Asian Fisheries Science **19**(2006):149-155
ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2006.19.2.007

Asian Fisheries Society, Manila, Philippines

Successful Artificial Breeding of the Mekong Giant Catfish (*Pangasianodon gigas*, Chevey) Reared in Earthen Ponds by Boostering with Gonadotropin Releasing Hormone Analogue (GnRHa)

K. MENG-UMPHAN[1], J. MANOSROI[2] AND A. MANOSROI[2*]

[1]Faculty of Fisheries Technology and Aquatic Resources,
Maejo University,
Sansai, Chiang Mai 50290, Thailand.

[2]Faculty of Pharmacy/ Pharmaceutical Cosmetic Raw Materials and Natural Products Research and Development Center (PCRNC),
Institute for Science and Technology Research and Development (IST),
Chiang Mai University,
Muang, Chiang Mai 50200, Thailand

Abstract

Ten-year-old Mekong giant catfish (MGC) held in captivity were divided in three treatments. Treatment 1 served as the control, treatment 2 was injected once a week with gonadotropin releasing hormone analog (GnRHa) 50 μg•kg^{-1} and treatment 3 was given 100 μg•kg^{-1} during the spawning season (May-July, 2002). The highest level of Testosterone (T) in male occurred seven days after injection, but the highest Estradiol (E2) level in female occurred fourteen days after the first injection. The T and E2 levels post-injection were respectively significantly different (P<0.05) compared with the levels before injection. Before the spawning season, T and E2 were 800 ng•ml^{-1} in the males and 3,179 pg•ml^{-1} in the females, respectively. During the first and second weeks after injection, the T and E2 levels continued to increase. The male MGC achieved greater and earlier maturation than the females. More sperm density was also observed in treatment 2 and 3 as compared with the control. Better fecundity was also observed in treatment 2. We succeeded in lowering the age at which spawning and fertilization occurred by injecting the fish weekly with GnRHa. This technique will prove useful in captive breeding of MGC to increase the wild population of this endangered species in the Mekong River.

Introduction

The Mekong Giant Catfish (MGC) population in the Mekong River has declined precipitously in recent years. Only two MGC were caught in 2000 and none in 2001 and 2002, in Chiang Kong District, Thailand. The MGC is being bred in captivity in several lakes and ponds, but the captive fish takes 16-17 years to reach maturity (Phonprasit and Thevarat 1998). Earlier sexual maturity and spawning can be induced by environmental manipulation and/or exogenous hormone administration (Prat et al. 1990). Hormone induction is necessary

* Corresponding author. Tel.: +66 539 44338, Fax: +66 538 94169
E-mail address: pmpti005@chiangmai.ac.th

for both conservation and economic aspects because the spawning time of the fish in captivity can be controlled and more fingerlings can be produced. The first successful attempt to induce a 16-year old MGC (57 kg of BW) from an earthen pond took place in June 2001 at Phayao Inland Fisheries Station, Department of Fisheries. The hormone used for induction was GnRHa at 30 μg•kg^{-1} in combination with domperidone (DOM) at 10 mg•kg^{-1}. This was followed by a second injection with pituitary gland extract. This approach achieved a fertilization rate of 65% (Department of Fisheries, 2002). The GnRHa was used to avoid possible enzymatic degradation on injection and produce a more prolonged stimulation of GTH release, compared with the native GnRH peptide (Van der Kraak et al. 1983; Zohar et al. 1989, 1995a, 1995b; Goren et al. 1990).

This work represents the use of aqueous solution of GnRHa to induce sexual maturation of the 10-year old MGC. The T and E2 levels, gonad development and spawning of MGC in ponds were investigated.

Materials and Methods

Fish samples

The study was conducted from May to July 2002, on 9 males and 9 females (ten years old, 16 kg mean weight) bred in earthen ponds (1,400 m^2) in Chiang Mai, Thailand. Sex identification was performed by physical observation and confirmed by biopsy of the gonads. Fish were marked by injecting Alcian blue dye into different parts of the fish. Prepared feed containing 20% protein (8.15% fishmeal, 4.9% soybean, 4% rice bran and 3% broken rice in combination with 1% premix (vitamins and minerals) and 1% tuna oil) were given daily at 2% of fish body weight. The feed formulation was the authors' recipes.

Effect of hormone administration on testosterone (T) and estradiol (E2) level in blood

Hormone treatment

Fish were randomly divided into 3 treatment groups, (T1= control; T2= 50 μg•kg^{-1}; T3= 100 μg•kg^{-1}) as shown in Table 1. The hormone was diluted with 0.9% NaCl. Each treatment group contained 3 males and 3 females. Treatment was done weekly for four consecutive weeks. After the initial dose, the hormone treatment dosage was reduced by 50% every week until the fourth week. Blood samples were then taken one week before and every week after every injection for hormone analysis.

Testosterone and Estradiol analysis

Blood sample (0.5 ml) was taken from the caudal vasculature of each fish before and after weekly injections. The serum sample was separated by centrifuging at 10,000 rpm for 5 minutes and stored at – 30 °C until future analysis. Fifty microliters of the serum was used to determine E2 and T contents by an electrochemiluminescence immunoassay analyzer (Elecsys1010, Roche Germany). A polyclonal and a monoclonal antibody from rabbit and mouse specifically directed against 17β- estradiol and testosterone were used for the assay.

The same experimental fish were utilized for spermatozoa and egg sampling.

Effect of hormone administration on fecundity and sperm density

After a week of GnRHa injection without DOM, a 2-3 mm (outer diameter) catheter tube was inserted into the urogenital papillary of the fish for sampling oocytes and sperms. Twenty hours before oocytes and sperms were stripped, the first injection (10 μg•kg^{-1} GnRHa and 2.5 mg•kg^{-1} DOM) was administered. After six hours, a second injection (20 μg•kg^{-1} GnRHa and 2.5 mg•kg^{-1} DOM) was administered. Eggs and sperms were stripped 20 hours after the first injection. One gram of eggs was counted for determining fecundity. The sperm volume was measured using a syringe and the densities were determined by a hemocytometer slide according to Aas et al. (1991).

$$\text{Number of sperm mm}^{-3} = \frac{\text{average counting number x dilution ration}}{0.02}$$

Data and statistical analysis

The fecundity of egg, sperm volume, T and E2 levels of the fish were presented as average values (mean ± standard error). One way analysis of variance (ANOVA, $p<0.05$), and Least Significance Difference test (LSD) of the average E2 and T level, were analyzed by Statistic Package for Social Science (SPSS).

Results

Testosterone (T) and Estradiol (E2) Level

The results of this experiment are presented in Figs. 1 and 2 and Tables 1 and 2. The Serum T and E2 levels in males and females were significantly higher one week after the initial injection compared with before injection (Figs. 1 and 2). After the second injection, the E2 level had increased to 3,179 pg•ml^{-1} in females but T level had decreased to 74 ng•ml^{-1}. After the third injection, the T in males slightly decreased but E2 in females increased to 7,272 pg•ml^{-1}. After the fourth injection, the T seemed to be stable but E2 had decreased in the males and females, respectively (Table 2, Figs. 1 and 2).

On June 15, 2002, exactly 40 days after the treatment began (May 6, 2002), the development of eggs and sperms were investigated. Sperms were stripped from all treated groups and had good motility. Moreover, the sperm density was observed to be higher in the treated group than in the control group (Table 2). On the other hand, only those females which had been treated with 50 μg•kg^{-1} of GnRHa could be stripped. The sperms were used to fertilize the eggs from the female fish. About 300 fingerlings of MGC were obtained. The eggs ranging from 1.2 to 1.4 mm in diameter were obtained from the 17 kg MGC treated with 50 μg•kg^{-1} of GnRHa. After two injections with 10 μg•kg^{-1} of GnRHa in combination with 10 mg•kg^{-1} DOM, 401 grams of eggs were stripped and fertilized with the diluted sperms (23 ml). Unfortunately, fertilization was unsuccessful. However, when fertilization was performed on the diluted sperms from MGC treated with 100 μg•kg^{-1} of GnRHa and about 300 MGC fingerlings were obtained.

After 75 days of the fourth injection, fish from the treatment group that was injected with GnRHa at 50 μg•kg^{-1} kg was injected four times with GnRHa at 6 h. intervals. These injections continued with GnRHa at 5, 10, 15 and 15 μg•kg^{-1} in combination with DOM (2.5 mg•kg^{-1}) for the 17 kg females, while the 16 kg male was injected with GnRHa at 5 μg•kg^{-1} in

combination with DOM at 2.5 mg•kg^{-1}. This led to the first successful fertilization of the 10-year old MGC reared in earthen ponds. Eleven MGC fingerlings were obtained. Cumulative spawning numbers for male and female from each treatment were obtained and the highest number was from those treated with 50 μg•kg^{-1} and the lowest was from those treated with 0.9% NaCl (Table 2).

Table 1. The dosage treatment of GnRHa in aqueous solution by weekly injection in fish.

Treatment No.	Sex	Number	Average Weight	Concentration of sample	Amount (ml.BW^{-1})
T1	Male	3	14.53±3.89	O.9% NaCl	2.07
	Female	3	16.77±5.33		2.39
T2	Male	3	16.40±0.20	50 μg•kg^{-1} GnRHa in 0.9% NaCl	2.34
	Female	3	16.67±4.04		2.38
T3	Male	3	16.00±3.12	100 μg•kg^{-1} GnRHa in 0.9% NaCl	1.86
	Female	3	16.83±4.65		2.40

Table 2. The sperm density and volume (June 15, 2002)

Treatments	Sex	June 15, 2002			July 26, 2002
		Sperm density•mm^{-3}			Cumulative No (N=3)
		Weight (kg)	Volume (ml•kg^{-1})	$\bar{X}$+SEM	(No)
T1 (NaCl 0.9%) (1 ml•kg^{-1} weekly)	male	14.1	1.41	3,567,000+171,208	2
T2 (GnRHa 50 μg•kg^{-1})	male	16.6	1.38	5,418,833+485,743	3
T3 (GnRHa 100 μg•kg^{-1} weekly)	male	14	2.13	5,084,000+226,042	3

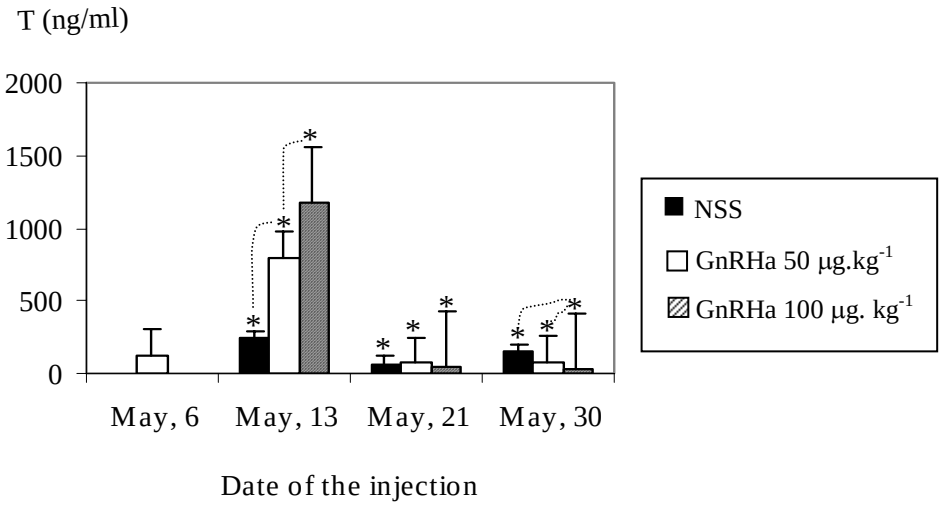

Fig. 1. The average serum testosterone (T) levels (ng•ml^{-1}) in male *P gigas* (10 years old, weighed 16 kg) reared in pond at Maejo University before (May 6, 2002) and after weekly injection (May 13, 21 and 30, 2003) of 0.9% NaCl solution (NSS, ■), the artifical hormone (GnRHa) at 50 (□) and 100 μg•kg^{-1} (▨) the data is represented as the mean ± SEM. Asterisks located on the broken line connecting two values indicate the differences between them at $^{*}P$ <0.05.

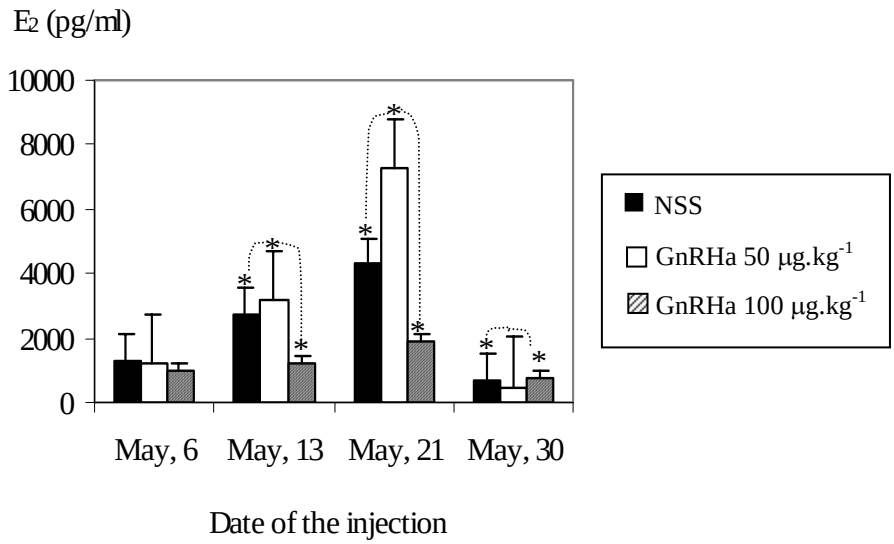

Fig. 2. The average serum estradiol levels (E2, pg•ml^{-1}) in female *P gigas* (10 years old, weighed 16 kg) reared in ponds at Maejo University before (May 6, 2002) and after weekly injection (May 13, 21 and 30, 2003) of 0.9% NaCl solution (NSS, ■), the artifical hormone (GnRHa) at 50 (□) and 100 μg•kg^{-1} (▨) the data is represented as mean ± SEM. Asterisks located on the broken line connecting two values indicate the differences between them at $^{*}P$ <0.05.

Discussion

The weekly GnRHa administration to the 10-year old, 16 kg MGC during the spawning season (May-July, 2002) affected the T and E2 levels both in male and female fish. The T and E2 levels of 800 ng•ml^{-1} and 3,179 pg•ml^{-1} respectively, peaked in May one week after injection. Levels of T and E2 in male and female fish were significantly higher than before injection. During the first and second weeks after injection with GnRHa at 50 μg•kg^{-1}, T and E2 levels increased sharply. The T and E2 levels in May in male and female fish (10 years old) were higher than what was obtained in the past of the eight and nine years old fish. The T levels of MGC had declined after the third week of injections, but the E2 level still increased. This may be due to that T is a precursor of E2 during vitellogenesis (Scott et. al. 1983). Similar results were previously reported by Nagahama (1987). Testosterone (T) was a precursor for E2 production in Chum Salmon (*Oncorhynchus keta*), but the T level decreased four weeks after the injection. It seemed to be higher in the treated group than in the control group. The treatment of Salmon gonadotropin (sGTH) and 17α-hydroxyprogesterone (17αP) in lyophilized gelatin emulsion (LG emulsion) to induce vitellogenesis and ovulation induction was studied by Sato et al. (2000). Weekly injections of Salmon gonadotropin (sGTH) in Japanese eel (*Anguillar japonica)* were more effective than biweekly injections in elevating T, E2 levels and inducing vitellogenesis. This was so even though the weekly dose (2 mg•kgl^{-1}) was half of the biweekly dose (4mg•kg^{-1}).

Concerning cumulative spawning, fish after the fourth injection of GnRHa (without DOM) during the spawning season (May-July) had greater spawning numbers than in the control group. Moreover, the MGC reared in earthen ponds are known to have longer period

of spawning from May to July compared with the MGC in the Mekong River. The spawning period occurs only in April and May (Phonprasit and Thevarat 1998). On the other hand, the MGC males responded to GnRHa and the spawning period was better and earlier than the females. Based on the cumulative spawning numbers, a maximum dose of GnRHa by half reduction of weekly injection should be 50 μg•kg^{-1}. Prat et al. (2001) reported that European Sea Bass (*Dicentrachus labrax* L) females in late vitellogenesis were treated with two injections of GnRH analogue alone (5 and 20 μg•kg^{-1} BW) given 12 hrs apart, or in combination with pimozide (PIM 10 mg•kg^{-1} BW) either in the first or second injection. The GnRHa alone or combined with PIM accelerated the final oocyte maturation and induced spawning.

The number of eggs spawned was the highest in the group that received GnRHa alone and lowest in the group that received PIM in the second injection. Estradiol (E2) increased sharply 12 hrs after the first injection of GnRHa alone or combined with PIM. Females that received PIM in the second injection still showed elevated plasma E2 for a longer period. Plasma testosterone (T) increase was parallel to the plasma E2 decrease and peaked earlier in the group that received GnRHa alone in both injections. Similarly, hormonal administration in Reeves Carp, a Chinese endangered species has improved oocyte development (Wang et al. 1998). However, the fertilization rate and hatching rate of MGC (10 years old) eggs were low. This may be due to the induced early maturation that eggs were not able to attain full maturity. The successful artificial breeding using a 16-year old MGC from earthen ponds at the Prayoa fisheries station was obtained. Also, a 1.5-year old *P. hypohthalmus* (1 kg weight) treated with 100-300 μg•kg^{-1}, of GnRHa and 5-20 mg•kg^{-1} of DOM in soybean oil has showed better development in sex hormone level, GSI value, development of oocytes and fertilization rate (Manosroi et al. 2004).

Conclusion

In conclusion, the hormone level (T and E2) of the MGC was elevated after the treatment with GnRHa. The fecundity of oocytes and the sperm density were also improved under the hormone treatment regimen. Fertilization was also achieved in our experiment, but it was low. This study has suggested a technique to lower the age at which spawning and fertilization occurred by injecting the fish weekly with GnRHa.

Acknowledgments

We would like to thank the Thailand Research Fund (TRF) for the funding. We appreciate the suggestion and help of Assoc. Prof. Dr. Padermsak Charayapan, Dr. Aphichart Termwitchakorn and Dr. Somsri Ngamwongchon for the useful suggestion and Mr. Rogelio Carandang Jr. for correcting the manuscript.

References

Aas, G.H., T. Rafstic and B. Gjarde. 1991. Evaluation of milt quality of Atlantic Salmon. Aquaculture 95:125-132.

Department of Fisheries. 2001. The first successful artificial propagation of the Maekong gaint catfish (*Pangasianodon gigas*, Chevey) from earthen pond. Bangkok. 45 pp.

Haddy, A.C. and N.W. Pankhurst. 1999. Stress- induced changes in concentration of plasma sex steroids in black bream. Journal of Fish Biology 55:1304-1316.

Goren, A., Y. Zohar, M. Fridkin, E. Elhanati and Y. Koch. 1990. Degradation of gonadotropin releasing hormone in the gilthead seabream, *Sparus aurata*; I. Cleavage of native salmon GnRH, mammalian LHRH and their analogs in the pituitary. General Comparative Endocrinology 79:291-305.

Manosroi, J., K. Meng-Umphan and A. Manosroi. 2004. Maturation induction of *Pangasius hypophthalmus* using gonadotropin releasing hormone analogue (GnRHa) in combination with domperidone , in oil suspension dosage forms. Asian Fisheries Science 17:39-49.

Nagahama, Y. 1987. Gonadotropin action on gametogenesis and steroidogenesis in teleost gonads. Zoology Science 4:209-222.

Prat, F., S. Zanuy, M. Carrillo, A. De Mones and A. Fostier. 1990. Seasonal changes in plasma levels of gonadal steroilds of sea bass, *Dicentrachus labrax* L. General Comparative Endocrinology 78:361-373.

Prat, F., S. Zanuy and M. Carrillo. 2001. Effect of gonadotropin-releasing hormone analoge (GnRHa) and pimozide on plama levels of sex steroids and ovarian development in sea bass (*Dicentrarchus labrax* L). Aquaculture 198:325-338.

Phonprasit, S. and P. Thevarat. 1998. The Maekong giant catfish biology and breeding. Thai Fisheries Gazette 51:107-115.

Scott, A.P., J.P. Sumpter and P.A. Hardiman. 1983. Hormone changes during ovulation in rainbow trout (*Salmo garirdneri*, Richardson*).* General Comparative Endocrinology 49:18-134.

Sato, N., I. Kawazoe, Y. Suzuki and K. Aida. 2000. Adequate interval of sGTH adninistration to induce vitellogenesis, and induction of ovulation by sumultaneous adninistration of sGTH and 17α-hydrosyprogesterone in the Japanese eel. Fisheries Science 66:644-654.

Van der Kraak, G., H.R. Lin, E.M. Donalson, H.M. Dye and G.A. Hunter. 1983. Effects of LH-RH and des-Gly^{10} –[$D\text{-}Ala^{6}$]-LH-RH ethylamide on plasma gonadotropin levels and oocyte maturation in adult female Silver carp (*Hypophthalmichthys molitrix*) and goldfish (*Carassius auratus*). Aquaculture 78:81-86.

Wang, H.P., K.J. Wei, H. Yao, J.J. Lin and J.B. Mai. 1998. Induced Ovarian Development, Maturation and Ovulation of domestic Reeves Shad by Hormone Implatation and Injection. Asian Fisheries Science 11:43-50.

Zohar, Y., M. Tosky, G. Pagelson and Y. Finkelman. 1989. Induction of spawning in the gilthead seabream, *Sparus aurata*, using [$D\text{-}Ala^{6}$-Pro^{9}Net]-LHRH: comparison with the use of HCG. Israeli Journal. Aquaculture 41:105-113.

Zohar, Y., A. Goren, M. Fridkin, E. Elhanati and Y. Koch. 1990. Degradation of gonadotropin-releasing hormones in the gilthead seabream *Sparus aurata*: II. Cleavage of native salmond GnRH, mammalian LHRH and their analogs in the pituitary, kidney and liver. General Comparative Endocrinol 79:306-331.

Zohar,Y., M. Harel, S. Hassin and A. Tandler. 1995a. Gilthead sea bream (*Sparus aurata*). In: Broodstock managemant and Egg and Larval Quality. (ed. N.R. Bromage and R.J. Roberts), pp. 94-117. Blackwell, Oxford.

Zohar,Y., A. Elizur, N.M. Sherwood, J.F. Rivier and N. Zmora. 1995b. Gonadotropin releasing potencies of the three native forms of gonadotropin releasing hormones present in the brain of gilthead seabrem (*Sparus aurata*). General Comparative Endocrinology 97:289-299.