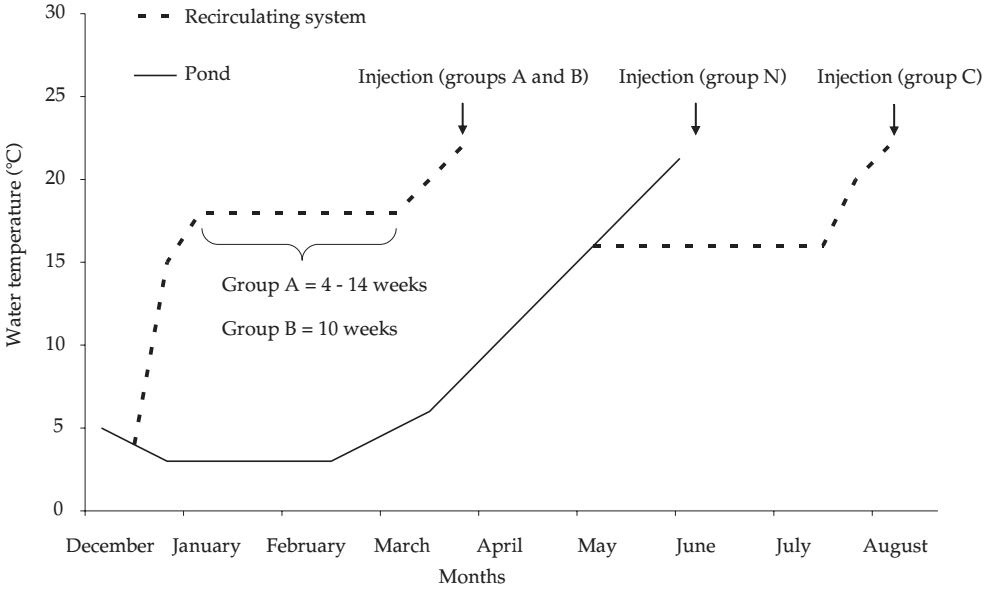

Fig. 1. Different variants of thermal stimulation in artificial European catfish reproduction. Fish groups are described in Materials and Metods.

been met, the fish were held for another week under the same conditions before repeating the oocyte investigation. When the proper degree of maturity was confirmed in the fish, the water temperature was raised to 20°C for a period of one week. Two days before the first hormone dose was administered, the water temperature was elevated to 22°C, and feeding was stopped.

In order to prepare the spawners for postponed reproduction after the natural spawning period (group C), they were collected from ponds when the water temperature reached 15°C (May) and placed in recirculating system. During the next two months the water temperature was maintained at 16 ± 1.5°C (Fig. 1), and fish were fed once a day *ad libitum* with trout granulate. Two days prior to the administration of the first hormone dose, the water temperature was raised to 22°C, and fish feeding was stopped.

The results of the induced and postponed reproduction were compared with those of artificial spawning obtained during the natural spawning period (group N). The European catfish spawners were held in the same ponds where they overwintered and were fed with trout granulate when the water temperature exceeded 16°C. The fish were moved to tanks in a recirculating system (Fig. 1) when the water temperature reached 22°C (June). The first hormonal injections were administered the following day.

HORMONAL STIMULATION

Two types of hormones were used to stimulate reproduction – Ovopel with the mammalian GnRH analog D-Ala^6, Pro^{9}Net-mGnRH (18-20 μg granule^{-1}) and the dopamine receptors blocker, metoclopramide (8-10 mg granule^{-1}) and carp pituitary extract (CPE). The Ovopel granules (average weight – 25 mg) were manufactured in Hungary and were prepared and injected similarly to CPE (Horvath et al. 1997). The hormones were administered to the females in two peritoneal injections in variants B, C, and N and in three doses in variant A (Table 1). The males were stimulated only once with a single dose of Ovopel (1 granule per kg of body weight) the day prior to spawning. After each hormone dose, the water temperature was raised by 0.5-1.0°C; following the final hormone injection, the water temperature was 23.5 ± 0.5°C.

TABLE 1

Hormonal stimulation of European catfish females – Ovopel (pellets) and carp pituitary extract CPE (mg per kg body weight). Latency – time (h) between subsequent doses. Groups are described in the Materials and Metods.

Groups	No. of females	Body weight of females (kg)	Hormonal doses			Latency
			Ovopel	Ovopel	CPE	
A	16	2.6-4.4	0.2	0.3	3	24
B	6	3.2-3.8	-	0.3	3	24
C	6	2.8-3.4	-	0.3	3	24
N	12	3.2-5.6	-	0.3	3	24

The readiness of the females to release eggs was checked every 0.5 h, starting 12 h after the final injection. All manipulation of the fish was done after they had been anaesthetized using a PROPISCIN (IFI Olsztyn) solution at a concentration of 1mg dm^{-3} (Kazun and Siwicki 2001). The eggs was fertilized with milt that had been collected from the testicles of two dead male specimens. The fertilized eggs were incubated in Weiss jars at a temperature of 23.5 ± 0.5°C. The following indexes were calculated to determine the effectiveness of reproduction: percentage of ovulating females (%); time from resolving injection to egg collection (h); number of eggs collected (% of female body weight); survival rate between spawn and hatch (%).

In order to determine the statistical significance of the differences between the averages, variance analyses and the Tukey test were performed for different N using STATISTICA 5.0 software at a significance level of $\alpha = 0.05$.

RESULTS

The application of thermal and hormonal stimulation in controlled European catfish reproduction facilitated successful spawning even five months (January 11) before and two months (August 4) after the natural spawning period of this species (Table 2). The highest treatment effectiveness, defined as the percentage of ovulating females, was obtained in group C, in which eggs were collected from all females in each of the three years of the study. In groups A and N the percentage of ovulating females was similar at 75 and 83%, respectively, while in group B eggs were obtained only in one year (from two females).

TABLE 2

Comparison of the results (average ± SD) of European catfish artificial reproduction in and outside of its natural reproductive season. Averages with the same indexes in the same column do not differ significantly statistically (P > 0.05). Groups are described in the Materials and Metods.

Group	Spawning date	Ovulating females (%)	Time from resolving injection to ovulation (h)	Relative number of eggs obtained (% of female body weight)	Survival of larvae until exogenous feeding (%)
A	Before season (11.01-20.04)	75	15.5 ± 1.0 B	13.4 ± 5.2 A	45.0 ± 16.0 A
B	Before season (26.03-12.04)	33	17.5 ± 0.5 A	7.2 ± 2.2 B	30.0*
C	After season (28.07-4.08)	100	13.5 ± 1.0 C	13.6 ± 3.8 A	48.0 ± 17.6 A
N	During season (8-16.06)	83	14.5 ± 1.0 BC	12.5 ± 2.5 A	50.0 ± 14.6 A

**including the 50% larvae with body deformations*

In comparison with group N, the time between the resolving injection and egg collection in groups A and B was longer by 1 and 3 h, respectively, and was 1 h shorter in group C (Table 2). The largest number of eggs, expressed as the percentage of body weight, was collected from group N (13.6 ± 4.5%); however, there were no statistically

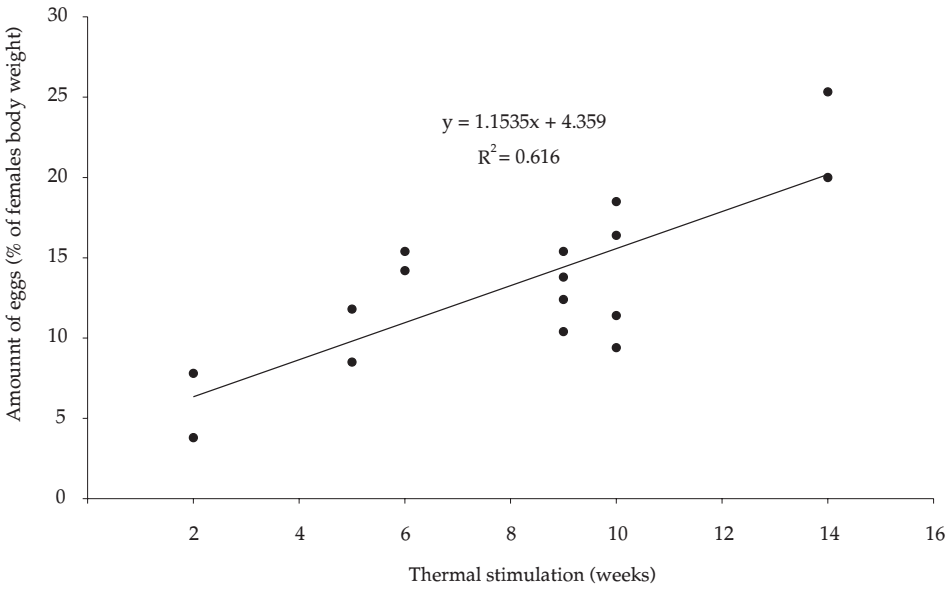

Fig. 2. Impact of different thermal stimulation periods of European catfish females on the number of eggs collected (% of female body weight) in preseason spawning.

significant differences ($P > 0.05$) between these results and those obtained for groups A and C. The number of eggs collected from group B was lower than that in groups A, C, and N (by 46, 39, and 47%, respectively); these differences were statistically significant ($P < 0.05$). The greatest variability in the number of eggs collected was observed in group A and was related to the longer maturation period of the females at temperatures of 18°C. The longer the maturation period was, the larger the number of eggs collected (Fig. 2). The largest number of eggs (average of 22.5% of female body weight) was obtained from the females that matured for the longest period of time (14 weeks).

Eggs survival to the hatch stage in groups A, C, and N was similar and higher than in group B (Table 2).

DISCUSSION

This study confirmed that the appropriate hormonal and thermal stimulation renders it possible to time European catfish reproduction between January and August. This is the foundation for developing new propagation methods and the production of stocking material of this valuable species (Ulikowski 2000). Similar propagation methods have recently been developed for several other species, such as ide, *Leuciscus idus* (L.), dace, *Leuciscus leuciscus* (L.), European perch, *Perca fluviatilis* L. or pikeperch, *Sander lucioperca* (L.) (Kucharczyk et al. 1998, 2000, Migaud et al. 2001, Kupren et al. 2003, Zakęś and Szczepkowski 2003). Light stimulation was also applied in the out-of-season propagation of perch and pikeperch. European catfish do not appear to require such stimulation as the current study was conducted in continual darkness and the results of propagation were good. This might be connected with the nocturnal life strategy of European catfish, and the fact that, in the wild, they always seek out darker rather than lighter areas. This type of life strategy means that sight is a less important sense than that of smell, taste or the lateral line (Horoszewicz 1971).

In the present study, the effectiveness of the hormonal stimulation of a combined injection of Ovopel and CPE, determined as the percentage of ovulating females, was similar to that obtained by other authors applying one type of hormone (Koldras et al. 1994, Linhart et al. 1997, Klodzinska and Okoniewski 1998, Brzuska and Adamek 1999, Brzuska 2000a, b, 2001). However, the application of only one stimulator is unsatisfactory in preseason European catfish reproduction (Ulikowski et al. 1998, Ulikowski 2003). The lower effectiveness and greater variability in reproduction results in subsequent years in group B as compared to group A indicates that one dose (0.3 granule per kg of body

weight) of Ovopel applied before CPE is not necessary to stimulate ovulation in preseason European catfish reproduction in each season. It is possible that the one-time Ovopel dose should be increased, or that the period between the Ovopel and CPE injections should be longer or even that thermal stimulation should be longer. This requires further study. Epler and Popek (2002) demonstrated that the success of stimulating fish reproduction can be affected by different environmental conditions that might increase dopamine inhibition thus resulting in ineffective GnRH interaction with the pituitary. The impact of these factors might be partially neutralized by the repeated doses of dopamine blockers; this might explain the better results obtained in group A, which was stimulated with a higher number of Ovopel doses than group B. Another very important factor that could be responsible for the positive reaction of females to hormonal stimulation might be the length of time they are held at temperatures that are conducive to ovulation. It has been demonstrated that with carp, *Cyprinus carpio* L., this period should not exceed 24-30 h (Epler et al. 1989b). With regard to European catfish, this requires further investigation.

The results of European catfish reproduction at different times of the year indicate that the period between the last injection and egg collection shortens during the season, *i.e.*, ovulation can be induced faster in summer than in the early months of the year. This period was also shortened in accelerated European catfish reproduction following the application of an additional dose of Ovopel (group A). In controlled European catfish reproduction the period between the last injection and egg collection varies depending on the type, number, and volume of the hormonal stimulator doses as well as water temperature, even during the natural spawning season. This period was twice as long with a single dose of CPE or Ovopel (Linhart et al. 1997, Brzuska 2000b) than it was with two doses of the same stimulator (Brzuska 2000a). The stimulation of European catfish females with different hormones revealed that the time between the resolving injection and egg collection was shorter after CPE had been administered than after analogue hormones (Linhart et al.1997, Brzuska and Adamek 1999, Brzuska 2000a). Yaron (1995) suggested that this period is always shorter among different fish species after hypophysation than after the administration of other stimulators and analogue hormones.

In preseason reproduction in group B, egg survival to hatch was lower than in the other groups, and about 50% of the hatch had body deformations (Table 2). Linhart and Billard (1995) reported that the ability of the oocytes to be fertilized decreases following ovulation and the number of deformed larvae increases the longer the eggs are stored in amniotic fluid prior to fertilization. Therefore, it is recommended that

egg storage does not exceed 3 h (Linhart et al. 1997). Perhaps the period during which the eggs can be fertilized in preseason reproduction is shorter than during the spawning season. This should be investigated during future studies.

CONCLUSIONS

1. It is possible to regulate European catfish reproduction between January and August with the application of special thermal and hormonal stimulation and without photoperiod stimulation.
2. During the season the time between the resolving injection and egg collection shortens; thus, ovulation can occur faster in summer than in the early months of the year.
3. During preseason European catfish reproduction, the number of eggs collected increases as the thermal stimulation period lengthens.
4. Administering Ovopel in two doses before hypophysation with CPE produces better preseason European catfish spawning effects than when only one dose is used.

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STRESZCZENIE

ROZRÓD SUMA EUROPEJSKIEGO (*SILURUS GLANIS* L.) POZA SEZONEM TARŁOWYM

Celem tej pracy było opracowanie metod rozrodu suma europejskiego poza jego naturalnym sezonem tarłowym z wykorzystaniem stymulacji termicznej i hormonalnej (rys. 1, tab. 1). Do wywołania owulacji zastosowano kombinowaną stymulację hormonalną – Ovopelu w iniekcji pobudzającej dojrzewanie oocytów (w jednej dawce 0,3 granulki lub w dwóch dawkach 0,2 i 0,3 granulki na kg masy ciała samic) i ekstraktu przysadki mózgowej karpia CPE w iniekcji wyzwalającej (3 mg na kg masy ciała samic). Odstęp

między iniekcjami wynosił 24 h. Samce stymulowano jedną dawką Ovopelu (1 granulka na kg masy ciała). Uzyskane wyniki wykazały, że przez odpowiednią stymulację termiczną i hormonalną, bez stosowania stymulacji świetlnej, można przeprowadzić sztuczny rozród suma europejskiego, w okresie od stycznia do sierpnia (tab. 2, rys. 2). W powyższym okresie stwierdzono, że czas od iniekcji uwalniającej do pobrania ikry ulegał skróceniu, czyli latem szybciej można było doprowadzić do owulacji niż zimą i wiosną. Natomiast ilość pozyskanej ikry w przedsezonowym rozrodzie była większa, gdy okres stymulacji termicznej był dłuższy. Uzyskane wyniki wykazały, że zastosowanie Ovopelu w dwóch, zamiast jednej, dawkach pobudzających wpływa korzystnie na efektywność rozrodu przedsezonowego suma wyrażoną liczbą owulujących samic i ilością pozyskiwanej ikry oraz jej przeżywalnością.