Pertanika J. Trop. Agric. Sci. 36 (1): 71 - 78 (2013)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Induced spawning of a river catfish *Hemibagrus nemurus* (Valenciennes, 1840)

Adebiyi, F. A.[1], Siraj, S. S.[1]*, Harmin, S. A.[2] and Christianus, A.[1]

[1]*Department of Aquaculture, Faculty of Agriculture, Universiti Putra Malaysia. 43400 UPM Serdang, Selangor, Malaysia*

[2]*Center for Land and Aquatic Technology. Faculty of Science and Biotechnology, Universiti Industri Selangor, 45600 Bestari Jaya, Selangor, Malaysia*

ABSTRACT

There is a developing interest in *Hemibagrus nemurus* culture. Most hatcheries depend on fry from the wild, which is under threat due to human activities. Hatchery production of *H. nemurus* is not well established due to problems associated with artificial propagation. Good egg quality is essential for fry production. This paper reports the findings of research conducted to determine the effects of mammalian gonadotropin releasing hormone analogue (GnRHa) and ovaprim on induced spawning performance and egg quality of *H. nemurus*. Mature female *H. nemurus* were treated with 0.5ml of 0.7% NaCl (control), 5µg/kg, 20µg/kg, 50µg/kg body weight (BW) of fish GnRHa and 0.5ml/kg BW of fish of ovaprim (20µg/ml sGnRHa and 10mg/ml domperidone). The results showed that the administration of 5µg/kg BW GnRHa is not sufficient to induce the spawning of *H. nemurus*. The egg quality of fish treated with GnRHa at doses 20µg/kg and 50µg/kg were numerically different, however, there was no significant difference (P>0.05). Ovaprim treated fish produced the best results. Ovulatory response ranged from 50.0% to 66.7% among all the treatments with high fertilization rate (94-95%). The highest hatching rate (78.3%) and percentage of normal larvae (68.8%) was observed from ovaprim injected fish. The results indicated that 20µg/kg and 50µg/kg BW GnRHa were effective in inducing ovulation and spawning in *H. nemurus* and ovaprim can be recommended to be used by hatcheries to induce *H. nemurus* for spawning. The major significance of this work was 5µg/kg dose of GnRHa is ineffective for ovulation in *H. nemurus*.

ARTICLE INFO

Article history:
Received: 24 May 2011
Accepted: 6 June 2011

Keywords: Catfish, Egg quality, *Hemibagrus nemurus*, Hormone, Induced spawning, Ovulation, GnRHa, Reproduction

E-mail addresses:
adebiyi_fatima@yahoo.com (Adebiyi, F. A.),
shapor@putra.upm.edu.my (Siraj, S. S.),
sharrazny@unisel.edu.my (Harmin, S. A.),
anniechr@yahoo.com (Christianus, A.)
* Corresponding author

ISSN: 1511-3701

INTRODUCTION

Gonadotropin releasing hormone analogue (GnRHa) has been successfully used in the induced spawning of several fish species (Zohar and Mylonas, 2001; Rainis and Ballestrazzi, 2005). In some fish, dopamine prevents the release of gonadotropin through inhibitory actions on the pituitary in the endocrine system, thereby making it impossible for fish to ovulate and spawn. Pre-spawn fish exhibiting dopaminergic tone were induced to spawn using GnRHa in combination with dopamine antagonist like metoclopramide, haloperidol, pimozide and domperidone. Successful spawning of some fish species were achieved using this technique. GnRHa and metoclopramide (Drori *et al.,*1994), GnRHa and haloperidol (Arabaci and Sari, 2004), GnRHa and pimozide (Tan-Fermin *et al*., 1997; Park *et al*., 2007) and GnRHa and domperidone (Manosroi *et al*., 2004; Sahoo *et al*. 2005) were reported to be successful to induce ovulation and spawning in fish.

Hemibagrus nemurus is a tropical freshwater catfish that belongs to the Family *Bagridae* and widely distributed in Asian countries which include: Thailand, Laos, Cambodia, Vietnam, Indonesia and Malaysia (Rainboth, 1996). It is economically important for aquaculture and fisheries; and nutritionally valued for low cholesterol, high protein and omega-3 poly unsaturated fatty acid (Mesomya *et al*., 2002). Due to the high demand of the fish for food, most of the natural stocks of fish species are being depleted as a result of man's activities and environmental degradation. There are few government and private hatcheries practising artificial propagation of this fish, and the broodstocks are mostly obtained from the wild. The intensive and commercial culture of *H. nemurus* is not well established due to the problems associated with induced spawning, some of which include simultaneous maturation of both male and female fish and availability of high quality broodstocks (Muchlisin *et al.,* 2004). Interest in the aquaculture industry of *H. nemurus* is developing (Khan *et al*., 1990), thus there is a need to intensify studies on the reproductive biology of *H. nemurus* so as to be able to use such information for aquaculture production and management.

Khan *et al*. (1990) provided baseline data for induced breeding of this fish, such as fecundity, gonadosomatic index (GSI) and seasonal variations of oocyte diameters. Besides, Christianus *et al*. (1998, 1999) presented information on the reproductive biology of male *H. nemurus* with respect to gonadal development.

Spawning performance is an important indicator for successful induced breeding programme. Thus, this study was attempted to determine the effect of GnRHa and ovaprim on the spawning performance and egg quality of *H. nemurus*.

MATERIALS AND METHODS

Experimental Animals and Maintenance

One hundred adult male and female *H. nemurus* collected from the wild (Pahang

River, Pahang, Malaysia) were used for this experiment. Fish body weight ranged from 440 - 900g and standard length measured ranged from 30.5 to 39.4cm. Upon arrival the fish were disinfected with potassium permanganate and kept in 40 tonnes flow-through concrete rectangular tanks under natural photoperiod and temperature for acclimatization. Fish were fed at a rate of 3% body weight to satiation with commercial pelleted feed containing 38 - 40% crude protein, 3% crude fat, 6% fiber and 13% moisture. Thirty females and 15 males were randomly selected and transferred into 2 tonnes round fibreglass tanks and were used for the following experiments. Physico-chemical parameters like temperature, dissolved oxygen and pH were monitored and recorded. These parameters were maintained at 27.0 - 27.5°C, 7.72 - 8.02mg/l and 7.74 - 7.80 respectively.

Hormone Preparation and Administration

Hormones (D-Ala^6-Pro^9-NET)-LHRH (GnRHa) and ovaprim (sGnRHa and Domperidone) were purchased from Syndel laboratories, Canada. 1mg GnRHa was dissolved in 10ml of 0.7% NaCl solution. The solution was divided into 10 aliquots and each aliquot was stored in 1ml glass vial. (1ml=100µg GnRHa). The hormone was stored in freezer at -20°C until used. Ovaprim was stored at room temperature. Hormones were administered by intramuscular injections below the dorsal fin of the fish.

Fish Selection and Identification

All fish were anaesthetized with clove oil (0.1ml/l) before handling. Mature fish were used for this experiment and were identified by the genital papillae. Males have elongated genital papillae with reddish tip and released milt upon pressing the abdomen, while the females have round genital papillae and were identified by ovarian biopsy. Eggs were collected and examined under microscope for the germinal vesicle (GV) position and oocyte diameter (OD). Fish having oocytes with the GV migrating towards the periphery were selected. These fish were then tagged with coloured plastic tags for easy identification based on treatment groups.

Experimental Design

Both the male and female fish were injected with hormone to induce spermiation and ovulation. The fish were divided into 5 groups. The first group was injected with 0.5ml of 0.7% saline solution and used as a negative control. The second group received 5µg/kg BW GnRHa hormone treatment, the third group 20µg/kg BW GnRHa, the fourth group 50µg/kg BW GnRHa hormone treatment and the fifth group 0.5ml/kg BW of ovaprim (20µg/ml sGnRHa and 10mg/ml domperidone) as a positive control. Each group comprised of 9 fish (6 females and 3 males).

Ovulation rate was measured as the percentage of females that ovulated in a treatment group. Ovulated eggs were checked under the microscope for germinal

vesicle breakdown. Artificial fertilization was carried out using the dry method. Artificial fertilization of spawn from each fish was carried out in triplicates for statistical reasons. Fertilized eggs were incubated in 5 litres plastic tanks. To determine the fertilization rate, 3 subsamples were collected (200 eggs per sample) and embryo at 4-cell division stage was characterized as fertilized. To determine the hatching rate, the subsamples of the fertilized eggs were collected, placed in 1000ml beakers with aeration and observed until hatching. Hatching occurred around 24-28 hours after fertilization. Normal larvae (Fig.1) and abnormal larvae (larvae with deformed body, curved spine, round yolk sac, short and thick body, enlarged fin folds, big yolk sac not proportional to the body) were counted and recorded. Survival rate was determined by counting the number of larvae after 4 days of hatching.

Fig.1. Normal Larvae of *H. nemurus*

Statistical Analysis

All data analyses were carried out using a computer Statistical Analysis Software (SAS). Data were expressed as mean ± standard error of mean. One way analysis of variance (ANOVA) followed by Duncan's New Multiple Range Test (DMRT) was used for the variation in treatment means and $P < 0.05$ was selected as the level of significance.

The following formulas were used to calculate the egg quality parameters:

$$\text{Ovulatory response} = \frac{\text{Number that spawn}}{\text{Number induced}} \times 100\%$$

$$\text{Fertilization rate} = \frac{\text{Number of fertilized eggs}}{\text{Number of eggs incubated}} \times 100\%$$

$$\text{Hatching rate} = \frac{\text{Number of hatched eggs}}{\text{Number of fertilized eggs}} \times 100\%$$

$$\text{Percentage of normal larvae} = \frac{\text{Number of normal larvae}}{\text{Number of hatched eggs}} \times 100\%$$

$$\text{Percentage of abnormal larvae} = \frac{\text{Number of abnormal larvae}}{\text{Number of hatched eggs}} \times 100\%$$

$$\text{Survival rate} = \frac{\text{Number of larvae after 4 days}}{\text{Number of hatched eggs}} \times 100\%$$

RESULTS AND DISCUSSION

Table 1 shows the number of fertilized eggs, hatched eggs, normal larvae, abnormal larvae and survived larvae of induced spawn *H. nemurus*.

There was no ovulatory response in the control and 5µg/kg BW GnRHa groups. This indicated that for a successful induce spawning using GnRHa, higher doses are required. Hence, based on these results, the major significance of this study was that 5µg/kg BW GnRHa dose is ineffective for ovulation in *H. nemurus*. Ovulatory

TABLE 1
Number of fertilized eggs, hatched eggs, normal, abnormal and survived larvae of induced spawn *H. nemurus*.

Treatments	Fertilized Eggs	Hatched Eggs	Normal Larvae	Abnormal Larvae	Survived Larvae
Control	0.00± 0.00 [b]	0.00± 0.00 [c]	0.00± 0.00 [c]	0.00 ±0.00 [c]	0.00±0.00 [c]
5µg/kg BW GnRHa	0.00± 0.00 [b]	0.00± 0.00 [c]	0.00 ±0.00 [c]	0.00 ±0.00 [c]	0.00±0.00 [c]
20µg/kg BW GnRHa	188.33±4.41[a]	107.72±1.28 [b]	58.54±0.72 [b]	49.15±0.59 [a]	46.00±1.06 [b]
50µg/kg BW GnRHa	187.91±0.80 [a]	100.96±0.55 [b]	58.69± 0.43 [b]	42.25±0.17 [b]	40.68±0.33 [b]
Ovaprim	189.99±0.96 [a]	148.76±0.66 [a]	102.30±0.48 [a]	46.41±0.29 [ab]	62.23±0.11 [a]

[a]Data are mean ± SEM and based on subsamples of 200 eggs.
[b]Numbers with the same superscripts in a column are not significantly different ($p > 0.05$)
Number of female fish used per treatment = 6

responses of 50% were observed in fish injected with 20µg/kg BW GnRHa and ovaprim which is the positive control group. When compared to the ovulatory response of wild catfish *Silurus asotus,* GnRHa at doses of 0.01 - 0.02µg/g induced a low ovulation response of 25.0 - 37.5% (Wen and Lin, 2004), but in catfish *Pangasius hypophthalmus*, a higher ovulatory response (88%) was obtained using ovaprim (Legendre *et al.,* 2000). Ovaprim (sGnRHa (20µg/kg) and domperidone (10mg/kg) is ideal for induction of spawning in the Asian catfish *Clarias batrachus* for high ovulatory response (Sahoo *et al*., 2005). In this study, *H. nemurus* produced a high ovulatory response of 66.7% when treated with 50µg/kg BW GnRHa. On the contrary, 50µg/kg BW GnRHa could not induce ovulation in the Asian catfish *Clarias macrocephalus* (Tan-Fermin *et al*., 1997) and in the stinging catfish *Heteropneustes fossilis* (Tharakan and Joy, 1996).

A high fertilization rate (94-95%) was obtained with fish injected with 20µg/kg GnRHa, 50µg/kg GnRHa and ovaprim; and fertilization rates were not significantly different ($p > 0.05$) among these treatments. In this study, ovaprim resulted in the highest fertilization rate (95%). Ovaprim was found to produce high fertilization rate of 98.31% in African catfish *Heterobranchus bidorsalis* (Nwokoye *et al.,* 2007) and 73% in *Pangasius sutchi* (Chand *et al*., 2011). Thus, indicating that, GnRHa is capable of producing high fertilization rates when used to induce spawn fish.

Hatching rate was significantly different ($P<0.05$) among all the treatments. A high hatching rate (78.3%) was obtained from fish treated with ovaprim. Ovaprim injected catfish *Pangasius hypophthalmus* resulted in 72 ± 25% hatching rate (Legendre *et al*., 2000). A high hatching rate (98.35%) was also reported by Nwokoye *et al*. (2007) in ovaprim treated African catfish

Heterobranchus bidorsalis. The same hormone produced hatching rate of 60% in Asian catfish *Heteropneustes fossilis* (Haniffa and Sridhar, 2002). This signifies that ovaprim has a high potential for induced breeding of catfishes producing high hatching rates. In this study, 20µg/kg GnRHa and 50µg/kg GnRHa resulted in hatching rates of 57.2% and 53.3% respectively and there was no significant difference ($P > 0.05$) in the hatching rate of *H. nemurus* treated with 20µg/kg and 50µg/kg GnRHa. Some catfishes require higher doses of GnRHa to spawn. A higher dose of 100µg/kg GnRHa was used in channel catfish *Ictalurus punctatus,* and hatching rate of 69±5% was recorded (Silverstein *et al*.,1999).

The percentage of normal larvae obtained from the different treatments was significantly different ($P<0.05$). The highest percentage of normal larvae (68.8%) was obtained using ovaprim, followed by 50µg/kg GnRHa (58.1%) and 20µg/kg GnRHa (53.4%). Results of 20µg/kg GnRHa and 50µg/kg GnRHa were not significantly different ($P>0.05$). This study showed that ovaprim produced the lowest percentage of abnormal larvae. A larval survival rate of 40 - 43% was observed for all the treatments. Similarly, a low survival rate (38%) was observed in ovaprim treated Asian catfish *Heteropneustes fossilis* (Haniffa and Sridhar, 2002). The low survival rate might be due to some external factors such as water quality, temperature and feeding of larvae. Further studies are needed to investigate the effect of these factors on larvae survival. Thus, based on the findings of this study, it is obvious that in terms of egg quality, ovaprim injected *H. nemurus* gave the best results. GnRHa injected fish at a dose of 5µg/kg, is not effective in inducing ovulation and spawning, and the results showed that there was no significant difference ($P>0.05$) in the egg quality of induced spawn *H. nemurus* using GnRHa at doses of 20µg/kg and 50µg/kg.

CONCLUSION

This study confirms that GnRHa is effective in inducing ovulation and spawning in *H. nemurus* and can be used in hatcheries for induced breeding. However, a low dose of GnRHa (5µg/kg) may not induce the fish to spawn. Thus, the significance of this research was that 5µg/kg GnRHa can not bring about ovulation in *H. nemurus*. Induced breeding of this fish in hatcheries will help to reduce pressure on the wild population due to over-harvesting. At the same time, *H. nemurus* fry produced in hatcheries can be used for wild restocking. Ovaprim injected *H. nemurus* produced the best spawning performance and egg quality and can be recommended to hatcheries. The spawning performance and egg quality of *H. nemurus* treated with 20µg/kg GnRHa and 50µg/kg GnRHa were not significantly different. Therefore, it is economical and cost effective to use 20µg/kg GnRHa.

ACKNOWLEDGEMENTS

This research was supported by internal grant of UPM. The authors are also grateful to the staff of UPM Aquaculture Research

Station, Puchong (Mr. Azmi Yaacob, Mr. Syah Rizan, Mr. Zainal Abidin, and Mr. Jasni Yusuf) for their assistance.

REFERENCES

Arabaci, M., & Sari, M. (2004). Induction of ovulation in endemic pearl mullet (*Chalcalburnus tarichi*) living in the highly alkaline Lake Van, using GnRHa ([D- Ser (tBu)^{6}, Pro^{9}-Net]-GnRH) combined with haloperidol. *Aquaculture, 238,* 529-535.

Chand, B. K., Singh, M. K., & Mandal, B. (2011). Studies on the breeding of *Pangasius sutchi* using different inducing agents. *Journal of Applied Aquaculture, 23,* 32-40.

Christianus, A., Siraj, S. S., Harmin, S. A., & Nordin, W. M. W. (1998). Morphology and histology of the gonad of matured river catfish, *Mystus nemurus* (Cuvier and Valenciennes). *Malaysian Applied Biology, 27*, 103-106.

Christianus, A., Siraj, S. S., Harmin, S. A., & Nordin, W. M. W. (1999). Gonadal development of the male river catfish *Mystus nemurus*. *Science International, 11,* 39-41.

Drori, S., Ofir, M., Levavi-Sivan, B., & Yaron, Z. (1994). Spawning induction in common carp (*Cyprinus carpio*) using pituitary extract or GnRH superactive analogue combined with metoclopramide: analysis of hormone profile, progress of oocyte maturation and dependence on temperature. *Aquaculture, 119*, 393-407.

Haniffa, M. A., & Sridhar, S. (2002). Induced spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using HCG and synthetic hormone (ovaprim).*Veterinarski Arhiv, 72*, 51-56.

Khan, M. S., Ambak, M. A., Ang, K. J., & Mohsin, A. K. (1990). Reproductive biology of a tropical catfish *Mystus nemurus* Cuvier and Valenciennes in Chendoroh reservoir, Malaysia. *Aquaculture and Fisheries Management, 21*, 173-179.

Legendre, M., Slembrouck, J., Subagja, J., & Kristanto, A. H. (2000). Ovulation rate, latency period and ova viability after GnRH or HCG induced breeding in the Asian catfish *Pangasius hypophthalmus (Siluriformes, Pangasiidae)*. *Aquatic Living Resources, 13 (3)*, 145-151.

Manosroi, J., Meng-Umphan, K., & Manosroi, A. (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.

Mesomya, W., Cuptapun, Y., Jittanoonta, P., Hengsawadi, D., Boonvisut, S., Huttayanon, P.,& Sriwatana, W. (2002). Nutritional evaluations of green catfish, *Mystus nemurus. Kasetsart Journal (Natural Science), 36*, 69-74.

Muchlisin, Z. A., Hashim, R., & Chong, A. S. C. (2004). Preliminary study on the cryopreservation of tropical bagrid catfish (*Mystus nemurus*) spermatozoa: The effect of extender and cryoprotectant on the motility after short term storage. *Theriogenology, 62,* 25-34.

Nwokoye, C. O., Nwuba, L. A., & Eyo, J. E. (2007). Induced propagation of African clariidcatfish *Heterobranchus bidorsalis* (Geoffrey Saint Hillarie, 1809) using synthetic and homoplastic hormones. *African Journal of Biotechnology, 6,* 2687-2693.

Park, W. D., Lee, C. H., Lee, C. S., Kim, D-J., Kin, J-H, Tamaru, C. S., & Sohn, Y. C. (2007). Effects of a gonadotropin–releasing hormone analog combined with pimozide on plasma sex steroid hormones, ovulation and egg quality in freshwater exposed female chum salmon (*Oncorhynchus keta)*. *Aquaculture, 271,* 488-497.

Rainboth, W. J. (1996). *Fishes of the Cambodian Mekong*. FAO species identification field guide for fishery purposes. FAO, Rome.

Rainis, S., & Ballestrazzi, R. (2005). The control of reproduction in finfish species through GnRH treatments. *Italian Journal of Animal Science, 4*, 345-353.

Sahoo, S. K., Giri, S. S., & Sahu, A. K. (2005). Induced spawning of Asian catfish *Clarias batrachus* (Linn.): Effects of various latency periods and SGnRHa and domperidone doses on spawning performance and egg quality. *Aquaculture Research, 36*, 1273-1278.

Silverstein, J. T., Bosworth, B. G., & Wolters, W. R. (1999). Evaluation of dual injection of LHRHa and the dopamine receptor antagonist pimozide in cage spawning of channel catfish *Ictalurus punctatus*. *Journal of the World Aquaculture Society, 30*, 263-268.

Tan-Fermin, J. D., Pagador, R. R., & Chavez, R. C. (1997). LHRHa and pimozide-induced spawning of Asian catfish *Clarias macrocephalus* at different times during an annual reproductive cycle. *Aquaculture, 148*, 323-331.

Tharakan, B., & Joy, K. P. C. (1996). Effects of mammalian gonadotropin releasing hormone analogue, pimozide and the combination on plasma gonadotropin levels in different seasons and induction of ovulation in female catfish. *Journal of Fish Biology, 48,* 623-632.

Wen, H. S., & Lin, H. R. (2004). Effects of exogenous neurohormone, gonadotropin GtH and dopaminergic drugs on the serum GtH content and ovulatory responsiveness of wild catfish, *Silurus asotus* (Linnaeus, 1758). *Aquaculture Research, 35*, 204-212.

Zohar, Y., & Mylonas, C. C. (2001). Endocrine manipulation of spawning in cultured fish: From hormones to genes. *Aquaculture, 197,* 99-136.