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RESEARCH PAPER

Hormone Induced Artificial Breeding of a Commercially Important Marine Finfish, Striped Mullet (*Mugil cephalus* L.) in Bangladesh

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Abstract

Artificial breeding trial of *Mugil cephalus* L. using hormones was conducted from August, 2014 to February, 2015 in a fish hatchery, Cox's Bazar, Bangladesh. In this experimental trial, average one kg sized *M. cephalus* were collected from wild and reared in saline-water ponds to breed in captivity through hormone induction. The salinity of rearing ponds was maintained between 20 – 25ppt and in hatchery 24 – 25ppt with 22 – 25ºC temperature. Carp pituitary glands (CPG), HCG (Human chorionic gonadotropin) and LRH-A_2 (Luteinizing releasing hormone) were used for hypophysation. The effective dosages of CPG 30mg/kg body weight, LRH-A_2 150µg/fish with combination of 0.3 mL Domperidone and 0.5 mL Calcium injection, and HCG dose of 30,000 IU in case of female and 5,000 IU in case of male resulted spawning success. The GSI value of fecund fishes ranged from 7.92 to 12.38, egg diameter of matured fish between 550 to 600µm and that of fertilized egg from 650 to 700µm. Fecundity was calculated as 735 to 900 nos/g. The fish started spawning between 44 – 48 h and cell division was observed after the first hour of spawning but severe mortality occurred after 6 h.

Keywords: Induced breeding, hormones, mullet, *Mugil cephalus.*

Introduction

Striped mullet (*Mugil cephalus*), locally called as Khorul bata/Bhangan bata is a commercially important, high priced marine finfish in Bangladesh coast. The species is euryhaline and eurythermal that contributes to sizable fisheries of estuarine and coastal regions not only in Bangladesh but also throughout the world including China (Chang *et al*., 2000), Egypt (Saleh, 2008), India (Barman *et al*., 2005), Italy (Luzzana *et al*., 2005), Sri Lanka (De Silva and Silva, 1979), Taiwan (Chang and Tzeng, 2000) and Tunisia (Kheriji *et al*., 2003) etc. Full-scale commercial production of the species is not common in the country, although the species is considered as demandable food for its size and taste.

In the Indian sub-continent, induced breeding trials of mullets by pituitary injection was first initiated in India during 1961 and success was achieved in breeding of *M. cephalus* by hypophysation (CIFRI, 1961). Another successful attempt of induced spawning of *M. cephalus* reared in captivity was by Yashouv (1969) using carp pituitary gland and luteinizing hormone, and HCG by Kuo *et al.* (1973). Kuo *et al.* (1974) also established spawning procedure for *M. cephelus.*

Although, the mullet fry and juveniles are abundant in the wild, coastal aquaculture cannot thrive depending on the wild fry supply. Mass production of mullet seed/fry will expand its widespread aquaculture in the coast. So we need to develop artificial breeding technology of mullet in captivity. Study on reproductive biology of *M. cephalus* in Bangladesh attempted (Das *et al.*, 2008), although induced breeding techniques of this species not yet developed. Thus to ensure adequate supply of seed for the culture, knowledge about its artificial breeding is prerequisite and hence the present experiment was conducted in a fish hatchery at Cox's Bazar, Bangladesh.

Materials and Methods

Brood Rearing

The experiment was conducted from August, 2014 to February, 2015 in a fish hatchery at Rejukhal under Cox's Bazar district, Bangladesh. Two earthen rearing ponds (each 2,400 m^2) equal in depth and configuration including well organized inlet and outlet system to maintain saline water level were used. The water depth was maintained at a maximum

of 1.4 m.

Adult/sub-adults of mullets, *M. cephalus* were collected from the wild and stocked in the ponds of Niribili Fish Farm, Rejukhal in the month of August, 2014. The fish were fed with commercial floating feed (Mega Feed) twice daily at 2 – 2.5 per cent of their body weight and Vitamin-E (Selvitdex) was added to feed during the month of October to November for gonadal maturation. Physicochemical parameters i.e. water temperature, salinity, dissolved oxygen, free carbon dioxide, alkalinity, pH and ammonia were monitored following standard methods (APHA, 1998) and fish's health were also checked fortnightly. The salinity of ponds was maintained between of 15 – 20ppt and 25ppt during October to February for brood development.

Hatchery Facility Development

As marine fish hatchery is not well established in the country, following facilities were developed for the purposes of brood conditioning and subsequent spawning, incubation, larval rearing and production of live feed. The arrangements of various types of breeding and rearing tanks are shown in Table 1.

Hormonal Treatment and Spawning

Induced breeding of reared *M. cephalus* were conducted during January-February, 2015. Fishes were captured by seine net and transported to the holding tanks by plastic drums using 2-phenoxyethanol (2mL/10 L water) as anesthesia. Each tank was provided with continuous water circulation and aeration.

After transportation of broods, they were treated with Furacin (50ppm) to avoid possible infection, females and males were separated and oocytes were sampled following LOB (Live Ovarian Biopsy) method. In this method, catheter (no. 6) gently pushed into the gonad then sucked by mouth for collecting gonad and collected sample observed under microscope to check the development of gonads. Hormone injections were initiated within 48 h after transportation and acclimatization for 24 h. Interval between injections varied from 24 to 36 h. Dry carp pituitaries (CPG) for 1st dose and LRH-A_2 (Luteinizing releasing hormone) for 2nd dose with the combinations of Domperidone and Calcium injections were injected in varied doses. HCG was also used for the 1st dose of male in the first trial and both male/female in the second trial. In case of both female and male, hormone was injected in deep muscle at base of the dorsal fin.

Natural spawning with two un-injected males in holding tanks was also performed. Spawning behavior in holding tank was closely monitored. The released eggs were floating and drifting in nature. In case of fecundity study, the spent females were dissected and eggs retaining in the abdomen were counted volumetrically for the measurement of fecundity. Released eggs were continuously monitored by using ocular and stage micrometer to estimate eggs diameter. Fertilized eggs were kept in the spawning tank for incubation after treated with streptomycin solution at 0.5ml/L and observation continued through microscope until the starting of cell division.

Results and Discussion

The biological features of mullets have been well documented (Thompson, 1966, Chubb *et al.*, 1981), although relatively less information is available on reproduction aspects in the wild (Render *et al.*, 1995). After four months of rearing in the coastal ponds, healthy and diseases free broods transferred to hatchery for breeding. Striped mullets are considered as winter breeder as well as isochronal spawning fish i.e. they have synchronous gamete development and individuals spawn all their

Table 1. Hatchery tank facilities with capacity used in the breeding trials

Stage	Facility	Stocking density	Volume (ton)	Unit vol. (ton)	No. unit	Structure
Adult	Holding tank	1 fish/ ton	50	25	2	Square concrete tank, 5m × 5m × 1.0m capacity of 25 tons with water & aeration system
Brood	Spawning tank	1 fish/5 tons	100	25	4	Square concrete tank, 5m × 5m × 1.0m capacity of 25 tons with water & aeration system
Egg	Incubation tank	100 eggs/ liter	8	1	8	Circular / conical shape bottom, 1 ton capacity fiber- glass tank
Larvae	Larval rearing tank	20–50 larvae per liter	10	1.5	6	Rectangular concrete tank (1m × 1.5m × 1.5m) with mild aeration
Phytoplankton	Algal (*Nano*) culture tank	-	8	0.5	8	Circular tank flat bottom 500 liters fiber-glass tank
Zooplankton	Rotifer culture tank	-	3	0.5	6	Circular tank flat bottom 500 liters fiber-glass tank
Live feed	*Artemia* culture tank	-	4	0.5	8	Circular tank flat bottom 500 liters fiber-glass tank

reproductive material at once (Render *et al.,* 1995; McDonough *et al.,* 2003). In the first week of January 2015 mullets captured by seine net showed only 70 – 80% matured stages of gonadal development through LOB method. Histological criteria used to determine reproductive and their sexual differentiation stage in female mullets are presented in Figure 1.

Gonadosomatic Index (GSI) values used to determine sexual differentiation stage in female mullets are given in Table 2. GSI values were positively correlated with oocyte diameter and negatively correlated with oocyte density. GSI of fecund fishes found ranges from 7.92 to 12.38 and final size of vitellogenetic oocytes before hydration (600µm) was corresponding to GSI between 11 – 12. Monthly GSI levels showed that the time period of *M. cephalus* reproductive activity was from October through April in South Carolina estuaries, USA (McDonough *et al.,* 2003).

The physicochemical parameters of the water in the hatchery were measured daily and the average values are shown in Table 3. The salinity and other parameters of this study were close to those have been reported by Kuo *et al*. (1974).

In all the trials, injections were initiated within 48 h after transportation and acclimatization for 24 h. The fishes started pairing just before they spawned; males were observed a little bit active than female in the time of mating. The first release of a small number of eggs stimulated the male to release spermatozoa. Interval for 1st and 2nd dose of injections was maintained at 24 h and also in case of 3rd dose. Dry CPG for 1st dose and LRH-A_2 for 2nd dose with the combinations of Domperidone and Ca- injections were injected in varied doses, and HCG also applied in the first trial for male and for both male and female in the second and third trials. The spawning responses of three breeding trials are summarized in Tables 4, 5 and 6.

In order to assess successful spawning performance, two female with two injected male and two un-injected male were kept in spawning tank for overnight. Eight from ten injected females responded with ovulation and spawning (four spawned in spawning tank and rest were stripped) indicating spawning success of 66 per cent. Rest of oocytes from stripped female resulted 34 per cent spawning success. Similar observation was reported by Kuo (1995) having spawning success of 66 per cent for *M. cephalus*. In this experimental trial, complete fertilization has been failed due to poor quality as well as quantity of milts or due to lack of good males, but single spawning produced more than 60 per cent fertilization which was calculated as the total number of fertilized eggs divided by the total sampled number (n=100) of eggs. These results were close to those have been reported by Greeley *et al.* (1987). Two females out of 10 did not respond to multiple injections and developed atresia* (Fig. 1). All the hormones administered separately or in combined dose produced the same results, both positive and negative. Hydration was also observed within 6–12 h after the injection of effective dose and spawning started within 6–8 h at beginning of hydration. Initial diameter of oocytes in all the females varied within a range of 550–600 µm (except of one case) showed no significant difference between females with positive and negative response. However, responded females

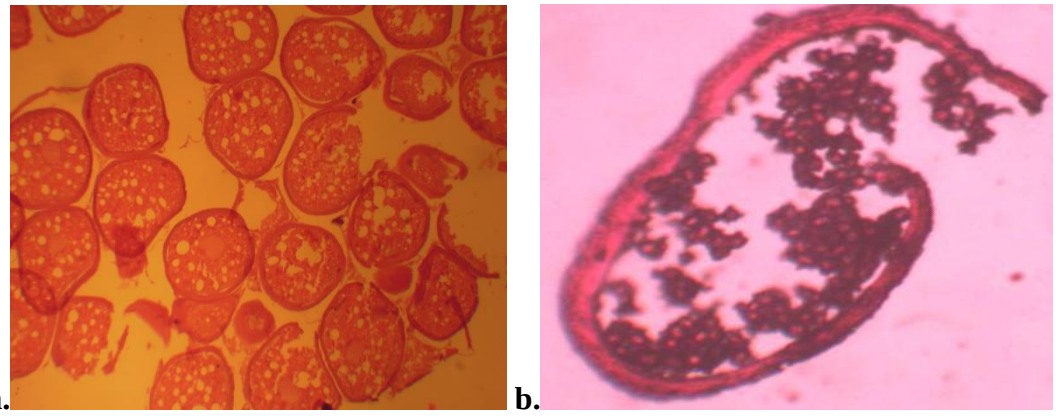

Figure 1. Histological observation of induced breed *Mugil cephalus*. a. Yolk granule compact and regular shaped before hormonal treatment. b. Yolk granule scattered and try to move outside of the layer after hormone injection.

Table 2. Gonadosomatic Index value of reared striped mullet (*Mugil cephalus*)

Month	Total weight	Total length	Gonad weight	GSI value	Maturity stages
November,14	1.63 Kg	50 cm	64 gm	7.92	Developing
Dececember,14	1.75 Kg	52 cm	85 gm	8.86	Yolk granule stage
January,15	2.2 Kg	57 cm	181 gm	12.38	Close to mature yolk stage (80%)

Table 3. Average value of the physicochemical parameters of Brood Holding (HT)/Breeding (BT)/Spawning Tank (ST) during January – Febuary, 2015

Tank no.	Temperature (ºC)		Salinity (ppt)		P^H	Dissolved oxygen (ppm)		Water exchange	Treatment With Chlorine
	8 AM	6 PM	8 AM	6 PM	6 PM	8 AM	6 PM	(%) daily	
HT-1	20.7	24.5	28.4	7.9	8.1	5.1	6.2	30	Initial
HT-2	20.5	23.8	28.6	7.6	8.0	5.2	6.3	30	do
BT-1	21.2	23.4	28.2	7.8	7.9	5.4	6.2	50	do
BT-2	21.7	23.8	27.7	7.8	8.0	5.3	6.1	50	do
ST-1	21.3	25.2	28.1	7.7	7.9	5.1	5.8	50	Every trial
ST-2	21.4	25.3	28.3	7.8	8.0	5.3	5.9	50	do
ST-3	21.6	25.8	28.5	7.9	8.0	5.5	5.7	50	do
ST-4	21.8	25.7	28.0	7.8	7.9	5.3	6.0	50	do

Table 4. First breeding response of *Mugil cephalus* during 1st week of January, 2015

Sex	Total body weight (kg)	Total length (cm)	Injection dose (unit/fish) Priming (mg/kg)	Resolving 1 (after 24 h)	Resolving 2 (after 24 h)	LP (h)	D_1 (µ)	D_2 (µ)	TSE (eggs/gm bw) /MC
Female	1.05	41	30 CPG	LRH-$A_2$100µg + 0.3mL Dom.+ 0.5 mL Ca- inj.	LRH-A_2 50µg + 0.3mL Dom.+ 0.2 mL Ca- inj	48	565	650	745
Female	1.30	44	40 CPG	LRH-$A_2$150µg + 0.3mL Dom.+ 0.5 mL Ca- inj	LRH-A_2 50µg + 0.3mL Dom.+ 0.2 mL Ca- inj	48	580	665	736
Female	1.40	46	45 CPG	LRH-$A_2$150µg + 0.3mLl Dom.+ 0.5 mL Ca- inj	LRH-A_2 50µg + 0.3mL Dom.+ 0.2 mL Ca- inj	48	560	655	821
Male	0.95	38	-	5,000 IU HCG	-	36	-		Less milt
Male	0.92	37	-	5,000 IU HCG	-	36	-		Less milt
Male	1.02	40	-	No dose	-	-	-		N/R
Male	1.06	41	-	No dose	-	-	-		N/R
Male	0.87	34	-	5,000 IU HCG	-	36	-		Less milt

LP = Latency period, D_1= Mean ova diameter before priming dose, D_2 = Mean ova diameter after spawning, TSE = Total spawned eggs in case of female, MC = Mt conditions in case of male projected by positive sign, LRH-A_2= Luteinizing Releasing Hormone, Dom.= Domperidone (Dopamine antagonist), Ca- inj.= Calcium Injection.

Table 5. Second breeding response of *Mugil cephalus* during last week of January, 2015

Sex	Total body weight (kg)	Total length (cm)	Injection Dose (unit/fish) Priming (mg/kg)	Resolving 1 (after 24 h)	Resolving 2 (after 24 h)	LP (h)	D_1 (µ)	D_2 (µ)	TSE (eggs/gm bwt) /MC
Female	1.85	53	60 CPG	LRH-A_2 300µg + 0.3mL Dom.+ 0.5 mL Ca- inj.	LRH-A_2 50µg + 0.3mL Dom.+ 0.2 mL Ca- inj	48	587	650	892
Female	1.65	49	50 CPG	30,000 IU HCG	-	36	563	665	865
Female	1.45	47	45 CPG	25,000 IU HCG	-	-	570	-	N/R
Female	1.70	51	55 CPG	LRH-A_2 250µg + 0.3mL Dom.+ 0.5 mL Ca- inj	LRH-A_2 50µg + 0.3mL Dom.+ 0.2 mL Ca- inj	48	570	670	780
Male	0.92	38	-	No dose	-	-			N/R
Male	0.82	33	-	No dose	-	-			N/R
Male	0.86	34	-	5,000 IU HCG	-	36			Less milt
Male	0.88	36	-	5,000 IU HCG	-	36			Less milt

had coalesced oil globule before injections and those which did not responded had partially or no fused oil droplets. Hypophysation produced positive result when the oocytes are at tertiary yolk stage with oil globule coalesced, nucleus migrated and diameter within 650–700 µm (Kuo *et al.*, 1974). The hypophysation results are presented in Table 7.

During first breeding trial, only single fertilization took place and the eggs hatch out after 44-48 h of spawning. James *et al.* (1982) reported that the ideal incubation period of *M. cephalus* is 36-48 h at 20-25°C of temperatures. All the eggs spawned,

fertilized and non had single oil globule. Eggs diameter varied within a range of 650–680 µm, and oil globules between 250– 280 µm (Table 8). Fecundity of those females has been determined as 735 –900 eggs per g of body weight.

After fertilization cell division started within an hour but the fertilized eggs were settled down before starting further segmentation. Finally huge mortality of fertilized eggs occurred and the dead cell were showed to float throughout the hatching tank (Table 9). Rapid fluctuation of temperature and shortage of required quality milts were the main reason for this type of mortality.

Conclusions

This was the first attempt in induced breeding of mullet fish in coastal Bangladesh. The results showed that induced breeding of *M. cephalus* is possible in our environment and also it could be successfully hypophyzed with hormone injections. Further study should be conducted using quality broods for fine tuning of hormone induced artificial breeding.

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Table 6. Third breeding response of *Mugil cephalus* during 1st week of February, 2015

Sex	Total body weight (kg)	Total length (cm)	Injection Dose (unit/fish) Priming (mg/kg)	Resolving 1 (after 24 h)	Resolving 2 (after 24 h)	LP (h)	D_1 (µ)	D_2 (µ)	TSE (eggs/gm bw)/MC
Female	1.25	42	40 CPG	LRH $A_2$100µg + 0.3mL Dom.+ 0.5 mL Ca- inj.	-	36	560	645	887
Female	1.48	45	45 CPG	LRH $A_2$150µg + 0.3mL Dom.+ 0.5 mL Ca- inj	LRH A_2 50µg + 5mL Dom.+ 0.2 mL Ca- inj	48	575	665	962
Female	1.67	49	50 CPG	30,000 IU HCG	-	36	570	-	N/R
Male	1.13	39	-	No dose	-	36	-	-	N/R
Male	0.96	37	-	5,000 IU HCG	-	36	-	-	N/R
Male	1.06	38	-	5,000 IU HCG	-	36	-	-	N/R
Male	0.85	32	-	No dose	-	36	-	-	N/R
Male	0.87	34	-	5,000 IU HCG	-	36	-	-	N/R

Table 7. Hypophyzation result of *Mugil cephalus* during induced breeding

Weight of fish (kg)	Length of fish (cm)	Initial diameter of oocytes (µm)	Total dose of hormones per fish PG (mg)	LRH-A_2 (µg)	Domperidone (µg)	HCG (IU)	Number of injections	Responded signs (++/+/-/(-)
1.05	41	565	30	100	0.3		3	+
1.30	44	580	40	150	0.3		3	+
1.40	46	560	45	150	0.3		3	++
1.85	53	587	60	300	0.3		3	+
1.65	49	563	50	-	-	30,000	2	+
1.45	47	570	45	-	-	25,000	2	(-)
1.70	51	570	55	250	0.3		3	+
1.25	42	560	40	100	0.3		2	+
1.48	45	575	45	150	0.3		3	+
1.67	49	570	50	-	-	30,000	2	(-)

++ spawned with fertilized eggs, + spawned, -/(-) did not spawned and atresia* checked

*atresia- the degenerative process that affects the majority of ovarian follicles

Table 8. Diameter of eggs and oil globules in *Mugil cephalus* hypophyzation

Weight of fish (kg)	Length of fish (cm)	Eggs ($\bar{X}$ ± SE) (µm)	Oil globule ($\bar{X}$ ± SE) (µm)
1.05	41.0	650 ± 8	260 ± 5
1.30	44.0	665 ± 6	255 ± 5
1.40	46.0	655 ± 7	267 ± 6
1.85	53.0	650 ± 8	265 ± 8
1.65	49.0	665 ± 10	270 ± 8
1.70	51.0	670 ± 7	265 ± 8
1.25	42.0	645 ± 8	255 ± 5
1.48	45.0	665 ± 10	257 ± 5

Table 9. Developmental stages of fertilization of *Mugil cephalus* in breeding trial

Time (h) after fertilization	Stages of development	Temperature of water (°C)
1.30	Two cell division	21.5
2.00	Four cell division	22.1
2.30	Sixteen Cell division	22.2
3.45	Cell division continued	22.2
6.00	Late segmentation stage, mortality started	23.2
12.00	Heavy mortality	25.8

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