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PROOF

Comparison of the Results from Induced Breeding of European Catfish (*Silurus glanis* L.) Broodstock Reared in an Intensive System or in Pond Conditions

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Abstract

European catfish, *Silurus glanis*, is an important species for aquaculture in temperate climates. At present, its production relies mainly on pond culture. However, European catfish could be a promising candidate for intensive aquaculture since it has a high growth rate on commercial diet, is resistant to handling and has relatively low requirement for water quality. In this study we compared the results from induced breeding of European catfish broodstock reared in an intensive system or in a pond environment.

Induced breeding experiments were conducted in two consecutive spawning seasons using European catfish broodstocks with different origins. One broodstock was cultured in an intensive system, while the other was reared in artificial pond conditions. One day before the experiments, fish were transferred to the hatchery and placed in 4.0 m3 polypropylene tanks with running water. One experimental group contained fish cultured in an intensive system, while the other consisted of fish reared in pond conditions. Fish in both groups received an injection of dry carp pituitary administered in 0.7% NaCl at a dose of 4.0 mg kg^{-1} body weight. Assessment of ovulation was carried out by determining the ovulation ratio (number of ovulated females / number injected) and by the pseudo-gonadosomatic index (PGSI) calculated as follows: (weight of stripped egg mass / body weight of the female before stripping) × 100. When ovulation was detected, the eggs were stripped and weighed. Fertilization rate of the eggs was used as a parameter for egg quality.

In both spawning seasons, five out of six females reared in pond conditions ovulated after the treatment, while pituitary injection induced ovulation in all females cultured in an intensive system. Mean fertilization rates for the two groups were high and statistically similar in both seasons ($P>0.05$). The mean PGSI values, however, were significantly higher ($P<0.05$) for the females cultured in the intensive system in both seasons. In conclusion, this study shows that European catfish females reared in intensive conditions from larval stage to maturity at constant temperature of 21.0 ± 1.0°C can be induced to ovulate by pituitary injection. The reason for the high relative fecundity of these females was probably the constant temperature that secured continuous ovarian development all year round. Results from the propagation of fish reared in pond conditions were similar to those from hatchery breeding of European catfish that has been carried out routinely for years.

Keywords: Induced breeding, Silurus glanis, broodstock, intensive rearing, pond culture.

Introduction

European catfish, *Silurus glanis*, is an important species for aquaculture in temperate climates. Earlier, its production relies mainly on pond culture (Horváth *et al.*, 2002). However, European catfish could be a promising candidate for intensive aquaculture since it has a high growth rate on commercial diet, is resistant to handling and has relatively low requirement for water quality. It has a white, tasty and boneless flesh with high protein content. Currently European catfish is cultured in closed thermoregulated small-scale systems in Czech Republic and France (Linhart *et al.*, 2002).

One of the prerequisites for sustainable aquaculture is the capacity to control reproductive processes of fish in captivity and to produce high quality stocking material. Induced breeding enables farmed broodstocks to ovulate under hatchery conditions, and allows the production of eggs to be adjusted as required to suit procedures on the farm. Over the last decades the techniques of induced breeding of European catfish have continuously been developed (Horváth, 1977; Brzuska and Adamek, 1999; Linhart *et al.*, 2004). Ulikowski (2004) induced out-of-season spawning in the European catfish between January and August by applying appropriate thermal and hormonal stimulation. At present, the production of European catfish relies mainly on

breeders cultured in earthen ponds.

For efficient fish culture in intensive aquaculture systems under controlled environmental conditions the fish life cycle must be fully closed in captivity, i.e. independent of breeders reared in pond conditions. In order to satisfy the market requirements, reproduction cycles should be controlled to obtain out-of-season spawning and produce fingerlings throughout the year (Migaud *et al.*, 2002).

The objective of this study was to test weather European catfish females reared in intensive conditions from larval stage to maturity at constant temperature of 21.0 ± 1.0°C can be induced to ovulate by pituitary injection and to compare the results to those from induced breeding of broodstock reared in a pond environment.

Materials and Methods

Induced breeding experiments were conducted in two consecutive spawning seasons using European catfish broodstocks with different origins. One broodstock was cultured all year round in artificial pond conditions and exposed to natural water temperature and photoperiod normal for the time of year in Hungary. Approximately 100 fish were kept in a 1000 m^2 pond with food fish. The weight of food fish stocked into the pond was three to four times of that of the European catfish broodstock. During the period of rising water temperatures in the spring, there is a danger of wild spawning. To avoid this, in the first half of April sexes were separated. In May fish were selected and transferred to the hatchery for propagation. The body weight (BW) of females (mean ± SD) selected for the breeding experiments in the two consecutive spawning seasons were 6180 ± 1535 g and 7962 ± 1310 g, respectively. Females were 6+ years of age.

The other group of broodstock were reared from larval stage at the fish culture station of Szarvas Fish Ltd. in Szarvas, Hungary. They were kept indoors in 120 m^3 concrete tanks supplied with running well water at temperature of 21.0 ± 1.0°C and exposed to a photoperiod normal for the time of year in Hungary. The animals were fed with dry pellets (protein content: 48 %) at a ration of 1.5 % of BW. Experiments were performed with three-year-old sexually mature female fish with a BW of 1959 ± 304 g in the first year and 2114 ± 270 g in the second.

One day before the experiments, the fish reared in the pond as well as in the indoor tanks were transported to the hatchery of Szarvas Fish Ltd. The number of females used in our experiments is shown in Table 1. Fish were placed in 4.0 m^3 polypropylene tanks with running well water at 23.0 ± 1.0°C. In the tanks the broodstock can become very aggressive and they will damage each other. To keep fish from fighting in the holding tanks their mouths were tied with twine. To induce ovulation in both groups, common carp pituitary extracts were prepared in 0.7 % NaCl at a dose of 4.0 mg kg^{-1} BW. This effective dose was divided into two parts. Ten percent was administered to the females as a preliminary injection. The resolving dose (90%) was injected 12 hours later. All injections were given intraperitoneally at a volume of 0.5 ml kg^{-1} BW. At 23.0 ± 1.0°C the maturation process was completed 12 hours after the second injection and the eggs were collected by stripping. To collect milt, two male fish were opened up, their testes were removed and passed through a fine mesh.

Eggs from each female were collected into a separate bowl. Into one bowl no more than 300 g of eggs were stripped. Eggs in each bowl were evenly mixed with approximately 5.0 ml of milt and then fertilization was initiated by adding 100 ml hatchery water. The mixed gametes were gently stirred for a few minutes before the complete batch of eggs was poured into a Zuger jar. The eggs stuck to each and to the wall of the jars and they swelled to many times their original volume. The protein layer of the eggs that causes stickiness was slowly digested by common water bacteria. 18 to 24 hours following their placement into the jars the eggs were gently stirred with a plastic rod after which the eggs separate freely in the flowing water.

Assessment of ovulation was carried out by determining the ovulation ratio (number of ovulated females / number injected) and by the pseudo-gonadosomatic index (PGSI) calculated as follows: (weight of stripped egg mass / BW of the female before stripping) × 100. Egg batches were incubated separately at 23.0 ± 1.0°C. Fertilization rates were determined under a dissecting microscope 48 hours after fertilization, when clear signs of embryonic movement could be observed. Ovulation ratio was analyzed by the chi-square test ($P<0.05$). The PGSI and fertilization rate data were subjected to one way analysis of variance (ANOVA) ($P<0.05$).

Results

Results of induced breeding of European catfish broodstock reared in intensive system or in pond environment are shown in Table 1. In both spawning seasons, five out of six females reared in pond conditions ovulated after the treatment, while pituitary injection induced ovulation in all females cultured in the intensive system. Ovulation rates were statistically similar between the two groups in both experimental years ($P>0.05$, Chi-square test).

Mean fertilization rates determined 48 hours after fertilization were relatively high and statistically similar between the two groups in both experimental years ($P > 0.05$, Two-sample t-test). The mean PGSI values, however, were significantly higher for the females cultured in the intensive system (ANOVA, $P<0.05$) in both seasons. The mean PGSI values for

Table 1. Results of induced breeding of European catfish (*Silurus glanis L.*) broodstock reared in intensive system or pond environment*

	Experiment 1		Experiment 1	
	broodstock reared in		broodstock reared in	
	pond environment	intensive system	pond environment	intensive system
BW (mean ± SD)	6180 ± 1535	1959 ± 304	7962 ± 1310	2114 ± 270
Ovulation rate	5/6	11/11	5/6	7/7
PGSI (mean±SD)	6.58 ± 2.06	18.03 ± 2.47	10.2 ± 1.42	19.63 ± 2.33
Fertilization rate (mean ± SD)	71.6 ± 5.32	67.5 ± 4.37	63.6 ± 6.69	69.6 ± 4.86

* Experiments were conducted in two consecutive spawning seasons. Ovulation was induced by intraperitoneal injection with a crude preparation of dried carp pituitary. Pituitary was administered in a 0.7 % NaCl saline at a dose of 4.0 mg kg^{-1} BW for both groups. Females received two injections: 0.4 mg kg^{-1} BW as a priming dose (10%) and 3.6 mg kg^{-1} BW as a resolving dose (90%).

BW: body weight of females at the time of the first injection (grams)

Ovulation rate: number of ovulated females / number injected

PGSI: (weight of stripped egg mass / BW of the female before stripping) × 100

Ovulation rates were similar between the two groups in both experimental years (Experiment 1: Chi-Sq = 1.948; Experiment 2: Chi-Sq = 1.264; Chi-square test)

The mean PGSI values were higher for the broodstock reared in intensive system in both experimental years (Experiment 1: T-Value = 9.02; Experiment 2: T-Value = 8.33; Two-sample t-test; $P < 0.01$)

The mean fertilization rate values determined 48 hours after fertilization were similar between the two groups in both experimental years (Experiment 1: T-Value = 1.52; P-Value = 0.18; Experiment 2: T-Value = 1.70; P-Value = 0.140; Two-sample t-test)

the group of females reared in pond conditions (experiment 1: 6.58 ± 2.06; experiment 2: 10.2 ± 1.42) are similar to those from routine hatchery breeding of the species. However, the mean PGSI values were unexpectedly high for the females reared in the intensive system (experiment 1: 18.03 ± 2.47; experiment 2: 19.63 ± 2.33).

Discussion

In our experiments ovulation rates of European catfish females reared in an intensive system or in a pond environment were similar after carp pituitary injections in both experimental years. In fact, all females cultured in intensive conditions responded to the hormonal treatment. These females reached sexual maturity at a relatively young age and small size.

Growth and age at maturity in European catfish is highly variable depending upon habitat (Harka, 1984). Hochman (1967) estimated that if all optimal conditions were met, then European catfish could reach a size of 1.2–2.5 kg after 3 years in a natural environment. In cool conditions, however, a 10-year-old European catfish may only weigh 2 kg. In the East Mediterranean region of Turkey females mature at age 4 and at a minimum size of 87.1 cm TL (Alp *et al.*, 2004). In Hungary with temperate climate females generally mature at age 5 with a minimum weight around 4 kg (Horváth *et al.*, 2002).

In our experiments, females reared in intensive conditions from larval stage were kept indoors in a flow-through system supplied with well water at temperature of 21.0 ± 1.0°C. They were fed with good quality dry food according to their needs. Due to the relatively high rearing temperature and adequate rate of feeding, females attained sexual maturity i.e. underwent puberty less than 3 years. Maturation occurring both at an earlier age and at a smaller body size was probably the result of improved growth conditions and feed availability. This is commonly observed in many farmed fish species (Taranger *et al.*, 2010).

Puberty i.e. the developmental period during which an individual becomes capable of reproducing sexually for the first time is regulated by environmental factors. A range of environmental cues such as photoperiod, water temperature, rainfall, food availability, water quality and water level play a role, however, in most fish species photoperiod and/or temperature variations are the main factors controlling this period (Bromage *et al.*, 2001; Taranger *et al.*, 2010). In salmonids photoperiod is the principal determinant of maturation (Bromage *et al.*, 2001). Photoperiodic and temperature variations appear to both play a crucial role in the control of maturation in perciform species (Wang *et al.*, 2010). Maturation of many cyprinids, catfish and other tropical and sub-tropical species is principally affected by temperature (Bromage *et al.*, 2001; Horváth, 1986).

It was proved that a decrease of temperature was required to proceed with first maturation in juvenile Eurasian perch (*Perca fluviatilis*) (Migaud *et al.*, 2002) and pikeperch (*Sander lucioperca*) (Hermelink et al., 2011). A constant high temperature inhibited gonadal maturation in both percid species. In our experiments the relatively high and constant temperature provided for European catfish reared in intensive conditions did not inhibit gametogenesis, puberty was completed and the females became capable of reproducing sexually. Common carp (*Cyprinus carpio*) females cultured under tropical climate and reared at temperature above 20ºC all year round reach their sexual maturity at age 1.0-1.5 (Jhingran and Pullin, 1985). Under temperate climate,

growing season lasting approximately 5 months with an average daily water temperature above 20ºC can only secure active gametogenesis in fish. Therefore, common carp females generally mature at age 5 in Central and Eastern Europe (Woynárovich and Horváth 1980). However, when common carp females were reared from advanced-fry stage in closed system at 25ºC maturation completed in females after 1.5 years and ovulation was successfully induced subsequently by hormonal treatment (Horváth, 1985).

Further question is how constant rearing temperature influences the annual sexual cycle of the European catfish. In nature European catfish, as a single-batch spawner with a group-synchronous oocyte development, reproduce only once a year when the water temperature has reached 22-23ºC in the spring (Copp *et al.*, 2009). Common carp has similar reproductive strategy under temperate climate. However, at constant rearing temperature of 20-24ºC which secure continuous oocyte development, common carp females can be spawned four times in one year (Horváth, 1996). African catfish (*Clarias gariepinus*) with a discontinuous reproductive cycle shows a seasonal gonadal maturation in nature. Oocyte development is mostly influenced by annual changes in water temperature and the final triggering of spawning is caused by a raise in water level due to rainfall (Van Den Hurk *et al*., 1985). African catfish reared under a constant water temperature of 25°C reach sexual maturity at the age of 6-9 months. They show a continuous ovarian cycle and can be induced to produce large quantities of viable eggs all year round (Richter *et al.*, 1987). Contrary to common carp and African catfish, in perciform species gametogenesis would be impaired at constant high rearing temperature. A wintering period with temperature below 10ºC would be required to complete gonadal cycle of Eurasian perch (Migaud *et al.*, 2002), yellow perch (*Perca flavescens*) (Heidinger and Kayes, 1986), walleye (*Sander vitreum*) (Hokanson, 1977) and pikeperch (Schlumberger and Proteau, 1996). Ulikowski (2008) successfully spawned European catfish females two times in one year under controlled conditions. To reveal the effects of water temperature on the complete reproductive cycle of European catfish further research are required.

Mean fertilization rates of eggs from European catfish females reared in an intensive system or in a pond environment were relatively high and similar in both experimental years. Several factors such as malnutrition, suboptimal temperatures or other stressors during broodstock culture can negatively impact egg quality (Bobe and Labbé, 2010). According to our results, artificial conditions at the fish culture station of Szarvas Fish Ltd. did not have any negative impact on egg quality. Mean fertilization rate values from our experiments are also comparable to those from other breeding experiments on the European catfish (Brzuska and Adamek, 1999; Brzuska, 2001; 2003).

The relative amount of stripped eggs (PGSI) for the group of females reared in pond conditions are comparable to those reported by other authors (Brzuska and Adamek, 1999; Brzuska, 2001; 2003). European catfish ovaries are relatively small in size in comparison with other fish species (Hochman, 1967). In adult European catfish females, the ovary represents only 9–15% of the total body weight, which is a relatively small proportion (Mihálik, 1982). According to Shikhshabekov (1978), European catfish females show gonadosomatic index (GSI) reaching 10-12 % before spawning.

Broodstock nutrition and feeding is an important factor to affect egg production in fish (Izquierdo *et al.*, 2001). The intensity of food intake and the rate of metabolism are mainly dependant on water temperature. The optimum temperature for growth and food conversion of European catfish is above 20ºC. Food assimilation is getting reduced as water temperature decreases and below 10ºC food digestion ceases (Hilge, 1985). In nature food intake is intensive during the spring and summer and generally falls to a minimum from September. The species practically does not feed from November to March (Omarov and Popova, 1985), when it hibernates in deep holes among tree roots.

In our experiments the mean PGSI values were unexpectedly high for the females reared in intensive conditions. These fish were kept indoors in a flow-through system supplied with water at a relatively high temperature of 21.0 ± 1.0°C. It was probably the constant high temperature which secured active metabolism, intensive food intake and continuous ovarian development all year round. High rearing temperature and adequate feeding resulted in mature females with high relative fecundity.

In conclusion, this study shows that European catfish females reared in intensive conditions from larval stage to maturity at constant temperature of 21.0 ± 1.0°C matured both at an earlier age (tree years of age) and at a smaller body size (around 2.0 kg BW). They could be induced to ovulate by pituitary injection. All injected females ovulated and they produced high quality eggs. The relative amount eggs from the females reared in intensive conditions was significantly higher than that from females cultured in pond conditions. The reason for the high relative fecundity was probably the constant temperature that secured continuous ovarian development all year round.

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