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INDUCED SPAWNING AND EMBRYONIC DEVELOPMENT OF ORNAMENTAL CARP (*CYPRINUS CARPIO*) TROUGH THE APPLICATION OF PITUITARY EXTRACT

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Abstract

The present research aims to investigate the effects of using carp pituitary extract on spawning performance parameters of koi carp. The experiment was performed during the breeding season (may, 2020). The stimulation of koi carp sexual maturation was performed by injecting the breeders with carp pituitary suspension, at a water temperature of 22°C. A single injection with carp hypophysis, 3 mg/kg body weight (BW) for female and 2 mg/kg BW for male, was used for spawning induction, thus, resulting a successful ovulation. The eggs treatment was performed by applying 5.8 ml malachite green/100 liters of water. The following developmental stages of koi carp were observed and characterized: embryonic, hatchling, larval, post larval, fry and fingerling. The recorded prolificacy was 7460 eggs/kg female BW, considering that the koi carp female was at the 3rd deposit during the year 2020. The hatching started 72 hours after fertilization. Results of the current study indicate the successful induction of koi carp spawning by using carp pituitary extract.

Key words*: breeders, hatching, koi carp, pituitary extract, spawning.*

INTRODUCTION

Common carp (together with koi carp) is one of the most important freshwater fish species in aquaculture (Kucharczyk et al., 2008). The Japanese ornamental carp (*Cyprinus carpio* var. Koi) is famous for multifarious colors and patterns, making it commonly culture and trade across the world (Xue et al., 2018).

Fish reproduction has a considerable interest for both basic and applied research given the importance of this process for the maintenance of species, the high number of fish species (more than 25 000, representing a half of all living vertebrates), exhibiting very different reproductive tactics and strategies, their key position in vertebrate phylogeny and the increasing importance of aquacultured fish as a food resource (Servili et al., 2020).

Among the most significant advancements in the field of aquaculture during recent times is the development of techniques to induce reproduction in fish (Mudasir et al., 2014). Reproduction in fishes is regulated by external environmental factors that trigger internal mechanisms into action. The final event of the reproductive cycle, the release of eggs and sperm resulting in spawning, can be controlled by either placing the fish in an appropriate environment or by changing the fish's internal regulating factors with injected hormones or other substances.

One of the most important problems in modern aquaculture is obtained of high-quality gametes. For this purpose, several hormonal treatments are used to stimulate the maturity of gametes in the main marketable freshwater species (cyprinids, perches).

Production in recirculating systems also requires the use of directed reproduction techniques in order to increase the economic sustainability. In intensive aquaculture, the application of a stimulation method is necessary in order to assure high-quality gametes.

The application of a maturing agent usually influences not only the timing of maturation, but also the percentage of fish that spawn, as well as the quality and quantity of gametes.

Fishes show the broadest range of sexual patterns of any group of vertebrates (Godwin & Phillips, 2018). Hormonal stimulation in

common carp is a routine practice to enhance sperm production and control gamete maturation (Dietrich et al., 2019). Proper selection of breeders can be also considered as one of the keys to success in induced breeding. Thus, the breeders should be healthy, fully ripe and of medium size (Panda, 2016). Knowledge of fish reproductive biology is very important and contributes to the conservation of wild fish stocks through sustainable production technologies.

Therefore, understanding the reproductive aspects of fish is also very important for providing sound scientific advice in fishery management (Tessema et al., 2020). The aim of this study is to analyse the response of ornamental carp to injection with pituitary extract by evaluating the timing and duration of embryonic development stages, as well as the breeding performance in terms of prolificacy.

MATERIALS AND METHODS

The experiment was carried out during the spring of year 2020, at the Research Center for Modeling of recirculating aquaculture systems - Food Science and Engineering Faculty, "Dunărea de Jos" University of Galați.

Healthy, disease free, fully mature ripe fish exemplars, at the age 5 years, were selected as follows: the males with an average body weight (BW) of 445 g and the female with a BW of 372 g. Females and males were differentiated by shape: in females (♀), the body is plump and the genital opening is situated above the genital papilla. In males (♂), the body is slender and the genital opening is found behind the genital papilla (FAO, 2015).

On Thursday, May 7, year 2020, the breeders were brought from a pond rearing system (water temperature of 15ºC) and introduced, separately, in aquariums, at a water temperature of 20ºC. It must be pointed out that, during year 2020, until harvesting from pond, the water temperature was not favorable for koi carp reproduction.

Before the beginning of the experiment, fish were acclimatized during 2 weeks to the new stocking conditions. The breeders were fed with pellets at a feeding ratio of 2% BW, three times per day (8:00, 13:00, and 18:00). The oxygen level was over the concentration of 6 mg L^{-1}. The fish were subjected to a natural photoperiod, and the average temperature of the water was 22°C.

During the acclimatization the female spawn twice, without any stimulation (on May 9 and May 13). However, the feeding process continued, and the breeders were kept separately, as mentioned previously.

The injection of breeders with pituitary extract (prepared with saline solution and applied in a single dose) was proceed on May 19, year 2021, at 7 pm. The breeder's individual biomass was determined, and the doses were calculated as follows: 3 mg/kg BW for the female and 2 mg/kg BW for the male. The manipulation of the breeders was done with a damp cloth, without anaesthesia.

Pituitary extract was injected with sterile 1 mL syringes. The syringe needle was inserted into the base of the anal fin and the piston the pituitary extract was inserted into the general body cavity.

To avoid backflow of pituitary extract, the syringe needle was suddenly removed. After injection, breeders were placed in an aquarium with a protection grid on the bottom. To prevent infection of the eggs with *Saprolegnia*, a harmful factor specific to embryonic development, and considering that koi carp is an ornamental fish species, long-term treatment was performed with 5.8 mL of malachite green at 100 L of water.

The fertilized eggs of koi carp were observed as highly adhesive, demersal and spherical. Eggs samples were taken before fertilization and one time each hour, after the fertilization. Descriptions of the developmental stages were made by examining living specimens under the Novex Holland microscope with photo-camera.

RESULTS AND DISCUSSIONS

Determination of correct dosage of pituitary extract to be given to the breeders is very important and depends upon the size and state of maturity of the breeders as well as upon the state of maturity of the donor for the glands (Monjit & Chanda, 2014).

The time of injection depends upon the water temperature. In our case, at a temperature of 22°C, the injection was made 12 hours before the expected time for spawning.

The spawning period for common carp is typically at a water temperature ranging between 18-28°C, although spawning has been observed at water temperatures as low as 15°C (Tempero et al., 2006). Considering that ornamental carp, is a descendant of the common carp, it is assumed that the breeding conditions are similar.

According to Billard (1999), pituitary in ornamental carp is done in the same way as in common carp, but the percentage of ovulation in females is lower, often 50%, and fertility significantly lower (<100,000 eggs/kg body weight). In present experiment, the recorded prolificacy was 7460 eggs/kg female BW. Haniffa et al. (2007) performed a study in which spawning of koi carp was induced by intra-peritoneal injections of Ovaprim at a dose of 0.3 mL/kg body weight and the spawning was noticed 6 hours after the injection, at a temperature variation range of 26-28ºC. In the present study, the spawning was noticed 12hours after fertilization, at a water temperature of 22°C.

Changes in structure emphasize the thresholds between embryonic, larval and post-larval development from the onset of cleavage or epiboly, or at the time of organogenesis, respectively.

It has been observed that the eggs became translucent as development progressed. The diameter of the fertilized egg capsule ranged between 0.8-1 mm.

The incubation period of eggs depends largely on water quality parameters such as salinity and temperature (Kuo et al., 1973; Liao, 1975). Thus, in present study, after 72 hours of incubation, at a water temperature of 22ºC, the embryonic development ended and the hatching began, which lasted about 6-7 hours.

According to Kimmel et al. (1995), the newly fertilized eggs are found in the zygote period, until the appearance of the first division, at about 45 minutes from fertilization (Figure 1). The end of the zygote is marked by the appearance of the first division fold, which occurs near the animal pole. A view of the animal pole shows that the blastodisk has an ellipsoidal shape, as can be seen in Figure 1.

According to Stevens et al. (1998), embryonic development in carp is similar to that thoroughly studied by Kimmel et al. (1995) in zebrafish, *Danio rerio*.

After closing of zygote, the most important processes that take place during the blastula period are the entry of the embryo in the middle blastula period and the beginning of the epibolia. The period of the gastrula is characterized by a continuation of the epibolia. By the end of the gastrulation period, the yolk sac becomes completely swallowed.

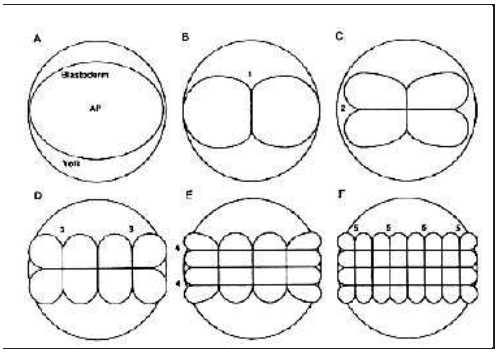

Figure 1. Graph of the animal pole during the first 5 divisions. The outer circle represents the vitellus, and the ellipsoidal ring in Fig. A represents the blastodisc before division. B-F shows the successive divisions, with the divisions seeming to cut the short axis of the blastodisc and the odd ones cutting the long axis (Kimmel et al., 1995)

In the present study, considering the description of Kimmel et al. (1995), the following stages were identified: segmentation, pharyngula and hatching period (Figure 2).

Epibolia closes because the blastoderm completely covers the yolk plug (100% epibolia) (6 hours after fertilization).

During the segmentation period the somites develop (10 hours after fertilization), the rudiments of the first organs become visible, the caudal buds become much more prominent and the embryo lengthens. The brain also acquires a tubular structure (16 hours after fertilization). The first morphologically differentiated cells and the first body movements appear (17 hours after fertilization). The somites appear consecutively on the trunk and tail (30 hours after fertilization). The rudiments of the eyes, the optical primordia (32 hours after fertilization), develop very early from the side walls of the diencephalon, giving the brain a arrowhead shape from the dorsal view (35 hours after fertilization). A characteristic strangulation that is important for

the 14-19 somite stage occurs in the posterior region of the yolk sac, where the tail buds end, and gives the vitellus a kidney shape (40 hours after fertilization). The rudiments of the crystalline and the otic ones appear digging the otic vesicle, which contains two very small otoliths at this stage (42 hours after fertilization).

This strangulation is quickly becoming more apparent. The strangled region develops into an elongation of the yolk sac, the extension of the yolk. The shape of the vitellus will continue to differ from the previous region, the vitellus ball (45 hours after fertilization). The caudal buds begin to project out of the embryo's body. The embryo enters the pharyngula period with a well-developed notochord and a recently completed set of somites extending to the end of the tail (50 hours after fertilization). In the hatching period, pigment cells differentiate; melanophores begin to differentiate at the beginning of this period, and the pigmentation looks much better during this period. The circulatory system is formed (60 hours after fertilization).

The heart starts beating right from the beginning of this period and has well-defined chamber formats. Blood begins to flow through a closed set of channels (63 hours after fertilization). During this period, the embryo continues to develop at about the same rate as in previous periods. The morphogenesis of many organs is now considerably complete and slowed down. The rapid development of the jaws, gills and pectoral fins can be observed. The development of pectoral fins is an important element especially for the beginning of the hatching period. Also specific to this period is the fact that the jaws develop, being visible even the very small mouth (70 hours after fertilization).

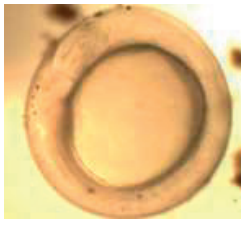

Figure 2.1. Gastrula

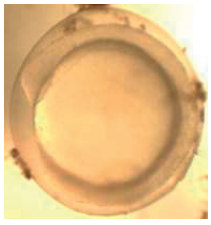

Figure 2.2. Segmentation (after 10 hours)

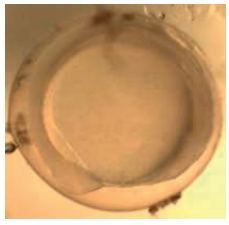

Figure 2.3. Segmentation (after 11 hours)

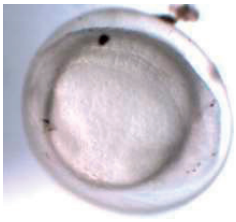

Figure 2.4. Segmentation (after 12 hours)

Figure 2.5. Segmentation (after 16 hours)

Figure 2.6. Segmentation (after 17 hours)

Figure 2.7. Segmentation (after 30 hours)

Figure 2.8. Segmentation (after 32 hours)

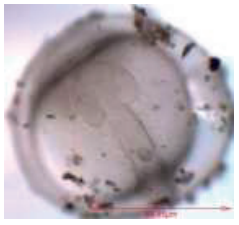

Figure 2.9. Segmentation (after 35 hours)

Figure 2.10. Segmentation (after 40 hours)

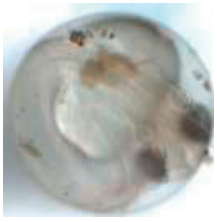

Figure 2.11. Segmentation (after 42 hours)

Figure 2.12. Segmentation (after 45 hours)

Figure 2.13. Segmentation (after 50 hours)

Figure 2.14. Hatching period (after 60 hours)

Figure 2.15. Hatching period (after 63 hours)

Figure 2. The development stages of ornamental carp

The length of larvae immediately after hatching was 6.3 mm, the mouth and anus were closed and the pectoral fin was formed (Figure 3).

Figure 3. The larva after hatch

The resorption of the yolk sac was observed 3 days after hatching (Figure 4). The larvae were fed egg yolk on the first day, *Artemia* nauplii the next day, then frozen rotifers.

Post larvae at 15 days after hatching had a total length of 12 mm, separate anal fin and dorsal membranes and fin ray. Since two month, the koi fry have been fed with frozen cyclops. On 06.08.2020, they had an average weight of 0.05 g/exp. Results of this study showed that successful induced spawning in ornamental carp was achieved by using a single dose of hypophysis, as described.

Figure 4. Larva after resorption of the yolk sac

CONCLUSIONS

The acclimatization of the female ornamental carp must be done with small variations in temperature in order not to spawn in the absence of the male.

Reproduction with pituitary extract can be used successfully in order to fructify the third deposit.

Further trials are now essential to standardize use of dosage and to gather additional information on the eggs and hatchlings of koi carp produced through pituitary extract treatment, such as their size, rate of growth, survival etc.

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REFERENCES

Billard, R. (1999). *Carp, biology and culture*. Paris, FR: INRA Publishing House.

Dietrich, M., Irnazarow, I., Inglot, M., Mikołaj, A., Jurecka, P., Steinhagen, D., & Ciereszko, A. (2019). Hormonal stimulation of carp is accompanied by changes in seminal plasma proteins associated with the immune and stress responses. *Journal of Proteomics,* 202, 103369.

FAO (2015). *Training manual on the artificial propagation of carps*. Second revised edition, ISBN 978-92-5-108689-6.

Godwin, J., & Phillips, M. (2018). Modes of Reproduction in Fishes. *Encyclopedia of Reproduction (Second Edition)*, 6, 23-31.

Haniffa, M.A., Allen, P.S., Benziger, A., Jesu, A., Nagarajan, M., & Siby, P. (2007). Breeding Behaviour and Embryonic Development of Koi Carp (*Cyprinus carpio*). *Taiwania*, 52(1), 93-99.

Kimmel, C., Ballard, W., Kimmel, S., Ullmann, B., & Schilling, T. (1995). Stages of Embryonic Development of the Zebrafish. *Developmental Dynamics*, 203, 255-310.

Kuo, C.M., Shehadeh, Z.H., & Milison, K.K. (1973). A preliminary report on the development, growth and survival of laboratory reared larvae of the grey mullet, *Mugil cephalus (L.). J. fish. Biol.,* 5, 459-470.

Kucharczyk, D., Targońska, K., Hliwa, P., Gomułka, P., Kwiatkowski, M., Krejszeff, S., & Perkowski, J. (2008). Reproductive parameters of common carp (*Cyprinus carpio L*) spawners during natural season and out-of-season spawning. *Reproductive Biology*, *8*(3).

Liao, I.C. (1975). Experiments on the induced breeding of the grey mullet in Taiwan from 1963-1973. *Aquaculture,* 6, 31-58.

Monjit, P., & Mukti, C. (2014). Induced breeding of carps. *https://www.researchgate.net/publication/261994048*

Mudasir, R., Masoodul, H. B., Gulzar, A.N., & Tamim, A. (2014). Induced Breeding of Grass Carp (*Ctenopharyngodon idella*) and Silver Carp (*Hypophthalmichthys molitrix*) Using Ovatide as Synthetic Hormone at National Fish Seed Farm (Nfsf) Manasbal, Kashmir, J&K. *Fish Aquac J*, *5*(4); ISSN: 2150-3508 FAJ.

Panda, S. (2016). A review on induced breeding in fishes. *International Journal of Bioassays,* 5.5, 4579-4588.

Servili, A., Canario, A., Mouchel, O., & Muñoz-Cueto, J. (2020). Climate change impacts on fish reproduction are mediated at multiple levels of the brain-pituitary-gonad axis. *General and Comparative Endocrinology,* 291, 113-439.

Stevens, K., Schipper, H., Samallo, J., & Stroband, H. (1998). Blastomeres and cells with mesendodermal fates of carp embryos express cth1, a member of the TIS11 family of primary response genes. *Int. Dev. Biol*., 42.

Tempero, G., Ling, N., Hicks, B., & Osborne, M. (2006). Age composition, growth, and reproduction of koi carp (*Cyprinus carpio*) in the lower Waikato region, New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 40, 4, 571-583.

Tessema, A., Abebe, G., Seyoum, M., Tadesse, F., & Eshete, D. (2020). Reproductive biology of common carp (*Cyprinus carpio Linnaeus, 1758*) in Lake Hayq, Ethiopia. *Fisheries and Aquatic Sciences*, 23, Article number: 16.

Xue, T., Xiaolei, P., Liangyan, W., Mengrong, L., Chuanju, D., Xiao, M., Lei, W., Dongying, S., Jianxin, F., Peng, X., Xuejun, L. (2018). Dynamic regulation of mRNA and miRNA associated with the developmental stages of skin pigmentation in Japanese ornamental carp. *Gene*, 666, 32-43, ISSN 0378-1119, https://doi.org/10.1016/j.gene.2018.04.054.