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PRELIMINARY SUCCESS ON HORMONE INDUCED BREEDING OF STRIPED MULLET (*MUGIL CEPHALUS* L.) IN BANGLADESH

Ehsanul Karim*, Jakia Hasan and M. Enamul Hoq

Marine Fisheries and Technology Station, Bangladesh Fisheries Research Institute
Cox's Bazar 4700, Bangladesh

Abstract: Induced breeding trial of two years old striped mullet (*Mugil cephalus* L.) was conducted during winter season in a commercial fish hatchery at Cox's Bazar, Bangladesh. Adult or sub-adults of *M. cephalus* were collected from the wild and reared in the saline ponds from August to December 2014. The broods were injected using carp PG, HCG and LRH-A_2 after 48 hrs of acclimatization in the hatchery tanks. The effective doses of carp PG 30mg/kg body weight, LRH-$A_2$150µg with a combination of 0.3ml 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 were found to spawning success. The GSI value ranged between 7.92-12.38 and egg diameter of matured fish ranged between 563-594µm and that of fertilized egg between 650-680µm with fecundity calculated as 780 to 900 nos/g. The fish started spawning between 44-48 hrs and cell division was observed after the first hour of spawning, however, mass mortality occurred after 6 hrs of spawning.

Key words: *Mugil cephalus,* breeding, spawning

INTRODUCTION

Striped mullet (*Mugil cephalus* L.) is traditionally cultured along with tiger shrimp in coastal shrimp farms of Bangladesh coast. This is a euryhaline and eurythermal species that contributes to coastal fisheries not only in Bangladesh but also in China (Chang *et al.* 2004), Egypt (Saleh 2008), India (Curian 1975 and Barman *et al.* 2005), Sri Lanka (De Silva and Silva 1979), Taiwan (Chang *et al.* 2000) etc. Although there is no deliberate practice to stock the shrimp farms with mullet fry but the species is cultured in the shrimp *ghers* in the coastal region of Bangladesh where they enter with the tidal water during water exchanges. They grow well with the shrimps but they fed on the juvenile shrimps and resulted lower shrimp yield. However, the species is considered as demandable fish for its taste and price.

Although, the mullet fry and juveniles are available in the wild, aquaculture sector cannot thrive depending on the wild seed supply. Study on reproductive biology of *M. cephalus* in Bangladesh attempted (Das *et al.* 2008), although induced breeding techniques of this species not yet developed. Studies on

* Corresponding author: hoq_me@yahoo.com

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induced breeding of mullets in Indian sub-continent by pituitary injection were initiated during 1961 in India and success was achieved by hypophysation in breeding of *M. cephalus* by CIFRI (1961) followed by induced spawning using carp pituitary gland and leutenizing hormone (Yashouv and Sampsonov 1970). Kuo *et al.* (1974) also established spawning procedures for *M. cephelus* in Israel. Thus to ensure adequate supply of mullet seed for coastal aquaculture, knowledge about its artificial breeding technology is prerequisite and hence the present induced breeding experiment was carried out in a fish hatchery at Cox's Bazar.

MATERIAL AND METHODS

Study area: The experiment was conducted from August 2014 to February 2015 in Niribili Fish Farm, Rejukhal, Cox's Bazar. Two (50-60 decimal sized) brood rearing ponds- equal in depth and configuration including water inlet and outlet system were used. The water depth was maintained at a maximum of 1.4 m.

Brood development: Adult or sub-adults of *M. cephalus* were collected from the wild and stocked in saline ponds (15-20 ppt) of Niribili Fish Farm in the month of August 2014. The fish were fed with commercially available floating feed twice daily @ 2-2.5% of their body weight and Vit-E (Selvitdex) was added with supplied feed in the month of October-November for their gonadal maturation. Water quality (water temperature, salinity, dissolved oxygen, pH, alkalinity and ammonia) and fish's health were monitored fortnightly. The salinity in the rearing ponds was maintained between of 15-20 ppt during August-October and 25 ppt during November-February which was the required range for satisfactory gonadal development of mullets.

Hatchery facility development: To develop marine fish hatchery system following facilities were established for broodstock conditioning and subsequent spawning, incubation, larval rearing, and production of live feed. The capacity of various types of holding tanks are shown in Table 1.

Table 1. Tank facilities and capacity used in breeding trials

Stage	Facility	Stocking density	Volume (ton)	Unit vol. (ton)	No. unit	Structure
Adult	Holding tank	1 fish/ ton	60	30	2	Square concrete tank, 6m× 5m×1.0m capacity of 30 tons with aeration- system
Broods	Spawning tank	1 fish/5 tons	120	30	4	Square concrete tank, 6m× 5m×1.0m capacity of 30 tons with aeration
Eggs	Incubation tank	100 eggs/ liter	8	1	8	Circular/conical shape 1000 l capacity fiber- glass tank

Stage	Facility	Stocking density	Volume (ton)	Unit vol. (ton)	No. unit	Structure
Larvae	Larval rearing tank	20–50 larvae per liter	30	5	6	Rectangular concrete tank (1m×1.5m×1.5m) of with aeration
Phytoplankton	Algal (*Nano*) culture tank		8	0.5	8	Flat bottom 500 liters fiberglass tank
Zooplankton	Rotifer culture tank		3	0.5	6	Flat bottom 500 liters fiberglass tank
Live feed	*Artemia* culture tank		4	0.5	8	Flat bottom 500 liters fiberglass tank

Hormonal treatment and spawning: Experiments on induced breeding were conducted during winter months of January-February 2015. Fishes were captured by seine net and transported to the holding tanks by plastic drums with anesthesia (phenoxy-ethanol, 2ml/10 l water). Each tank was provided with continuous water circulation and aeration.

After transportation of broods, they were treated with Furacin (50 ppm for 5 min.), and females and males were separated, followed by sampled of oocytes using Live Ovarian Biopsy (LOB) method. Injections were initiated within 48 hrs after transportation and acclimatization in holding tanks. Interval between injections varied from 24 to 36 hrs. Dry carp pituitaries for 1st dose and LRH-A_2 (2nd dose) with the combination of Domperidone and Calcium injections were injected in varied doses. HCG was also used in the 1st dose for male in the first trial and for both male and female in the second breeding trial.

Natural spawning in holding tanks with un-injected males was also performed and spawning behavior was closely observed. The 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 for estimation 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 0.5ml/l streptomycin solution and observation continued through microscope until the starting of cell division.

RESULTS AND DISCUSSION

The biological features of mullets has been well documented (Thompson 1966, Chubb *et al.* 1981), although relatively less information are available on the reproductive biology of mullets (Render *et al.* 1995). After four months of rearing in the saline ponds, healthy and diseases free broods transferred to hatchery tanks for breeding trials. Striped mullets are considered as winter

breeder as well as isochronal spawned fish i.e. they have synchronous gamete development and individual spawned at once (Render *et al.* 1995, McDonough *et al.* 2003) and spawning population is not uniform in size distribution. In the first week of January 2015 mullets captured by seine net showed only 70-80% matured stages of gonadal development through LOB 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. Gonado-Somatic 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 ranged from 7.92 to 12.38 and final size of vitellogenetic oocytes before hydration (600µm) was corresponding to GSI between 11-12.

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

Month	Total wt.	Total L	Gonad wt.	GSI value	Maturity stages
Nov, 2014	1.63 kg	50 cm	64 g	7.92	Developing
Dec, 2014	1.75 kg	52 cm	85 g	8.86	Yolk granule stage
Jan, 2015	2.2 kg	57 cm	181 g	12.38	Close to mature yolk stage (80%)

The physicochemical parameters of the water in the hatchery tanks were measured daily following standard methods (APHA 1998) and the average values are given in Table 3. The salinity and other parameters of this study were close to those have been reported by Kuo *et al.* (1974).

Table 3. Average value of the physicochemical parameters of brood holding (HT)/ breeding (BT)/ spawning tank (ST) during January-February 2015

Tank nos.	Temperature (°C)		Salinity (ppt)	PH		DO (ppm)		Water exchange	Treatment with chlorine
	9 AM	6 PM	9 AM	9 AM	6 PM	9 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

In all the breeding trials attempted, injections were initiated within 48 hrs after transportation and acclimatization. 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 between 1st and 2nd dose of injections was maintained at 24 hrs and also in case of 3rd dose. The response of 3 breeding trials are summarized in Tables 4-6.

In order to assess successful spawning performance, two females with two injected males and two un-injected male were kept in holding tanks for overnight. Eight from ten injected females responded with ovulation and spawning (four spawned in tank and rest were stripped) as spawning rate close to 66%. Rest of oocytes from female stripped produced 34% fertilization. Similar observation was reported by Kuo (1995), having spawning rate of 66% for *M. cephalus*. From this experimental trials, in most cases fertilization has 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% fertilization rate 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 fishes did not respond to multiple injections and developed Atresia. All the hormones administered separately or combined produced the same results, both positive and negative. Hydration was observed within 6–12 hrs after the injection of effective dose and spawning within 6–8 hrs after beginning of hydration. Initial diameter of oocytes in all the females varied within a range of 550–600 µm (except of one case) showing no significant difference between females with positive and negative response. However, responded females had coalesced oil globule before injections and those which did not respond had partially or not fused oil droplets. Hypophysation produces positive result when the oocytes are at tertiary yolk stage with oil globule coalesced, nucleus migrated and diameter of 650–700 µm (Kuo *et al.* 1974). The hypophysation results are presented in Table 7.

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Table 4. 1st Breeding response of *Mugil cephalus* during 1st week of January 2015

Sex	Body wt (kg)	Length (cm)	Injection dose (unit/fish)			LP (hrs)	D_1 (µ)	D_2 (µ)	TSE (eggs/g bwt) /MC
			Priming (mg/kg)	Resolving 1 (after 24 hrs)	Resolving 2 (after 24 hrs)				
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 + 0.3ml 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	-	5000 IU HCG	-	36			Less milt
Male	0.92	37	-	5000 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	-	5000 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= Milt conditions in case of male projected by positive sign, cPG= Carp pituitary gland, LRH-A_2= Leutinizing Releaseing Hormone, Dom.= Domperidone (Dopamine), Ca- inj.= Calcium injection, N/R= Not responding

Table 5. 2nd Breeding response of *Mugil cephalus* during last week of January 2015

Sex	Body wt (kg)	Length (cm)	Injection dose (unit/fish)			LP (hrs)	D_1 (µ)	D_2 (µ)	TSE (eggs/g bwt) /MC
			Priming (mg/kg)	Resolving 1 (after 24 hrs)	Resolving 2 (after 24 hrs)				
Female	1.85	53	60 cPG	LRH-$A_2$300µg + 0.3ml Dom.+ 0.5 ml Ca- inj.	LRH-$A_2$50µg + 0.3ml Dom.+ 0.2 ml Ca- inj.	48	587	650	892
Female	1.65	49	50 cPG	30000 IU HCG	-	36	563	665	865
Female	1.45	47	45 cPG	25000 IU HCG	-	-	570	-	N/R
Female	1.70	51	55 cPG	LRH-$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	-	5000 IU HCG	-	36			Less milt
Male	0.88	36	-	5000 IU HCG	-	36			Less milt

Table 6. 3rd Breeding response of *Mugil cephalus* in the 1st week of February 2015

Sex	Body wt (kg)	Length (cm)	Injection dose (unit/fish)			LP (hrs)	D_1 (µ)	D_2 (µ)	TSE (eggs/ g bwt) /MC
			Priming (mg/kg)	Resolving 1 (after 24 hrs)	Resolving 2 (after 24 hrs)				
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	30000 IU HCG	-	36	570	-	N/R
Male	1.13	39	-	No dose	-	36			N/R
Male	0.96	37	-	5000 IU HCG	-	36			N/R
Male	1.06	38	-	5000 IU HCG	-	36			N/R
Male	0.85	32	-	No dose	-	36			N/R
Male	0.87	34	-	5000 IU HCG	-	36			N/R

Table 7. Hypophyzation of *Mugil cephalus* in the breeding trials

Weight of fish (kg)	Length of fish (cm)	Initial diameter of oocytes (µm)	Total dose of hormones per fish				Number of injections	Responded signs (+)/(-)
			PG (mg)	LRH (µg)	Domperidone (µg)	HCG (IU)		
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	-	-	30000	2	+
1.45	47	570	45	-	-	25000	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	-	-	30000	2	(-)

(++) spawned with fertilized eggs, (+) spawned, (-) did not spawned & atresia checked

In the first trial, only single fertilization took place and the eggs took 44-48 hrs after spawning to hatch out. James *et al.* (1982) reported that the ideal incubation period of *M. cephalus* was 36-48 hrs at 20-25°C of temperatures. All the eggs spawned, fertilized and none had single oil globule. Egg diameter varied within a range of 650–680 µm, and oil globules had 250–280 µm. Fecundity of those females was determined as 735–900 eggs per gm of body weight. After fertilization cell division started within an hour but the fertilized eggs were settled down before starting further segmentation and finally huge mortality occurred and then the dead eggs were floating throughout the tank. Rapid fluctuation of temperature and poor milt quality as well as quantity of milts might be the main reason of mass egg mortality (Table 8).

Table 8. Stages of development *Mugil cephalus* in breeding trials

Age (hrs.) after fertilization	Stage of development	Temperature of water (°C)
1.00	Two cells division	21.5
2.00	Cell division continued	22.2
6.00	Beginning of segmentation, mortality started	24.2
12.00	High mortality	25.8

This is the first attempt of induced breeding of *Mugil cephalus* in a modified marine fish hatchery at Cox's Bazar. This indicates that induced breeding of

striped mullet is possible and also it could be successfully hypophyzed with hormone injection. Further breeding trials should be conducted using quality broods especially male for fine tuning of hormone induced artificial breeding.

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