Int. J.MAr.Sci.Eng., 3(3), 153-158, Summer 2013
ISSN 2251-6743

The integrated effect of LHRHA$_2$ and pituitary extract on maturation of *Barbus xanthopterus*

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Received 1 March 2013; revised 23 March 2013; accepted 24 May 2013

ABSTRACT: The purpose of this project is the investigation of LHRHa$_2$ integrated effect with carp pituitary extract (CPE) in three stages injection on sexual indexes (fecundity, fertilization, hatch and larval survival) in artificial propagation of *Barbus xanthopterus*. 20 fish were divided in to 4 treatment and injected interpritoneal as follows 4mgkg^{-1} b.w.of CPE as positive control, 4 µg/kg +4 mgkg^{-1} b.w. of LHRH-A $_2$ +CPE, 7 µg/kg^{-1} b.w. + 4mgkg^{-1} b.w. of LHRH-A $_2$ + CPE , 10 µg/kg^{-1} b.w. + 4mg kg^{-1} b.w. of LHRH-A $_2$ +CPE. Results showed that the broodstocks of control treatment were answered no positive to injecting 4 mg/lit pituitary extract and second treatment (7 µg LHRHa2 + 4 mg CPE) showed highest weight of extracted egg ,spawning success, fertilization success, hatching rate and larvae survival, in comparison with other groups. None of fish were ovulated in the group of positive control. Therefore, this dose and injection method can be introduced as the most successful and practical method for artificial propagation of *Barbus xanthopterus*.

Keywords: *Artificial; CPE; Fecundity; Fertilization; Hatching; Larval survival; Propagation*

INTRODUCTION

Cyprinidae are formed a very large family of fishes and over 15 species of this genus have been identified in Khuzestan province (Najafpour *et al.,* 1996) that its important species are *Barbus grypus*, *Barbus sharpey* and *Barbus xanthopterus*. *Barbus xanthopterus* is among valuable and economic fishes of region and studies of Ramin (1999) show that main transmittal of this fish in Hur Al-Azim scope of Karkheh River until Hamidiyeh Dam and Arvand River. One of the problems related to propagation of *Barbus xanthopterus* is lack of accountability of broodstocks to pituitary extract injection in center propagation of Susangerd local fishes that is the only propagation place of this species.

The purpose of propagation of this fish is achievement to mass production and release the fish juvenile in water resources of province. According to physiology viewpoint the hormones have an important role in spawning of fishes (Sattari, 2002).

The final maturation of ovules is one of important phenomena that have the very effective role at yield and oocytes task for transmission of genetic characteristics and species traits to new generation. This stage has caused the type of polarity in cell that is associated with completion of meiosis so that oocytes nucleus has gone to one side of cell and at the same time, also ovules size quickly is increased. Oocytes begin to absorb water in many fishes at this stage that their content sometime is increased to 300-400 and this also is caused fast increase of gonad weight. It seems that this content increase be accompanied with effect of specific hormones (Babiker and Ibrahim, 1979).

Many local and valuable fishes of earth are destroy because population increase, fishing intensity, environment pollution, limited spawning areas, overfishing, nonconformity of fishing appropriate time and use of tools and equipment of destructive fishing. For this reason, artificial propagation and breeding of valuable species *B.xanthopterus* is performed in Dashte Azadegan Fish Culture Farm that it has been expressed as the main solution to increase and recreation of declining storages.

The use of hormones is played the essential role at spawning induction and acceleration of sexual maturation in some cases (Nandisha *et al.,* 1990).

Some of made hormones such as LHRHa (analogues of Gonadotropin releasing hormone) are effective at sexual maturation of fish. The purpose of this study is investigation of artificial propagation and determination of injection appropriate dose at artificial propagation of *B.xanthopterus* of *cyprinidae* family namely *Barbus xanthopterus* species that is from genus of *Barbus* fishes. The maximum results

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and best and most effective method to induction of sexual maturation of *Hypophthalmichthys molitrix* has been obtained from integration of 50 IU HCG synthetic hormone and 5 mg pituitary extract in 3 stages by examination of HCG hormone effect with pituitary extract on *Hypophthalmichthys molitrrix* (Sahafizadeh, 2001). The maximum results at integrated method of hormone and pituitary extract has been achieved in the test of GnRH effect compared with pituitary extract in artificial propagation of Common carp (Darafshan, 2003). In another research, analog effect of GnRH hormone with Domperidone Anti-dopamine (ova-fact) was examined by Linpe method with pituitary extract of Common carp on reproductive index of *Barbussharpey*i (Mohammadian *et al.,* 2009). Also pituitary extract effect with Prostaglandin was investigated at artificial propagation of *Acipenser persicus* (Karami, 2009). The study of combined effect of two GnRH and Prostaglandin hormones on semi-artificial propagation of *Carassius auratus* has shown the maximum result (Parciani *et al.,* 2009). The maximum results at injection of 4 mg pituitary extract has reported in biotechnology of *Barbus xanthopterus* propagation and comparison of $LHRH_{a2}$ and GnRH synthetic hormones effect and pituitary extract on artificial propagation of *Barbus xanthopterus* (Mortazavi *et al.,* 2010).

According to importance of object, the purpose of this project is the investigation of $LHRHa_2$ integrated effect with pituitary extract in three stages injection on sexual indexes (weigth of extract eggs, fertilization, hatch and larval survival) in artificial propagation of this valuable species.

MATERIALS AND METHODS

This study was performed in Susangerd local fish development workshop in 40 km of Ahvaz-Susangerd road in 2013. All female broodstocks randomly were selected from broodstock maintenance pools and 20 female broodstocks selected in 4 treatment groups (5 broodstocks in each group). First treatment was divided by pituitary extract dose and 4 µg $LHRHa_2$, second treatment by pituitary extract and 7 µg $LHRHa_2$, third treatment by 4 mg pituitary extract and 10 µg $LHRHa_2$ and control treatment by injection of 4 mg pituitary extract. After fishing, broodstocks were transferred into propagation salon by special tub of broodstocks shipment with oxygen and maintained in equilibrium basins to remove stress for 8 hours. Before injection, the fishes was examined based on appearance, weight, size and healthy then hormone therapy operation was injected for female broodstocks during three stages (first stage by $LHRHa_2$, second stage by 10% pituitary extract after 24 hours and third stage 90% pituitary extract after 12 hours for each kg of body weight). Female broodstocks of control treatment also was injected by 4 mg of pituitary for each kg of body weight in two stages. Hormone injection to fishes was performed intraperitoneally and under pectoral fins with angle about 45 degrees (Mouazedi *et al.,* 2001). In this research, ethylene glycol (20 ppm) was used for anesthesia of broodstocks (Gres well, 1992) and eggs extraction was done after 12 hours of final injection. In this research the dry method was used for fertilization, thus that the mix of sperm and ovum was blended with feather without water.

Incubation stages were done in Veis incubators after fertilization and washing. Hatching took place after 3-4 days and then the exited larvae were held in zook incubators (150 liter). After the absorbtion of 2/3 yolk sac, the larvae was accounted and transferred to soil pools of larvae maintenance.

SPSS software was used in order to data analysis. One-way ANOVA was used for the results and Duncan test for means comparison at 95% level.

RESULTS AND DISCUSSION

Examinations were showed that the broodstocks of control treatment were answered no positive to injecting 4 mg/lit pituitary extract also in comparison of achieved egg rate, there is no significant difference (P>0.05) between second treatment (4 mg/lit pituitary and 7 µg $LHRHa_2$ hormone) and third treatment (4 mg/lit extract and 10 µg $LHRHa_2$ hormone) and second treatment showed highest weight of extracted egg (Figure 1). First treatment (4 mg/kg pituitary and 4 µg $LHRHa_2$ hormone) had the lowest weight of extracted egg which it had significant difference with second and third treatments (P>0.05).

The maximum percent of fertilization was found at second treatment that has not observed significant difference with the results of third treatment (P>0.05). The comparison of second and third treatments results with firs treatment showed that there is a significant difference (P>0.05) (Fig. 2).

The results of hatching percent were showed no significant difference between second and third treatments of integrated method at 0.05 level (P>0.05) and second treatment has the maximum percent of hatching (Fig. 3).

There is no significant difference between second and third treatment based on the results of extracted larvae (P>0.05) and second treatment showed the maximum number of extracted larvae (Fig. 4).

The research results showed the extracted egg rate, larva number, fertilization percent and hatching percent had the maximum amount in second treatment by integrated injection of $LHRH_{A2}$ hormone and pituitary extract that based on obtained results in this test, 4 µg/kg + 7 mg/kg of hormone and pituitary

extract is effective dose and best method for induction of sexual maturation in *B. xanthopterus*. In same test that was performed by Hosseinzadeh Sahafi (2001), integration of 50 IU of HCG synthetic hormone and 5 mg pituitary extract in three stages showed the maximum results as best and effective method at induction of sexual maturation in *Hypophthalmichthys molitrix* that according to the determination of proper dose and injection method (integration of two materials in three stages), it is same with the results of this study. Investigation and comparison of pituitary injection treatments effect with HCG and metoclopramide in efficiency of artificial propagation of *Abramis brama orientalis* in a study by Dadrass *et al.* (2009) showed in response percent of broodstocks, fertilization percent, hatching percent and larval survival has observed no significant difference in treatment of HCG hormone injection along metoclopramide with control treatment and the reason of this difference with present research is species different and type of injection material. Examination of pituitary extract effect with prostaglandin in artificial propagation of *Acipenser persicus* showed 10 mg pituitary extract with propagation PGF2a is the most effective dose in sexual induction of female broodstocks and all injected broodstocks have spawned by this method (Akrami, 2009). The nearest study to this research was performed by Kahkesh and *et al.* (2010) by investigation of the effect of the types of synthetic hormones such as HCG, $LHRH_{A2}$, ovaprim, ovotide and integrated method of hormone, HCG and pituitary extract on sexual indexes of *Barbus sharpeyi* and response percent of broodstocks was obtained 87%, 94.57 ± 0.99 fertilization percent and 78.42 ± 1.65 hatching percent that the highest results have been related to integrated treatment of hormone and pituitary that corresponded with the results of present study. Mortazavi *et al.* (2010) was concluded that the best results have related to pituitary extract treatment by test of $LHRH_{A2}$ and GnRH synthetic hormones in comparison to pituitary extract that no corresponded with achieved results of this study. The lack of broodstocks response in control treatment that injected with pituitary extract is caused the environmental conditions difference of fish maintenance and the type of their nutrition. Kahkash et al. (2011) was obtained the best results in integrated method by examination of synthetic hormones effect such as HCG, $LHRH_{A2}$, ovaprim, ovotide and integrated method of $LHRH_{A2}$ and pituitary extract on sexual indexes of *Barbus grypus* that the response percent of broodstocks was 100%, 86.58 ± 0.6 fertilization percent, 81.35 ± 0.95 hatching percent and larval survival 79.23 ± 0.82 that is corresponded with achieved results of this study.

CONCLUSION

According to these results, is concluded that injected dose in second treatment has shown the best results by 7 µg LHRHa2 and 4 mg pituitary extract. Therefore, this dose and injection method can be introduced as the most successful and practical method for artificial propagation of *B. xanthopterus* that can be used for all propagation centers of province and removed many propagation problems of this fish such as lack of broodstocks response, reduction of fertilization percent rate, hatching percent and survival of larvae and according to sever need of province water sources to restructuring storages and appropriation of adequate budget for production of this species from Khuzestan Department of Fisheries, the production of this species would increased to mass production and to prevent the extinction of this species by release the juveniles in water resources.

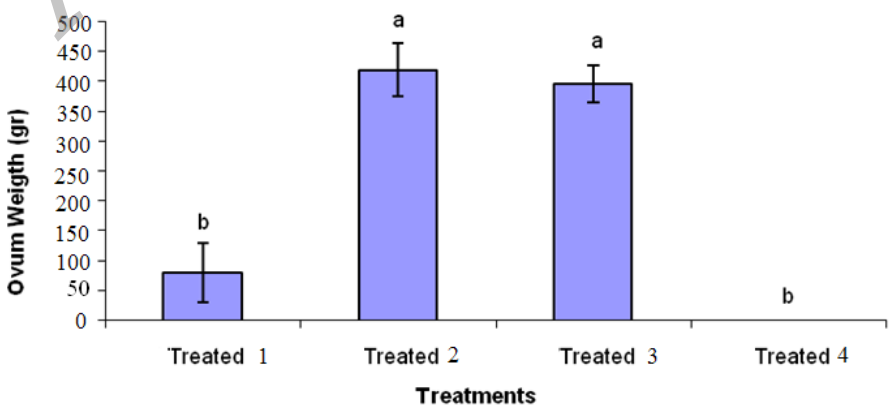

Fig. 1: effect of different hormonal treatments on extracted oocyte weight (g)

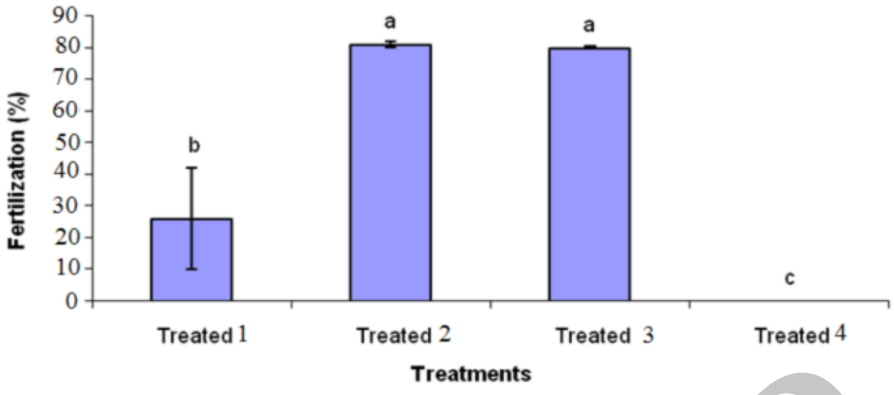

Fig. 2: effect of different hormonal treatments on Fertilization (%)

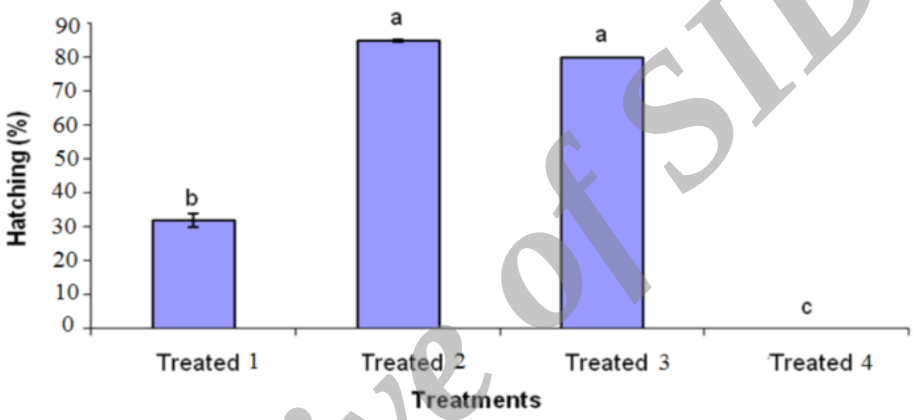

Fig. 3: effect of different hormonal treatments on hatching (%)

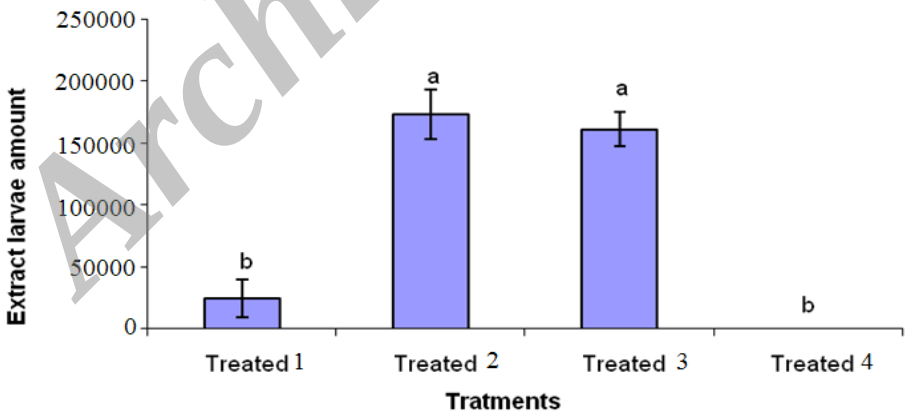

Fig. 4: effect of different hormonal treatments on extract larvae amount

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How to cite this article: (Harvard style)

Mabudi, H.; Savari, A.; Javadzadeh, N., (2013). The integrated effect of LHRHA$_2$ and pituitary extract on maturation of Barbus xanthopterus. Int. J. Mar. Sci. Eng., 3 (3), 153-158.