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Enhancing Ovulation of Malaysian Mahseer (*Tor tambroides*) in Captivity by Removal of Dopaminergic Inhibition

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ABSTRACT

Natural spawning ground of most Malaysian streams are undergoing extreme changes as a result of human activities, pollution and climate change (extreme temperature fluctuation) which lead to dramatic decrease in mahseer (*Tor tambroides*, Cyprinidae) populations. An experiment was conducted to evaluate the effectiveness of salmon gonadotropin releasing hormone analogue (sGnRHa) alone or in combination with dopamine antagonist domperidone (DOM) on breeding performances of female breeders. Sixty females *T. tambroides* ranging in weight 2.01 to 3.96 kg while thirty males with average weight 1.38 to 1.72 kg were used in this study. The females were given pretreatment with Ovaplant (23.4-44.9 $\mu\text{g kg}^{-1}$) for 6 weeks prior to induction. Females were tested with various treatments such as Ovatide, sGnRHa, sGnRHa+DOM and DOM. sGnRH analogue alone does not contain dopamine antagonist to block dopaminergic inhibition action. Females were given single intramuscular injection of Ovatide (0.5 mL kg^{-1} BW), sGnRHa (10 $\mu\text{g kg}^{-1}$ BW), sGnRHa+DOM (10 $\mu\text{g kg}^{-1}$ BW+5 mg kg^{-1} BW) or DOM (5 mg kg^{-1} BW). The control group was administered with 0.9% NaCl. The fish did not spawn spontaneously but through hand stripping to release eggs. Results showed that Ovatide significantly produced highest ovulation rate, total stripped eggs and stripped fecundity. Interestingly, we observed that broodstock groups injected with sGnRHa+DOM produced highest fertilization, hatching and survival rates compared to other treatments. Good egg and larval quality were recorded in the group treated with sGnRHa+DOM. No females ovulated in the group receiving DOM alone and saline solution. The combination treatment was found to be more potent in inducing ovulation as compared to sGnRHa alone or DOM alone. Our results suggest that dopaminergic inhibition is a major barrier in inducing spontaneous spawning in captive mahseer and we successfully produced progeny generation of *T. tambroides* larvae.

Key words: Dopamine antagonist, F2 generation, induced ovulation, pretreatment, *Tor tambroides*.

INTRODUCTION

Tor tambroides (Bleeker, 1854) is a cyprinid commonly known as mahseer and locally referred to as kelah, empurau or pelian. It inhabits rivers and lakes, ascending to the rapid river as well

rocky bottom to breed. Some of the *Tor* species such as *T. khudree* are described by Ogale (2002) as a sport fish that provides unbeatable recreation to fishing lovers around the world, better than salmon. In recent years due to human interference, kela stocks have been threatened with multi-sided dangers such as overfishing, industrial pollution, deforestation which disrupt natural spawning ground of riverine fish populations. Deterioration of environmental conditions and demand of this species as food and game fish resulting in over exploitation of natural stocks lead to noticeable decline of mahseers in the wild (Ogale, 2002).

During the last 20 years, one of the major developments in fish culture is captive breeding. There has been a rising application of modern and efficient techniques to induce final oocyte maturation, ovulation, spawning and spermiation. These methods engage the use of synthetic agonists of gonadotropin-releasing hormone. Gonadotropin-releasing hormone (GnRH) treatment is gaining reception throughout the world, surpass the hypophysation techniques such as pituitary treatment that has been used for decennary (Yaron, 1995) in induced spawning of fish. Earlier studies on induced ovulation and spawning in *Clarias* species by administration of Luteinizing Hormone Releasing Hormone (LHRH) analogue alone or together with a dopamine antagonist (Ngamvongchon *et al.*, 1988; Manickam and Joy, 1989; Tan-Fermin and Emata, 1993), sGnRH analogue(s) exhibited maximum activity when evaluating the structure-activity relationship of GnRH peptides.

Inducing final oocyte maturation and ovulation by using a type of GnRH analogues proved to be effective; however, fish biological potencies differ greatly. Salmon GnRH (sGnRH) analogue and mammalian GnRH analogue (mGnRH) or LHRHa are the most common GnRH analogues used in fish spawning induction. sGnRH was found to be more effective than mGnRH in rousing serum gonadotropin (GtH) levels and ovulation in goldfish *Carassius auratus* (Peter *et al.*, 1985) common carp *Cyprinus carpio* and Chinese loach *Paramisgurnus dabryanus* (Lin *et al.*, 1988, 1991) and African catfish *Clarias gariepinus* (Szabo *et al.*, 2007). Dual control (positive and negative) are demonstrated in many teleost fish. Gonadotropin (GtH) discharge is stimulated by gonadotropin releasing hormones (GnRH) and is repressed by dopamine (DA) (Chang and Peter, 1983; De Leeuw *et al.*, 1985). The evidence concerning the dopaminergic inhibition of GtH release frequently comes from study reported in goldfish *Carassius auratus* and other cyprinid species (Peter *et al.*, 1986) and in African catfish *Clarias gariepinus* (Van Oordt and Goos, 1987). In goldfish, dopamine can eradicate the pituitary's reaction to Luteinizing Hormone-releasing Hormone Analogue (LHRHa) or to lesion of dopaminergic tracts in the pituitary gland and preoptic nucleus (Chang and Peter, 1983). In the other hand, dopamine antagonists such as pimozide (PIM) and domperidone (DOM) can boost plasma GtH levels or, if administered in conjunction with LHRHa and sGnRHa, can markedly potentiate the response to LHRHa and sGnRHa.

Successful induced ovulation and multiple breeding in captivity by artificial propagation have been achieved in *Tor tambroides* (Ingram *et al.*, 2005). Ovaprim that consist sGnRHa and domperidone is the most successful hormone used to induce ovulation in mahseer (Ingram *et al.*, 2005). Ovulation induction using 0.5 mL Ovaprim in one dose proved to have highest fertilization rate compared to frog pituitary extract (FPE) and *Clarias* pituitary extract (CPE) (Adebayo and Popoola, 2008). Nowadays, larval production cost always concerned by hatcheries. They are looking for ways to reduce induction work cost while getting higher larval production. Adebayo and Popoola (2008) reported that production cost in African catfish was highest using CPE followed by Ovaprim and FPE. Popularity of newly formulated hormone such as Ovatide arises because of the affordable price and effectiveness as inducing agents compare to other hormones. The active

ingredients in Ovatide and Ovaprim are almost the same but Ovatide has low cost and low viscosity compared to Ovaprim. The effectiveness of Ovatide is already tested in spotted snakehead *Channa punctatus* (Marimuthu *et al.*, 2009), walking catfish *Clarias batrachus* (Sahoo *et al.*, 2005) and Indian major carp *Catla catla*. Higher spawning fecundity and fertilization rate were reported in Indian major carps *Cirrhina mrigala* and *Labeo rohita* when spawning was induced with Ovatide compared to Ovaprim.

Domperidone is commonly use in combination with other hormones to induce spawning in many species such as nase *Chondrostoma nasus*, bighead carp, grey mullet, silver perch, African catfish, rohu, common carp and many other teleost fish. In some fishes there is a strong inhibition of basal and GnRH stimulated discharge of LH by Dopamine (DA) (Van der Kraak *et al.*, 1988). Consequently administration of DA antagonists (e.g: Domperidone (DOM), reserpine, pimozide or metoclopramide) prior to the treatment with GnRH_a (Ovaplant) removes the inhibition on gonadotrophs and enhanced the stimulatory effect for GnRH_a on LH release. Domperidone has equal or somewhat greater potency than Pimozide in potentiating the action of releasing hormone agonist in loach; however, DOM had markedly greater efficiency in common carp (Lin *et al.*, 1988).

Nutrition and food for breeders is a major concern in breeding activity. High protein requirement is important for development of eggs and production of good egg quality. Homemade food main ingredients using natural source such as soy bean, angkabang fruit, beans, ficus and palm oil fruit as main ingredients. In this study high protein fruit (angkabang) was used to feed broodstocks before induction work. High amounts of protein, carbohydrate and micro and macro elements in the breeder diet showed improvement in reproductive performance compare to other treatment diets (Dada and Ogunduyile, 2011).

This study aimed to compare effectiveness of dopamine antagonist on breeding performance in filial 1 generation of *Tor tambroides* females in captive condition.

MATERIALS AND METHODS

Experimental fish: F₁ generation of broodfish (*Tor tambroides*) used in this study was obtained from Tarat Indigenous Fisheries Production and Research Center (IFPRC) farm, Serian, Sarawak. These fish were reared for 4-5 years in concrete recirculating aquaculture system (RAS) tanks at the farm facility of Lu Thian Tack (LTT) Aquaculture Farm, Asajaya, Sarawak. These fishes were fed at 5% body weight twice daily. In the morning, the fish were fed with self made feed (angkabang fruit, emperang fish, squid oil, vitamin C, flour) containing 62.6% crude protein, 17.6% crude lipid, 1.8% crude fibre, 12.7% ash, 5.3% carbohydrate and 4770.5 kal g⁻¹ energy. In the evening, the fish were fed with commercial tilapia pellet (Cargill, Malaysia) at different protein contents according to their age.

Broodstock selection: Matured F₁ broodfish comprised of 60 females and 30 males were selected from 124 m³ concrete tanks and acclimatized in separate 20 m³ concrete RAS tanks and maintained at ambient temperature of 26.0-27.3°C. The females were selected on the basis of having round and soft abdomen with swollen, protruding and reddish vents. For identification purposes, all the breeders were implanted with Passive Implant Transponders (PIT Tags) (American Veterinary Identification Devices (AVID), Norco, CA, USA). The female and male breeders with weight ranging from 2.01-3.96 and 1.38-1.72 kg, respectively were selected for induced breeding.

Hormone preparation and artificial propagation: Ovaplant (Syndel Laboratories, Vancouver, Canada) (75 and 150 µg pellets, 26.04-50.00 µg kg⁻¹) was implanted 6 weeks before ovulation

induction trials. Salmon gonadotropin releasing hormone analogue (sGnRHa) and domperidone (Sigma, St. Louis, MO, USA) were used. The sGnRHa and DOM were dissolved in acidified 0.9% saline solution (Chang *et al.*, 1984a,b) before used. The various treatments and dosages used were: Ovatide (0.5 mL kg⁻¹), sGnRHa (10 µg kg⁻¹), sGnRHa+DOM (10 µg kg⁻¹+5 mg kg⁻¹), DOM (0.5 mL kg⁻¹) and 0.9% saline solution (0.5 mL kg⁻¹). Ovatide (Hemmo Pharma, Mumbai, India) supplied in liquid form containing 20 µg of sGnRHa and 10 mg of domperidone in 1.0 mL. Ovatide was injected at 0.5 mL kg⁻¹ BW as recommended by distributor and used as a positive control. Fish were anesthetized with MS222 before implantation or injection to avoid stress and for ease of handling. After these procedures the fish were hold in 0.2% acriflavine solution to treat injury and reduce infection. The length and weight of the fish were recorded before the hormone administration. Ovulation rate was evaluated in the morning 24 h post induction by gently stripping the female's abdomen. The female breeders were anesthetized with MS222 (1:1000) and slight pressure was applied to the abdomen to determine whether eggs could be stripped from the fish. Eggs were stripped directly into a plastic jar. Volume of the eggs was recorded and 1 mL of the eggs was taken as subsample for fecundity estimation. Two matured males on the basis of having viscous and creamy milt were selected to fertilize eggs of a female. The same stripping procedure was applied on the male for milt. The milt was then stripped into the jar and mixed for 3-7 min to optimize fertilization capacity (dry method) as described by Joshi *et al.* (2002). Water was added slowly into the jar to initiate fertilization and these eggs were then washed with fresh water. The water hardened eggs were incubated in separated plastic mesh trays according to broodfish. A water level of 5 cm above the eggs with water discharge at 1-2 L min was maintained. The eggs were completely covered with dark plastic with small holes to avoid direct exposure to the light. Unfertilized eggs turned opaque and were removed twice a day (7:00 am.. and 6:00 pm.) to avoid any fungal infection.

Eggs and larval quality measurements: Small aliquots of 1 mL eggs (n = 3) were sampled and used to calculate total stripped eggs. As for egg quality evaluation, fertilization and hatching rates were estimated. Fertilization rate was determined by observing at least 50 eggs displaying 32-cell stage which was 24 h Post Fertilization (PF). Unfertilized eggs were removed to prevent fungal infection. At fourth days PF, the eggs started to hatch and all the eggs hatched at 6 days PF. Hatching rate was determined as time from fertilization until 50% of the eggs hatched. Eggs were sampled by placing 100-200 eggs on a petri disk then the numbers of hatched larvae were counted. During the larval rearing period, 40 larvae from each replicated tank per egg clutch were taken from the rearing tanks, anesthetized with MS222 (1:10,000) and standard length measurements will be recorded on day 1 to 7 Post Hatching (PH). Deformed larvae were recorded. These were done in triplicates and survival rate was calculated. Average of 40 eggs and larval were measured under stereomicroscope, SMZ-168 (2x magnification, 34.5 mm working distance) to determine egg diameter and larval length, respectively. Duration of embryogenesis was determined as the time between fertilization and hatching rate.

Water quality parameters: Water quality parameters such as water temperature, dissolved oxygen, pH, conductivity, turbidity, salt and total dissolve solid were monitored daily using multiparameter water quality apparatus, WQC 24-1-2.

Statistical analysis: Data were expressed as Mean±Standard error of the mean (SEM). Differences in the mean between treatments were tested using one-way analysis of variance

(ANOVA), $p < 0.05$, followed by Duncan multiple comparison test. Differences in ovulation success were tested using Chi-Square test for independence. These tests were carried out using SPSS 16.0 software.

RESULTS

Breeding performances: Daily water temperature, dissolved oxygen, pH, conductivity, turbidity, salt and total dissolved solid ($26.1-27.4^{\circ}\text{C}$, $5.95-13.4 \text{ mg L}^{-1}$, $7.43-8.0$, $8.6-13.9\%$ FS, 0% FS, 0% and $0-0.1 \text{ g L}^{-1}$, respectively) were recorded. This condition is suitable for ovulation induction activity. Ovulation in female broostocks was detected 24 h post administration. The ovulatory performance, egg quality and larval production of *T. tambroides* at different treatments were summarized in Table 1. Ovatide treatment showed the most significant ($p < 0.05$) ovulation rate (50% ovulated) among all the treatments. The numbers of ovulated fish in sGnRHa alone (17% ovulated) and sGnRHa+DOM (25% ovulated) were not different from each other but significantly higher than DOM alone and saline treatment. Unovulated fish were observed in the DOM and saline treatments. The total stripped eggs and fecundity was significantly highest ($p < 0.05$) when females were injected with Ovatide. Injection with sGnRHa+DOM ($10 \mu\text{g kg}^{-1} + 5 \text{ mg kg}^{-1} \text{ BW}$) resulted in lowest stripped egg yield. Significantly highest ($p < 0.05$) fertilization, hatching and survival rates were obtained in fish administered with sGnRHa+DOM compared to those with Ovatide and sGnRHa. Deterioration of egg quality was observed at sGnRHa treatment leading to low fertilization and hatching percentages. Normal larval production rate was significantly highest in the group treated with sGnRHa+DOM but the highest total larval production was highest from Ovatide treatment. Significantly highest ($p < 0.05$) abnormally was observed the group treated with sGnRHa alone than Ovatide and sGnRHa+DOM treatment. Types of abnormalities are shown in Fig. 2.

Egg and larvae quality: The egg diameter ranged from 2.92 to 3.3, 2.74 to 3.12 and 2.92 to 3.43 mm in Ovatide, sGnRHa and sGnRHa+DOM treatment, respectively. The mean egg diameter was

Table 1: Effectiveness of dopamine antagonist (DOM) on ovulation, total stripped eggs, fecundity, fertilization, hatching, survival and larval production of *T. tambroides*. The observation were based on $n=3$ of twelve replicates

Parameters	Treatments				
	Ovatide (0.5 mL kg^{-1})	sGnRHa ($10 \mu\text{g kg}^{-1}$)	sGnRHa + DOM ($10 \mu\text{g kg} + 5 \text{ mg kg}^{-1}$)	DOM (5 mg kg^{-1})	Saline (0.5 mL kg^{-1})
Weight of females (kg)	2.84±0.14 ^a	2.83±0.08 ^a	2.79±0.13 ^a	2.65±0.17 ^a	2.64±0.12 ^a
Fork length of females (cm)	50.2±1.4 ^b	49.8±1.1 ^b	47.5±0.6 ^{ab}	47.8±1.2 ^{ab}	46.5±0.6 ^a
Total length of females (cm)	54.0±0.7 ^a	53.3±0.8 ^a	54.5±0.6 ^a	53.8±0.7 ^a	53.5±0.7 ^a
Ovulation success	6/12 [*]	2/12	3/12	0/12	0/12
Total stripped eggs	2674±361 ^c	1479±21 ^b	1081±108 ^b	000±0 ^a	000±0 ^a
Stripped fecundity (eggs kg)	891±127 ^c	514±1 ^b	368±44 ^b	000±0 ^a	000±0 ^a
Fertilization rate (%)	84.4±4.0 ^b	21.4±9.5 ^a	94.3±1.4 ^b	---	---
Hatching rate (%)	81.6±2.3 ^b	50.8±0.6 ^a	90.8±1.2 ^c	---	---
Survival rate (%)	80.7±2.1 ^b	53.7±0.9 ^a	90.8±2.2 ^c	---	---
Deformed larvae (%)	12.1±0.3 ^b	25.2±3.1 ^c	3.1±0.6 ^a	---	---
Normal larvae (%)	70.8±1.7 ^b	40.1±1.6 ^a	87.7±2.3 ^c	---	---

Means value with the different superscript was significantly different ($p < 0.05$) evaluated using one-way ANOVA followed by Duncan's post-hoc test, *Significantly greater than the other treatments ($p < 0.05$, χ^2 test)

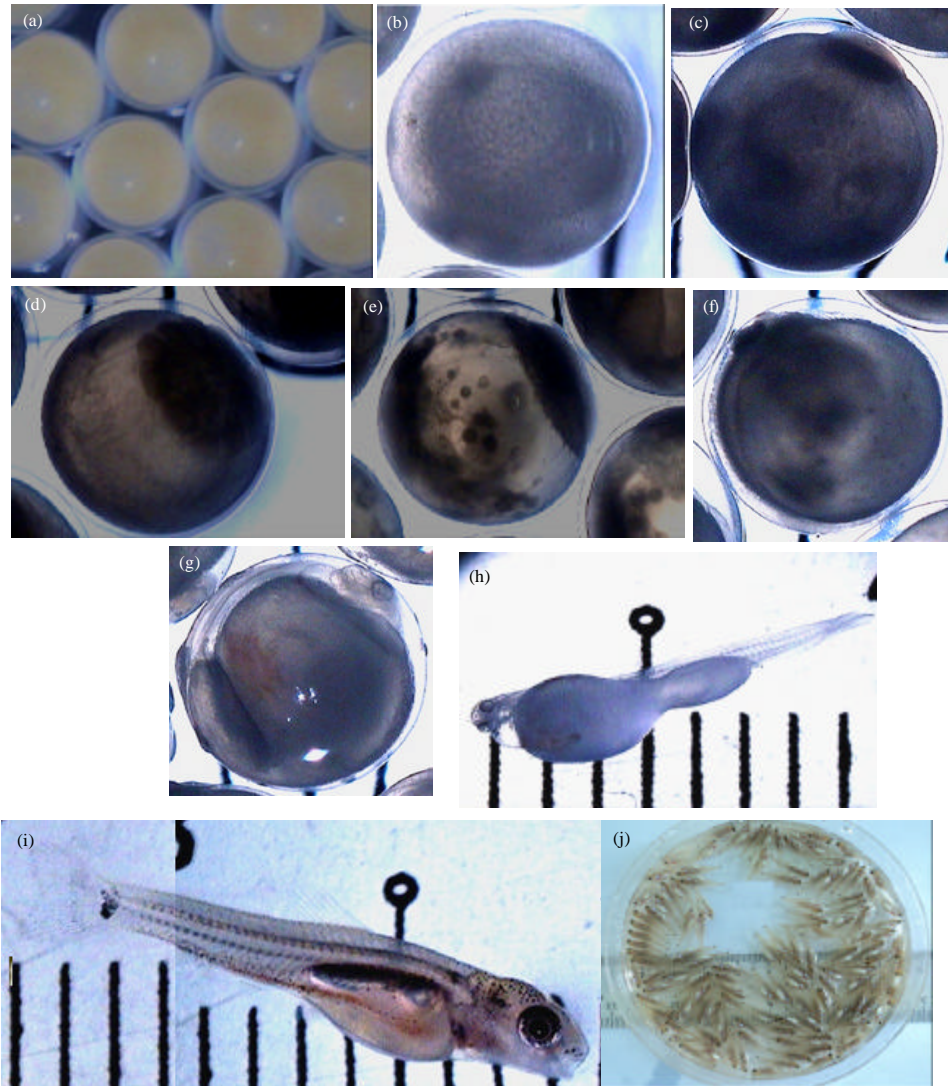


Fig. 1(a-j): Embryonic and larval development in *Tor tambroides*, (a): Water hardened eggs, (b): Fertilized egg, (c): One cell stage, (d): 4 h PF egg (16 cell stage), (e): 9 h PF egg (morula stage) and (f): 32 h PF egg (eyed stage). (g): 70 h PF egg (ready to hatch). (h): Just hatch larvae. I: Seven day old larvae. J: Fourteen days old fry

significantly higher ($p < 0.05$) in group treated with sGnRHa+DOM than Ovatide and sGnRHa egg. A similar trend was observed in diameter of the water hardened eggs. Duration for embryogenesis process significantly faster ($p < 0.05$) in the group treated with sGnRHa+DOM (80 h 0 min \pm 8 min) compare to the group treated with Ovatide and sGnRHa alone (81 24 \pm 7 and 83 h 24 min \pm 26 min, respectively) Table 2. Development of egg from PF until 7 days old larva is shown in Fig. 1a-f. Eggs in the sGnRHa treated group were the last to hatch and produced higher number of deformed larvae. Larval length for the 1 day Post Hatch (PH) was significantly longest in the group treated with sGnRHa+DOM (8.6 to 9.5 mm). Nonsignificant larval length was observed in group treated with Ovatide (7-8.3 mm) and sGnRHa alone (6.4-7.5 mm). At 2 day PH, larval length increased

Table 2: Eggs and larval qualities of Malaysian mahseer (*Tor tambroides*) injected with positive control of Ovatide or sGnRH_a alone or DOM alone or combined sGnRH_a + DOM or negative control of 0.9% saline solution

Parameters	Treatments				
	Ovatide (0.5 mL kg ⁻¹)	sGnRH _a (10 µg kg ⁻¹)	sGnRH _a + DOM (10 µg kg + 5 mg kg ⁻¹)	DOM (5 mg kg ⁻¹)	Saline (0.5 mL kg ⁻¹)
Stripped eggs diameter (mm)	3.10±0.63 ^{ab}	2.91±0.06 ^a	3.19±0.07 ^c	---	---
Fertilization rate (%)	98.3±0.38 ^b	0.1±0.03 ^a	99.5±0.15 ^c	---	---
Water-hardened eggs diameter (µm)	3.70±0.07 ^{ab}	3.48±0.07 ^a	3.81±0.09 ^c	---	---
Survival to eyed stage (%)	85±2 ^b	47±1 ^a	94±2 ^c	---	---
Embryogenesis duration (h-min)	81 h 24±7 min ^b	83 h 24±26 min ^c	80 h 0±8 min ^a	---	---
Hatching rate (%)	81.6±2.3 ^b	50.8±0.6 ^a	90.8±1.2 ^c	---	---
Length of larvae day 1 (mm)	7.6±0.20 ^a	7.05±0.23 ^a	9.17±0.16 ^b	---	---
Length of larvae day 2 (mm)	8.92±0.24 ^b	7.42±0.21 ^a	10.30±0.22 ^c	---	---
Length of larvae day 3 (mm)	9.45±0.26 ^b	7.93±0.20 ^a	10.73±0.19 ^c	---	---
Length of larvae day 4 (mm)	9.73±0.23 ^b	8.45±0.21 ^a	10.85±0.2 ^c	---	---
Length of larvae day 5 (mm)	10.55±0.22 ^b	9.07±0.24 ^a	11.63±0.12 ^c	---	---
Length of larvae day 6 (mm)	10.95±0.15 ^b	9.7±0.17 ^a	12.07±0.13 ^c	---	---
Length of larvae day 7 (mm)	11.31±0.14 ^b	10.08±0.20 ^a	12.87±0.10 ^c	---	---

Means values with the different superscript were significantly different (p<0.05) evaluated using one-way ANOVA followed by Duncan's post-hoc test

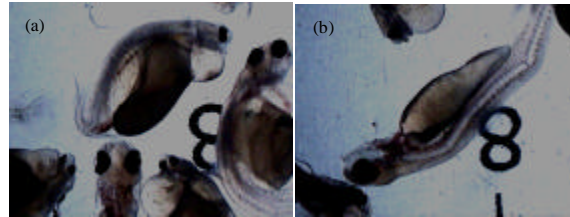


Fig. 2(a-b): Deformed larvae collected from the group treated with sGnRH_a alone

and showed significantly longest (p<0.05) in the group treated sGnRH_a+DOM (9.8-11 mm) followed by Ovatide (8-9.5 mm) and sGnRH_a alone (6.8-7.8 mm). This pattern was similar until day 7 PH. At 7 day PH, the increment of larval length continued and showed significantly longest in the group treated with sGnRH_a + DOM (12.5-13.2 mm) followed by Ovatide (11-12 mm) and sGnRH_a (9.5-10.8 mm). Increment of the larvae length is shown in the Fig. 1.

DISCUSSION

In this experiment, viable hatched larvae were produced from female broodstock weighing 2.78 kg. Ingram *et al.* (2007) reported that over 2.5 kg female broodfish were sexually matured and can produce hatchable eggs. While, in peninsular Malaysia, only female breeders of *T. tambroides* over 4.5 kg were found to be sexually matured (Tan, 1980). In Andang region of Sarawak, Sungan (1991) observed that females *T. douronensis* as small as 0.625 kg are able to release free-flowing eggs which are considered as matured. No females found ovulated in the control group probably because as observed in earlier finding that mahseer did not spawn in captivity naturally (Ingram *et al.*, 2005). Similar result was noted by Taufek *et al.* (2009) when African catfish reared

in captivity did not ovulate when induced with saline solution. The highest ovulation rate was observed in 0.5 mL kg⁻¹ BW Ovatide treatment. Ovatide successfully induced spawning in several fish species such as; stinging catfish, *Heteropneustes fossilis* (Marimuthu *et al.*, 2000); spotted snakehead, *Channa Punctatus* (Marimuthu *et al.*, 2009); walking catfish, *Clarias batrachus* (Sahoo *et al.*, 2005) and several cyprinid species like silver carp, *Hypophthalmichthys molitrix* and common carp, *Cyprinus carpio* (Horvath *et al.*, 1997).

DOM alone failed to induce ovulation in Malaysian mahseer females because it was not able to trigger complete ovulation thus resulting in nonresponse to stripping. This might be due to insufficient release of gonadotropin which slows the egg maturation process (Van der Kraak *et al.*, 1983; Billard *et al.*, 1984; Sahoo *et al.*, 2005). DOM without sGnRHa cannot help in oocyte maturation and induce ovulation. Higher stripping pressure and egg plunging was observed when sGnRHa was administered resulting in blood tinged eggs. Blood on the stripped egg coagulated and clogged the micropile which led to poor fertilization. Treatment with sGnRHa alone caused low egg production and egg quality which affect larval production. Low egg quality may lead to higher larval deformities. Highest deformity in sGnRHa alone treatment may be attributed to the fertilization of unripe ova which leading to abnormal embryonic development. Combination of DOM and GnRHa accelerates oocyte development and increases plasma estradiol levels (Aizen *et al.*, 2005). The highest production of larvae was obtained when treated with Ovatide but fertilization, hatching and larval survival rates were highest when treated with sGnRHa+DOM. This could be due to highest production of eggs in Ovatide treatment but lower fertilization, hatching and survival rates compared to sGnRHa+DOM treatment. Treatment with sGnRHa+DOM enhances fertilization, hatching and survival rates. In this group, egg diameter and water hardened eggs were significantly higher compared to other groups. This situation led to production of longer and healthy larvae compared to other treatments. Previous study showed that striped seabass injected with triiodothyronine (T3) prior to spawning, produced eggs and larvae, consequently increasing growth, swimbladder inflation and larval/fingerling survival.

Low effect of sGnRHa alone or DOM alone on oocyte maturation and ovulation probably because of inadequate and failure of GtH levels increment in circulation (Szabo *et al.*, 2002). sGnRHa and DOM alone were not effective to trigger oocyte maturation and ovulation in females and need to combine. Combination of both also will stimulate adequate increase in GtH level in the circulation. Dopamine is released by the hypothalamus and acts as a GtH release inhibitor in many teleosts and has been shown to directly inhibit basal and GnRH-stimulated GtH release (Peter *et al.*, 1991). Combination of dopamine antagonist and sGnRHa was highly effective in increasing circulating GtH levels and inducing ovulation in goldfish and common carp (Szabo *et al.*, 2002). Similar to other cyprinids, the results of this study showed that dopamine acts as an inhibitory factor on GtH releasing. DOM alone was unable to induce ovulation. Combination of sGnRHa and dopamine antagonist domperidone proves effective in inducing ovulation. In some fish, strong dopaminergic inhibition has been demonstrated, consequently dopamine receptor antagonists often combined with GnRHa (Szabo, 2003). Pimozide, domperidone and metoclopramide are the most commonly used dopamine antagonists which potentiate the effects of GnRHa (Yaron, 1995).

The present study demonstrated that Ovatide (0.5 mL kg⁻¹) treatment was the most successful in inducing ovulation followed by sGnRHa+DOM (10 µg kg⁻¹+5 mg kg⁻¹) and sGnRHa (10 µg kg⁻¹). Five mg kg⁻¹ DOM alone and the control treatment showed no potential at all. Combination of

sGnRHa with dopamine antagonist (DOM) showed more potent than sGnRHa alone or DOM alone in inducing ovulation. This study is very important in providing baseline information to help commercial hatcheries to produce optimum collection of good quality eggs leading to higher larval production.

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