Project ID: 305

Competitive Research Grant

Sub-Project Completion Report

on

Breeding Biology and Induced Breeding Technique of the Freshwater Gangmagur, *Hemibagrus menoda* (Hamilton, 1822)

Project Duration

May 2017 to September 2018

Department of Fisheries Management
Bangladesh Agricultural University
Mymensingh-2202, Bangladesh

Submitted to

Project Implementation Unit-BARC, NATP 2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215

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Project Implementation Unit
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Bangladesh Agricultural Research Council (BARC)
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Acronyms

ANOVA	=	Analysis of variance
BARC	=	Bangladesh Agricultural Research Council
BAU	=	Bangladesh Agricultural University
BW	=	Body Weight
BW	=	Body weight
CM	=	Centimeter
Co-PI	=	Co-Principal Investigator
DCP	=	Depth of Caudal Peduncle
DoF	=	Department of Fisheries
DPX	=	Distyrene, plasticizer xylene
EpNO	=	Early verinucleortar stage
et al.	=	Associates
F	=	Fecundity
FAO	=	Food and Agricultural Organization
FL	=	Fork length
Frss	=	Fast response survey system
g	=	Gram
GDP	=	Gross Domestic Product
GDP	=	Gross Domestic Product
GoB	=	Government of Bangladesh
GSI	=	Gonadosomatic Index
GW	=	Gonad weight
h	=	Hour
ha	=	Hectare
HCG	=	Human Chorionic Gonadotropine
HL	=	Head Length
Ho	=	Null Hypothesis
IUCN	=	International Union for Conservation of Nature
kg	=	Kilogram
l	=	Litre

LoA	=	Letter of Agreement
LpNr	=	Late perinucleolar stage
m	=	Meter
m	=	meter
mg	=	Milligram
mm	=	Millimeter
mt	=	Metric ton
N	=	North
NATP	=	National Agricultural Technology Program
No.	=	Number
OW	=	Ovary Weight
OW	=	Ovary weight
Pg	=	Pituitary gland
PG	=	Pituitary Gland
pH	=	Potential hydrogen
PI	=	Principal Investigator
PIU	=	Project Implementation Unit
ppm	=	Parts per million
pvc	=	Packed cell volume
S	=	Second
SE	=	Standard Error
SL	=	Standard Length
SL	=	Standard length
Tk	=	Taka
TL	=	Total length
TSP	=	Triple Super Phosphate
UO	=	Undeveloped oocyte
Wt.		Weight
YV	=	Yolk vesicle stage

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Executive Summary

The research was undertaken to develop environment friendly breeding technology of freshwater Gangmagur and thus to protect and conserve this endangered species through propagation and culture. Considering this aim, the reproductive biology and induced breeding protocol of Gangmagur. *Hemibagrus menoda* were carried in the Field Laboratory Complex at the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh. Reproductive parameters such as sex ratio, gonadosomatic index (GSI), ova diameter, fecundity and gonad histology data of the species were obtained from Kangsha River Netrakona, Bangladesh. Double hormone doses of PG and HCG extracts at 2♂:1♀ ratios were tested to evaluate their efficacy on ovulation, fertilization, hatching and survival rates of *H. menoda* using the induced breeding method. The ovulated eggs were placed in convex slides and the embryonic & larval developmental stages were studied under the microscope. Sex ratio (female: male) was found to be female biased (1.03:0.97). The monthly mean GSI of female *H. menoda* started to increase from May to June and reached the peak (12.50±4.97) in July, indicating the peak spawning season of the fish. Mean fecundity estimates based on mature females was 77273.77±276.82 for fishes with mean length of 31.85±2.39 cm, and lowest fecundity (22954.99; 25.00 cm) was in May and with the highest (222171.8; 40.20 cm) in July. Fecundity correlated positively with standard length (Log F = 0.0135 + 4.399 log SL; r^2 = 0.774), body weight (F = 198.7 BW - 47602; r^2= 0.805) and ovary weight (F = 1066 OW + 6124; r^2 = 0.832). In the mature ova, the ova diameter ranged from 0.77mm (standard length = 31.5± 2.15 cm) in May to 1.66mm (standard length = 40.2± 3.54 cm) in July, and spawning takes place once in a year but with longer duration from May to July. Oocytes in the Premature (PM) and mature (M) stages were abundant from April to August samples of ovary, indicating the spawning season. Successful ovulation occurred in the female's injected 24, 25, 26, and 27 mg PG, 3500, 4500, 5500 and 6500 IU HCG, 4, 6 and 7 ml Ovupin, 7, 5 and 3 ml Ovatide/kg body weight. Optimum doses of hormones were obtained from spawners injected double dose of 3500IU, 26 mg, 6 ml and 5 ml HCG, PG, ovupin and ovatide/kg body weight, respectively, in a 2♂:1♀, ratio. Unfertilized eggs were sticky, mean egg diameter increased from 0.8 ± 0.00 mm to 1.10 ± 0.01 mm and hatching occurred at 19.30 hr after fertilization at 24.1 °C water temperature. The egg yolk became completely absorbed within 68 hours at mean length of 6.5 ± 0.05 mm. Among the different hormones trialed, performance of PG extract was the best. Striping method is not applicable for this fish species. Water quality variables were within acceptable ranges for larval rearing. Finally, spawning period and breeding technique of Gangmagur, *H. menoda* had been identified.

CRG Sub-Project Completion Report (PCR)

A. Subject-project Description

1. **Title of the CRG sub-project: Breeding biology and induced breeding technique of the freshwater Gangmagur, *Hemibagrus menoda* (Hamilton, 1822)**

2. **Implementing Organization:**
   Department of Fisheries Management
   Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

3. **Name and full address with phone, cell and E-mail of PI and Co-PI:**
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   Co-Principal Investigator (Co-PI):
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4. **Sub-project budget (Tk):**
   4.1 Total : 2000000/=
   4.2 Revised (if any) : N/A
   Fund received : 1865048/=
   Fund spent : 1723462/=
   Unspent fund (Tk.) : 141586/=

5. **Duration:** From May 2017 to September 2018
   5.1 Start date (based on LoA signed): 9 May 2017
   5.2 End date : 30 September 2018

6. **Justification of undertaking the sub-project**

Fisheries and aquaculture play significant role in eliminating hunger, promoting health and reducing poverty for more than 800 million people who suffer from chronic malnourishment throughout the world (FAO, 2014). In Bangladesh, fisheries play an important role in the national economy and account for 4.7% of GDP, 9.1% of the export earnings (second largest, next to Ready-Made Garments) and 60% of the supply of protein (DoF, 2015). This might not be unconnected with the fact that Bangladesh is the third largest source of freshwater in the world (4708193 ha) and possesses vast inland water areas-impoundments, lakes, reservoirs, haors, ox-bow lakes, ponds, inundated paddy fields, flood plains and estuaries which have rich and extensive fishery resources (Rahman and Akhter, 2015). Although Bangladesh's resources for fish production are vast, there are biological, social and economic constraints where research needs to be strengthened to harness this potential (Hossain, 2015). Catches from capture fisheries are declining, species disappearing due to chronic over fishing and various anthropogenic and environmental causes (Welcomme *et al.,* 2010).

The supply of fishes that was found a plenty once in the country's rivers and rivulets, is not increasing to match the growing domestic demand (FE, 2013). Among the 276 fish species of Bangladesh, 56 are considered to have disappeared from the rivers, haors and beels of Bangladesh (Rahman and Akhter, 2015). The open water fish diversity of Bangladesh has been negatively impacted by a series of natural and anthropogenic actions ranging from siltation of water bodies, over-exploitation of natural fisheries to changing the habitats from small-to large-scale development interventions.

The catfish family Bagridae, commonly found throughout fresh- and brackish-water bodies in Asia and Africa, includes more than 200 species in 17 genera and is one of the largest catfish families presently recognized (Ferraris, 2007). At least 55 species of the Catfishes belonging to 35 genera have been recorded so far in Bangladesh (Rahman, 2005) and though the total production of these Catfishes has increased in recent years, the availability of some of them is declining day by day (Bashar *et al.,* 2009). As like other fish, many catfish species, like *Hemibagrus menoda* have become locally endangered (DoE, 2015). This fish is well known for its good taste and high market value.

In the past, fish farmers collected seeds from natural sources for aquaculture but its supply gradually declined due to some natural and manmade factors. The natural sources of seeds has declined to a critically low level over the last decades and surprisingly only 1% of the total seed is reportedly caught from rivers (FRSS, 2010-11). The rest 99% of the seeds are produced in 76 government and 845 private hatcheries (DoF, 2012). Although the seed production in the hatcheries is enough to fulfill the farmers demand, but the quality of seed has been deteriorated to a great extent and a considerable percentage of hatchery-produced seeds have shown low growth.

According to IUCN Bangladesh (2003) recorded a total 54 threatened indigenous fish species in the country including 14 vulnerable, 28 endangered and 12 critically endangered fish species. Our present knowledge about breeding biology: fecundity, GSI, gonadal histology, induced breeding and culture technique of Gangmagur, *Hemibagrus menoda* is very little. To the best of my knowledge, no work has been done on breeding biology, fecundity, GSI, gonadal histology, induced breeding and culture technique of Gangmagur. We believe that it will be possible to develop an appropriate potential mechanism for induced breeding, culture technique of this species through hormonal manipulation and use of various hatchery devices. Well-planned research activities may lead to resolve the complicated reproductive process and propagation of Gangmagur. This will make possible the way for the development of pond culture techniques of this species for our culture system too. This is a new work. So, no such scientific work has been conducted at BAU, Mymensingh or elsewhere in Bangladesh.

Hemibagrus menoda (Hamilton, 1822), a catfish commonly named Menoda catfish and locally called Gang tengra, Golsa-tengra, Arwari, Gangmagur, Koune magur in Bangladesh, is a demersal freshwater Catfish that dwells in the Ganges, Brahmaputra, Mohanad and Godavari river drainages in Bangladesh and northern India. *H. menoda* belongs to the Class Actinopterygii (Ray-finned fishes), Order Siluriformes, Family Bagridae, Genus Hemibagrus and Species *H. menoda* (Hamilton, 1822). Its synonyms include *Pimelodus menoda* Hamilton, 1822, *Macrones menoda* (Hamilton, 1822) and *Mystus menoda* (Hamilton, 1822). *H. menoda is* distinguished from its congeners by a unique combination of characters prominent of which is having a pattern of dark dots arranged in vertical columns on the sides of the body, a convex snout and a broad shallowly incised humeral process (Ng and Ferraris, 2000).

H. menoda have been utilized as experimental animals and are valuable food fishes because of their large size (450 mm or 17.7" SL, but can attain up to 800 mm), tasty flesh and high market value but they are less frequently encountered in markets than other genera of large Bagrid Catfishes such as *Rita* and *Sperata* (Hoque *et al.,* 1998; Ng and Ferraris 2000). There has been an alarming trend of the conservation status of *H. menoda.* The IUCN (2015) assessed 253 freshwater fishes in Bangladesh and *H. menoda* was among the 26 species categorized as near threatened. Besides, Hossain (2014)

reported that as a consequence of natural and anthropogenic induced changes, many Bangladeshi species have become critically endangered, such as *H. menoda.* There is therefore the need for the domestication and captive production of this valuable fish to avert its imminent shortage and looming extinction.

Some catfish cultures are well established and popular due to the availability of broodstock, fry and fingerlings. In Bangladesh, to date, about 22 indigenous fish species have been domesticated and their breeding and rearing protocols developed. These include fish in the order Siluriformes such as *Ompok bimaculatus, O. pabda, Mystus vittatus, M. gulio, Clarias batrachus* and *Heteropneustes fossilis* (Hussain, 2014). The rural people of Bangladesh are largely dependent on indigenous fish species for animal protein but there is lack of concern over the decline of indigenous species diversity (Johan *et al.,* 2014). Study of the *H. menoda will* aid in increasing the efficiency of aquaculture in Bangladesh. Catfishes in particular do not readily reproduce in fish ponds. Induced breeding therefore offers a reliable solution to the non-availability of the *H. menoda* fingerlings and for success in induced breeding, there is the need for understanding of the reproductive physiology of the fish so as to supply the farmers the much needed seed in large numbers. Moreover seed production is one of the key biological areas that must be addressed in the development of a viable aquaculture system (Harvey and Carolsfield, 1993).

Considering these realities, the present research work will attempt to explore some conservation and management measures of the Menoda catfish. This will involve studies on the age at sexual maturity, spawning season and fecundity so as to assess the reproductive potential and to evaluate the viability for commercial production of the fish. Induced breeding trials for the production of fry and fingerlings of this fish using different doses of hormones will also be conducted. Ration and stocking density plays a crucial role in determining the growth and survival, and ultimately the seed production, in an intensive system (Sahoo *et al.,* 2010). This research will also focus on the determination of suitable larval rearing and culture technique using different feeds and stocking density so as to develop an aquaculture package of the fish with potential of reproducibility.

7. Sub-project goal:

Development of environmental friendly breeding technique for protection, conservation and sustainable aquaculture of endangered high valued freshwater Gangmagur

8. Sub-project Objective(s):

The aim of this research work is to develop suitable technology for breeding, larval rearing and culture management of the Menoda catfish, *Hemibagrus Menoda,* which could be disseminated among the hatchery managers, nursery operators and fish farmers. To achieve this goal the proposed research has been designed with the following objectives:

i. To study the reproductive cycle of *H. menoda* by fecundity, gonado somatic index (GSI) and gonadal histology; and
ii. To develop induced breeding technique of *H. menoda* using inducing hormones.

9. Implementation location(s):

Fisheries Field Laboratory Complex, Department of Fisheries Management, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh-2202.

Methodology:

Experiment 1: Study of reproductive cycle of Menoda by Gonado Somatic Index (GSI), fecundity and gonadal histology

1.1 Specific objectives

(i) To determine the gonadal maturity of male and female Menoda;
(ii) To determine periodicity of spawning season;
(iii) To ascertain actual pattern of gonadal maturation;

The reproductive cycle of the fish was studied with a view to identifying the time of spawning, when fully developed gametes are released. The experiment was conducted at the Water Quality and Pond Dynamics Laboratory and the Aquaculture Laboratory of Bangladesh Agricultural University, Mymensingh, Bangladesh, during from May 2017 to April 2018.

1.2.1 Sampling site

Samples of *H. menoda* were caught from the Kangsha river located at Jaria – Jhanjail, Latitude 25^0 0′ 41.10" N and Longitude 9^0 0 38’ 27.16" E in the Netrakona district, Bangladesh (Fig. 1).

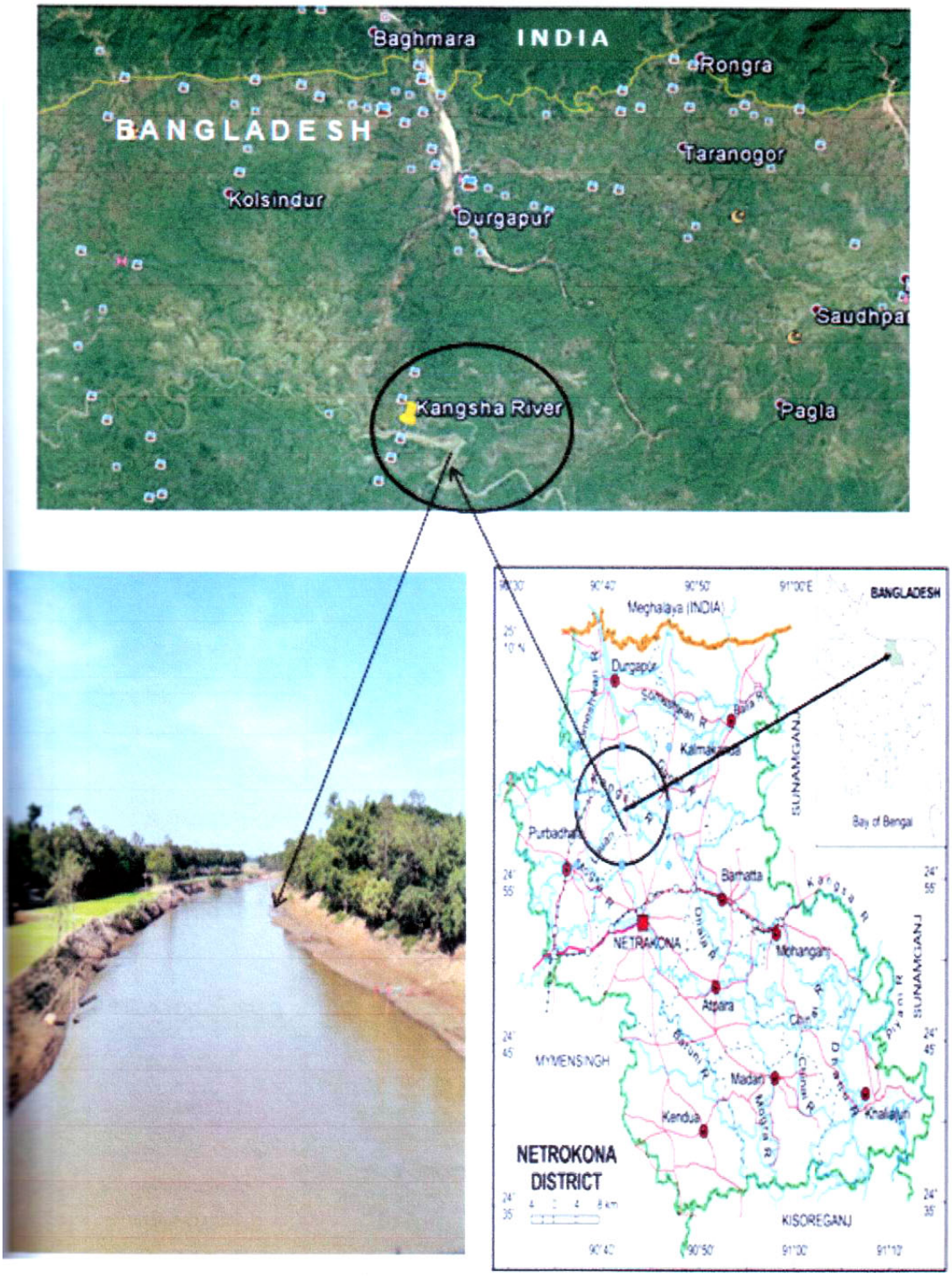


Fig. 1 Map of the study area showing the sampling site in the Netrokona district, Bangladesh

1.2.2 Sample collection

A total of 79 fish samples of *H. menoda* were collected from the landing sites at Kangsha River Netrakona district on monthly basis from May 2017 to September 2018. Live fishes were carried in jerry cans half filled with water. Care was taken to change the water intermittently during transportation. The dead fishes were chilled in ice and transported to the laboratory.

1.2.3 Observation and preservation of fish samples

Preliminary identification of the fish in terms of colour, fins, spots and abdomen status and genital types were carried out. The live fish was stunned while the dead chilled ones were thawed and preserved in 10% formalin, properly labeled and stored in the Aquatic Ecology Laboratory of the Department of fisheries Management, Bangladesh Agricultural University, Mymensingh.

1.2.4 Sex differentiation and sex ratio

Sex differentiation of the samples was carried out by adopting the method for distinguishing male from the female of Bagridae catfish (Norton et al., 2011). Males have single urogenital opening and a genital papilla just before the anal fin; females have two openings partitioned by a septum and are deeper bellied in comparison to slender males. The fish was dissected, number of female (♀) and male (♂) recorded and sex ratio was calculated by dividing the number of females with the number of males using the formula:

$$\text{Sex ratio} = \frac{\text{No. of } (♀)}{\text{No. of } (♂)}$$

The chi-square test was adopted to determine whether the proportion of females differ from the proportion of males using the formula:

$$\chi^2 = \sum (O-E)/E,$$

whereby; χ^2 = Chi-square test; O = observed values; E = expected values.

The null hypothesis (H_O) that there is no difference between the proportion of females and the proportion of males was tested at 5% alpha level (Jega *et al.,* 2017).

1.2.5 Measurement of some morphometric character

The preserved samples were properly washed with tap water and tissue paper used to absorb moisture on the surface of the body. The Standard length (SL) was measured from the tip of the snout to the base of the caudal fin, or end of the spine (Plate 1), Fork length (FL) was measured from the anterior part of the head with jaws closed, to the middle of the caudal fin, Total length (TL) was measured from the anterior part of the head with jaws closed, to the middle of the caudal fin, Head length (HL) was measured from the tip of the snout to the dorsal-most end of the gill opening, Head depth (HD) represents distance along the vertical axis of the eye, Depth of caudal peduncle (DCP) was measured from the end of the anal fin to the base of the median caudal rays. SL, FL and TL were measured using meter scale mounted on a wooden support. HL, HD and DCP were measured using a slide caliper. The body weight was taken using a digital balance with a precision of 0.01 g (Model: HL – 300A AND company limited).

Plate 1: Dorsal part of *H. menoda* showing standard length measurement

1.2.6 Gonad collection

The ventral side of the fish was cut and opened from the anus towards the lower jaw by using scissors. The whole mass (fat tissues, stomach, liver, ovary, liver, etc) were removed and the gonads were carefully detached from the other visceral organs by the use of needles and forceps. The gonads were then cleaned with tap water and wiped with blotting paper. The testes (Plate 2) and ovary (Plates 3) weight and length were then taken before being kept in 10% buffered formalin for study of fecundity and gonadosomatic index.

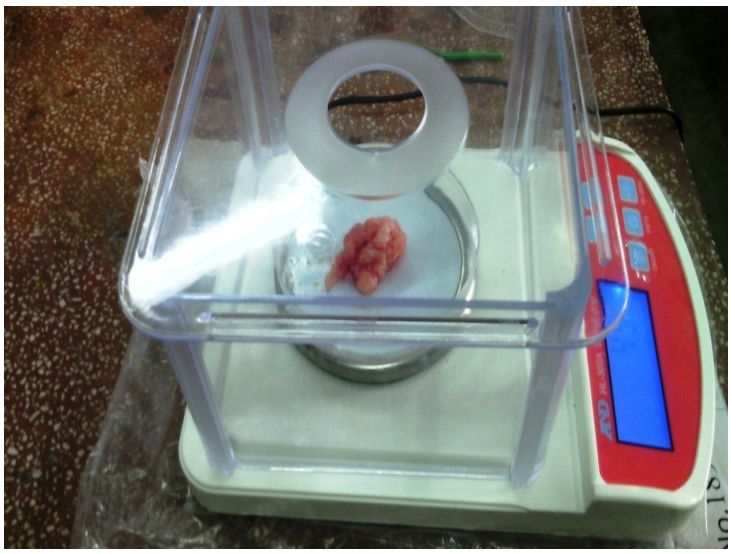


Plate 2: Weighing of testes of *H. menoda*

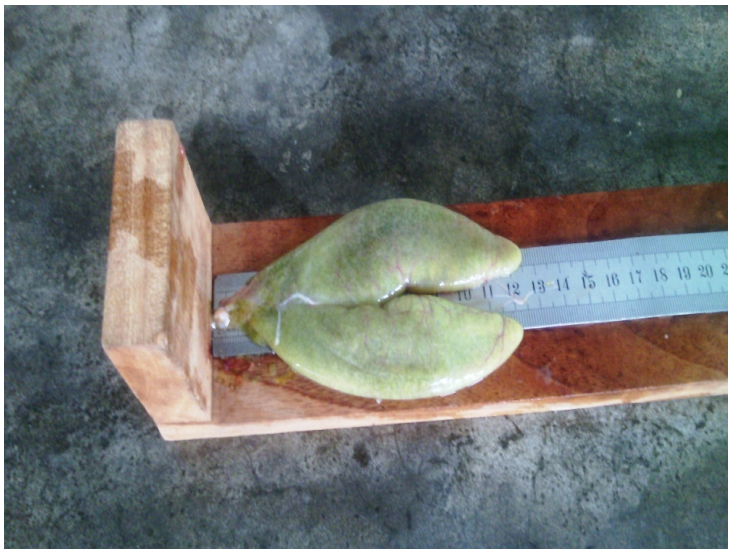


Plate 3: Length measurement of ovary of *H. menoda*

1.2.7 Determination of Gonado Somatic Index (GSI)

The study of GSI determines the state of maturity in terms of gonadal development and onset or periodicity of spawning season. GSI assumes that the gonad increases in size with increasing development comparing with the mass of the gonad (GW) to the mass of the fish (BW). To determine the sexual maturity for male and female, their gonado somatic index was calculated using the formula:

$$GSI = \frac{\text{Gonad weight (GW)}}{\text{Body weight (BW)}} \times 100 \qquad \text{(DeVlaming, 1972)}$$

Monthly average GSI values of male and females will be graphically presented.

1.2.8 Estimation of ova diameter

Ova diameter at different stages of maturity was measured with the help of Image J Software. Formalin was rinsed off the hardened eggs and moisture absorbed with a blotting paper. Ten to 12 eggs from each sample were captured under a microscope with camera attached. A stage micrometer was placed under the microscope, captured and calibrated. The scale was used to estimate the diameter of the eggs following the procedure in the Image J Software.

The month-wise diameter ranges and size frequency percentages of ova in the mature ovaries during the study period were estimated.

1.2.9 Fecundity estimation

For fecundity determination, the gravimetric method (Doha and Hai, 1970; Blay, 1981; Islam *et al*., 2008) was followed whereby the external connective tissues were removed from the surface of the ovaries and blotting paper used to remove moisture from the surface of the ovaries. A sub sample of about 1 -2 mm and 0.20 mg of the gonad was cut from the anterior, middle and posterior parts of the ovary and separately put in a petri dish. The eggs were separated by using needle and forceps and counted with the aid of microscope or magnifying glass. Care was taken to sort the mature and immature eggs. The total number of matured and immature eggs of each ovary sub sample was sorted out and counted with the help of a needle and magnifying glass and estimated using the equation:

$$F_1 = \frac{\text{Gonad weight}}{\text{Sub samle weight (BW)}} \times \text{Number of eggs in sub sample} \qquad \text{(Yelden and Avsar 2000)}$$

Where, F_1 = Fecundity of fish

By taking the mean number of three sub sample fecundities (F1 + F2 + F3), the Absolute (total) fecundity F_T, for each female fish was then determined using the formula as thus:

$$F_T = \frac{F_1+F_2+F_3}{3} \qquad \text{(Hossain } \textit{et al.,} \text{ 2012)}$$

1.2.10 Histological examination of oocytes of threatened menoda catfish

Ovaries belonging to a range of developmental stages were prepared for histological study by fixing in 10% buffered formalin for at least 3 days. They were then taken out from vials, washed in running water and cross sections from different parts of the ovaries were cut and placed on tissue paper so as to absorb the moisture. Each cross section of an ovary was separately put into one cassette and labeled with a pencil accordingly. Standard histological processes involving dehydration, clearing, infiltration, embedding, trimming and blocking, sectioning, staining, mounting and microscopic observation of slides for identification of gametogenic cell types were performed as follows:

Dehydration

First the gonads (tissue) were dehydrated to remove the water which was present. The dehydration process was carried out by immersing the gonad tissue in graded series of alcohol solutions until 100% water-free alcohol was reached as shown in the following schedule:

Sl. No.	Solution	Time
1	80% Ethyl alcohol	12-14 hours
2	95% Ethyl alcohol	1 hour
3	95% Ethyl alcohol	1 hour
4	100% Ethyl alcohol	1 hour
5	100% Ethyl alcohol	1 hour
6	100% Ethyl alcohol	1 hour

Clearing

Following dehydration, the gonad was immersed in two different benzine immersions in which the alcohol was gradually replaced with benzine. Because alcohol and wax don't mix, clearing was carried out using benzine. This made the tissue transparent and clearer and consistent paraffin blocks were obtained.

SL no.	Solution	Time
1	Benzine	1 hour
2	Benzine	1 hour

Infiltration

From Benzene, the samples were taken out from the cassettes and placed in paraffin sequentially in incubator (EL - 450B) following these steps:

Sl. No.	Solution	Time
1	Paraffin	40 minutes
2	Paraffin	40 minutes

Embedding

After infiltration, the cassettes were taken out from automatic tissue processor one after the other, opened and samples settled in the middle of a small paper-box previously marked as in the cassette and filled with melted paraffin from wax dispenser. Then the paper-boxes were allowed to cool at room temperature. Thus the embedded blocks containing the sample were formed which allowed smooth sectioning.

Trimming and blocking

A sharp knife was used for the trimming process whereby the undesirable wax layers of the embedded blocks are trimmed to obtain suitable blocks.

Plate 4: Microtome machine used in sectioning of eggs

Sectioning

Paraffin embedded blocks was cut by microtome knife in microtome machine (KEDEE KD-3358, China) at 5µm thick size (Plate 4). The sections were placed on lower part of a glass slide previously tagged and filled with water drops. The sections were kept overnight at room temperature for proper drying.

Staining

Staining is a process by which samples are stained with various dyes and staining materials such as Haematoxylene and Eosin, so that their components become visible under microscope. The sectioned ovaries were placed on a slide warmer so as to dry the slides before staining (Plate 5). Staining was done following the schedule in described below:

Plate 5: Slide warmer in the staining process

The staining schedule

Sl. No.	Solution	Time	Process
1.	Xylene	10 minutes	Clearing
2.	Xylene	10 minutes	Clearing (1-3)
3.	Xylene	10 minutes	Clearing (1-3)
4.	100% Ethyl alcohol	5 minutes	Rehydration
5.	100% Ethyl alcohol	5 minutes	Rehydration (4-8)
6.	90% Ethyl alcohol	3 minutes	Rehydration (4-8)
7.	80% Ethyl alcohol	3 minutes	Rehydration (4-8)
8.	70% Ethyl alcohol	3 minutes	Rehydration (4-8)
9.	50% Ethyl alcohol	2 minutes	Staining
10.	Distilled water	15 dips	Staining (9 - 15)
11.	Hematoxylene (Harris)	3-5 minutes	Staining (9 - 15)
12.	Washing in tap water	15 minutes	Staining (9 - 15)
13.	50% Ethyl alcohol	10-15 dips	Staining (9 - 15)
14.	95% Ethyl alcohol	30 seconds	Staining (9 - 15)
15.	Eosin Y	1-2 minutes	Staining (9 - 15)
16.	95% Ethyl alcohol	2 minutes	Dehydration
17.	100% Ethyl alcohol	1 minute	Dehydration (16-19)
18.	100% Ethyl alcohol	3 minutes	Dehydration (16-19)
19.	100% Ethyl alcohol	1 minute	Dehydration (16-19)
20.	Xylene	20 minutes	Clearing
21.	Xylene	20 minutes	Clearing (20-21)
22.	Drying at room temperature	Over night	Drying

Mounting

In this process the tissue sections on the slides were covered with a cover slip. DPX was used for mounting as a mounting agent. A drop of DPX was put on each slide followed by attachment of cover slip (22mm × 22mm). After mounting the slides were kept for several hours at room temperature (Plate 6).

Plate 6: Mounting of the slides using DPX with cover slip

Microscopic view of the tissue sections

The mounted slides were observed under a microscope (OLYMPUS microscope; Model: CX21, Japan) which was connected to computer with OPTIKA MB3 Digital camera (3.14 Mega pixel). By the help of this mechanism numerous photographs were taken at different magnifications (Plate 7).

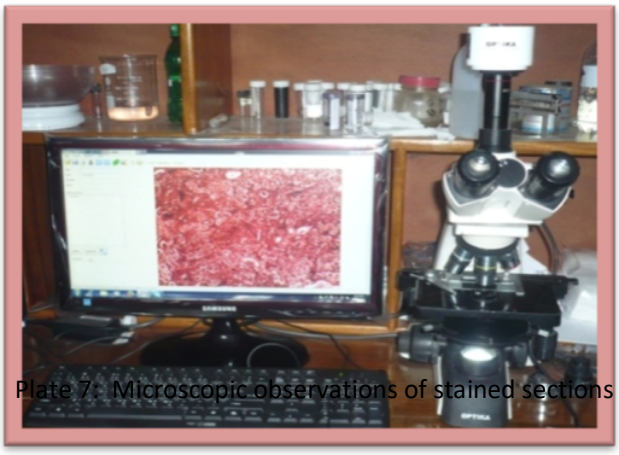


Plate 7: Microscopic observations of stained sections

Experiment 2: Induced breeding trial of Menoda through the use of different inducing hormones

2.1 Specific objectives

i. To determine optimum dose of PG extract, HCG , Ovupin and ovatide hormones for the induced breeding of the Menoda catfish;
ii. To develop induced breeding protocol for *H. menoda*

This experiment was carried out in the fish hatchery of the Field Laboratory Complex and the Aquaculture Laboratory, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh during May 2017 to July 2018 and it involved the determination of optimum dosage of PG (Pituitary gland, HCG (Human Chorionic Gonadotropine) extracts, ovupin and ovatide for the induced breeding of the menoda catfish.

2.2.1 Collection and management of broodstock

The wild fishes collected from the Kangsha River, Netrakona, and reared in brood ponds, situated in the Field Laboratory Complex, BAU, were used for this experiment.

Plate 8: Gang magur, *Hemibagrus menoda*

2.2.2 Brood selection

To carry out the induced breeding trial, male and female broods were caught from the brood rearing ponds using a cast net. The broods were examined and healthy looking, ready to spawn male and female were selected based on the following criteria: Males have swollen and reddish urogenital papillae (Plate 8), body relatively smaller, elongated and slender in shape while the females were identified by having rounded and protruding abdomen (Plate 9) which is soft when touched with fingers and the genital opening swollen and sometimes reddish in colour.

Plate 9: Ventral view of mature male *H. menoda* showing swollen urogenital papilla reddish at the tip

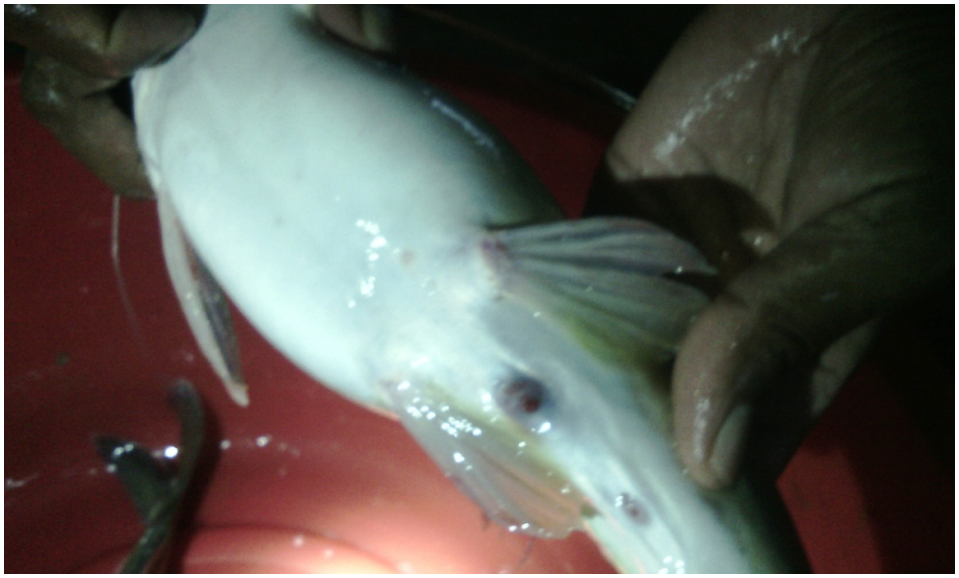


Plate 10: Ventral view of gravid ready to spawn female *H. menoda* showing swollen abdomen

2.2.3 Conditioning

The selected broods were transferred to the circular tank in the hatchery of the Field Lab of the Faculty of Fisheries, BAU, Mymensingh, where they were separately kept in cemented tanks with continuous water showering for about five hours prior to administering of hormones. Males and females were kept off-feed during conditioning.

2.2.4 Collection and preparation of PG, HCG, Ovupin and Ovatide

PG and HCG vials (Plate 10) bought from a reputable shop in Mymensingh town were used as inducing agents. For PG, the proper dosage of hormone injected into the fishes was calculated based on the recommended dose and body weight of the brood fish using the formula;

$$\text{Weight of PG(mg)} = \frac{\text{Wt x Pt}}{100},$$

Where,

Wt = total body weight of all the fishes injected

Pt = the rate in mg PG injected/kg body weight under a particular treatment.

The PG was weighed and then transferred into a tissue homogenizer with small amount of distilled water (Plate 11). The suspension of the homogenized glands was poured into a cubate and diluted with distilled water to dissolve it and centrifuged for 10 minutes at about 2000 rpm. The supernatant solution was then taken out in a 5ml hypodermic syringe for injection. For HCG preparation, 5 ml distilled water was used to dissolve 5000 IU contained in a single vial. Then based on fish weight, 1 ml was collected in a syringe which represents 1000 IU HCG to be injected to the fish. The remaining HCG i.e 4000 IU was collected in a syringe and preserved in a refrigerator (not chilled) for further use (2^{nd} dose).

For ovupin preparation, the powder form synthetic hormone was diluted with distilled water to dissolve it in the contained vial and shaked for a few minutes. The prepared solution was then taken out in a 1 ml hypodermic syringe for injection.

The ovatide hormone was weighed the solution was then taken out in a 1ml hypodermic syringe for injection.

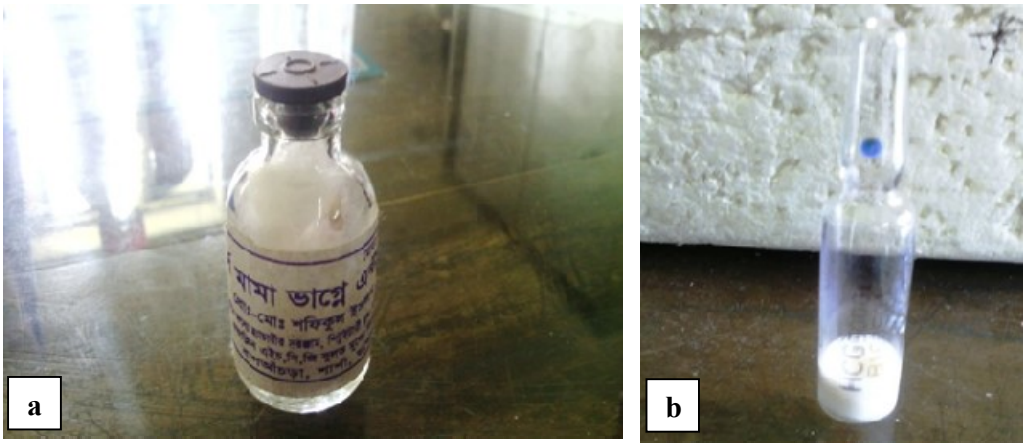


Plate 11: a) PG and b) HCG vials containing the hormones used for the induced breeding of the menoda catfish

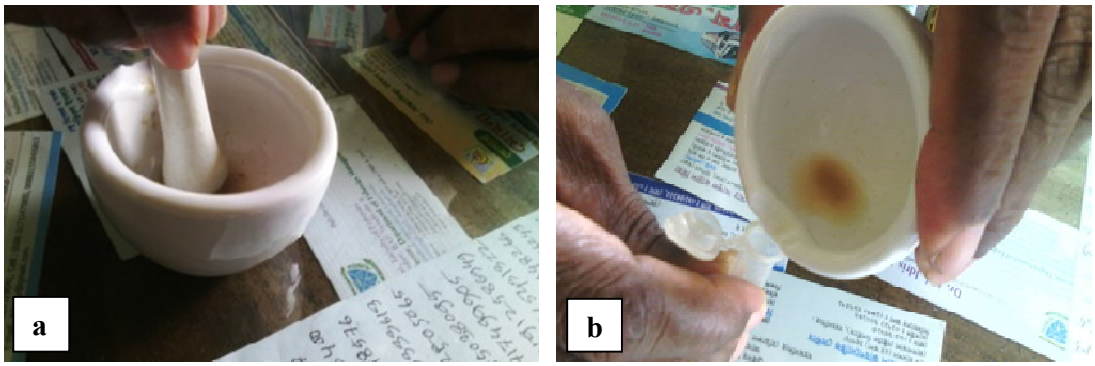


Plate 12: a) Crushing of the PG particles and b) pouring the diluted PG into vials before centrifuging

2.2.5 Hormone injection

The selected brood female fish weights were taken before being injected with appropriate quantity of PG or HCG solution, accordingly. One ml disposable syringe was used for injecting the hormone to the recipient fish in which appropriate amount of diluted hormone solution was drawn from a crucible. The fish were caught from the spawning tank with net and placed on soft foam on a table. Wet clean towel was used to cover the fish head and then the required amount of the extract (1^{st} dose) was administered intramuscularly to the female below the dorsal fin at an angle of about 45^{0}C with the body using a graduated syringe (Plate 12). The 2^{nd} dose was given to the female 6 hours after the 1^{st} dose. The male fish was given only one dose at the time when the female was given the 2^{nd} dose. The injected fish were then released into breeding hapa placed in tanks for synchronized spawning.

Plate 13: *H. menoda* being injected with hormone

2.2.6 Observation of reproductive behavior

After injection the male and female broods were kept together in the spawning tank (temperature 25.5 ^{0}C; pH 7.3; dissolved oxygen 6.5) and their reproductive behavior was closely monitored. It was observed that the males were in constant pursuit of the females though most often they closely remained together under the shower.

2.2.7 Experimental design

This experiment was conducted mainly using three trials each of PG (Table 1) and HCG (Table 2), each trial consist of three treatments, T_1, T_2 and T_3, with three replications each in a Complete Randomized Design. For each treatment, a female to male ratio of 1:2 (based on preliminary results) was adopted. A total of 27 brood fish which were obtained from the wild and reared in the pond were used for each trial. The effective dose for induced breeding of *H. menoda* was determined based ovulation, fertilization, hatching and survival rates.

Table 1. Layout of experiment for induced breeding of *H. menoda* with different doses of PG hormone at 1♀:2♂ ratio

Trial	Treatments	Brood fish ratio	Brood fish weight (g)	PG dose (mg/kg body weight)	1st dose (mg PG/kg body weight)	Time interval (hrs)	2nd dose (mg PG/kg body weight)
Trial -1 (July 2016)	T_1	Female	1080	25	10	6	15
		Male	540		-		10
		Male	300		-		10
	T_2	Female	380	35	15		20
		Male	378		-		15
		Male	300		-		15
	T_3	Female	390	45	20		25
		Male	300		-		20
		Male	300		-		20
Trial 2 (May 2017)	T_1	Female	400	22	08		14
		Male	475		-		08
		Male	290		-		08
	T_2	Female	1200	26	10	6	16
		Male	550		-		10
		Male	345		-		10
	T_3	Female	800	28	12		16
		Male	392		-		12
		Male	346		-		12
Trial 3 (June 2017)	T_1	Female	390	24	10		14
		Male	300		-		10
		Male	300		-		10
	T_2	Female	380	27	12	6	15
		Male	300		-		12
		Male	300		-		12
	T_3	Female	1080	30	12		18
		Male	540		-		12
		Male	300		-		12

Semi artificial (or induced natural) propagation method was adopted which involved synchronized spawning in breeding hapas whereby the injected broodfish (male and female spawners) were placed into a breeding hapa fixed in circular and rectangular tanks (dimensions: 1.22 x 2.74 x 0.37 m)according to hormone and dosage administration on trial and error basis. In this method, the males were not killed after injection. Double hapa (upper and lower) were used with the mesh size of the upper hapa (dimensions: 1.35 x 1.12 x 0.36 m, mesh size) being larger than the lower hapa (1.24 x 1.02 x 0.36 m). After ovulation and fertilization, the upper hapa was then removed along with the spent spawners while the fertilized eggs settled in the lower hapa which served as a shelter during the incubation period. Larvae also hatched in the hapa.

Table 2. Layout of experiment for induced breeding of *H. menoda* with different doses of HCG hormone at 1♀:2♂ ratio

Trial	Treatment (HCG, IU/kg bw)	Fish body weight (g)		1st dose HCG (IU/kg body weight)	2nd dose HCG (IU/kg body weight)	1st dose HCG (IU/kg body weight)
		♀	♂	♀	♀	♂
Trial -1	T_1 (600)	678	540	200	400	200
	T_2 (900)	477	528	300	600	300
	T_3 (1200)	654	359	400	800	400
Trial -2	T_1 (1500)	545	350	500	1000	500
	T_2 (2500)	534	437	1000	1500	1000
	T_3 (3500)	692	568	1500	2000	1500
Trial -3	T_1 (4500)	745	689	2000	2500	2000
	T_2 (5500)	757	740	2500	3000	2500
	T_3 (6500)	961	764	3000	3500	3000

Table 3. Layout of experiment for induced breeding of *H. menoda* with different doses of ovupin hormone

Treatments	Brood fish ratio	Brood fish weight (g)	Ovupin dose (ml/kg body weight)
T_1	Female	890	4
	Male	570	1.5
	Male	388	1.5
T_2	Female	850	6
	Male	358	2
	Male	320	2
T_3	Female	485	7
	Male	290	3
	Male	310	3

Table 4. Layout of experiment for induced breeding of *H. menoda* with different doses of Ovatide hormone at 1♀:2♂ ratio

Trial	Treatments	Brood fish weight (g)		dose (ml Ovatide/kg body weight)
Trial -1	T_1	Female	1020	**7**
		Male	570	3
		Male	388	3
	T_2	Female	840	5
		Male	368	2.5
		Male	310	2.5
	T_3	Female	475	3
		Male	295	1.5
		Male	295	1.5

2.2.8 Spawning, estimation of fertilization rate and incubation of eggs

Ovulation and fertilization occurred in the spawning hapa. After ovulation the upper hapa was removed along with the spent spawners leaving the fertilized eggs. Fertilization rate was determined by taking 100 eggs from each treatment and then incubated in 1.5 litres bowls each having sufficient water flow via a PVC pipe with inlet and outlet. The number of fertilized and unfertilized eggs in each bowl (treatment) was counted with a magnifying glass. The unfertilized eggs were whitish and opaque while the fertilized eggs were slightly transparent and showed evidence of cell division (when examined under the microscope). Incubation of the eggs occurred in the spawning tank with continuous water supply.

2.2.9 Care of hatchlings and determination of hatching rates

Care of hatchlings started from the moment the eggs began to hatch. Separation of larvae from unhatched eggs was achieved by siphoning with a 1.5mm rubber hose. The larvae were closely monitored so as to observe the time of yolk sac absorption for first feeding of larvae. Aeration was provided using aerators and flow through systems. After completion of hatching, about 6 hours post hatching, the number of larvae/hatchlings in each bowl were counted by siphoning them out.

2.2.10 Determination of survival rates

Survival rate was determined by randomly collecting 200 hatchlings from each replication under a particular treatment and stocked in trays containing water for three days (without feeding?).
Percent ovulation, fertilization, hatching (hatchability) and survival rates were determined as follows:

$$\% \text{ ovulation} = \frac{\text{No. of fish ovulated}}{\text{Total number of fish injected}} \times 100$$

$$\% \text{ fertilization} = \frac{\text{No. of fertilized eggs}}{\text{Total number of eggs (fertilized and fertilized)}} \times 100$$

$$\% \text{ hatching rate} = \frac{\text{No. of eggs hatched}}{\text{Total number of fertilized eggs}} \times 100$$

$$\% \text{ survival rate} = \frac{\text{No. of live hatchlings 72 hours after hatching}}{\text{Total number live hatchlings just after hatching}} \times 100$$

2.2.11 First feeding

Having observed that the hatchlings completely absorbed their yolk sac 68 hours after hatching and swam horizontally, egg yolk emulsion (Plate 13) was given to them as first feeding. Hard boiled chicken egg yolk was wrapped in a glass nylon cloth (Plate 14), immersed in water contained in an enamel plate and sieved by pressing the cloth in the water. Showering and aeration was stopped for 10 minutes during which the fish was fed the egg yolk emulsion, showering continued thereafter.

Plate 14: View of a) egg yolk and b) egg yolk emulsion prepared as first feed for the *H menoda* hatchlings

2.2.12 Physico-chemical parameters

The water quality variables *viz* temperature (^{0}C), pH and dissolved oxygen (mg/l) in the spawning tanks were monitored throughout the period of the experiment.

2.2.13 Statistical analysis

Data collected was recorded in MS Excel. Analysis of variance (ANOVA) in SPSS version 20 software for windows was used to compare treatment means and Tukey HSD used to test for significant difference.

11. Results and Discussion

Experiment 1: Study of reproductive cycle of Menoda by Gonado Somatic Index (GSI), fecundity, and gonadal histology

In order to identify the maturity stages, spawning season and periodicity of spawning of *H. menoda*, aspects of its reproductive biology such as the sex ratio, gonadosomaticc index, fecundity, ova diameter and gonadal histology were studied.

Sex ratio

A total of 79 samples, including 40 females and 39 males were used to examine the sex ratio of *H. menoda* over the 12 months study period in the Kangsha River, Bangladesh**.** The annual sex ratio of females to males was found to be 1.03:0.97, in favour of females (Table 5). Though the sexes were found be unequal in the months of March, April, June, August, September, December and January, Chi–square test indicated a non-significant difference ($p > 0.05$) in the sex ratio, with Chi–square values less than the critical values (3.84).

Table 5. Sex ratio of *Hemibagrus menoda* collected from the Kangsha River at Zanzail in the Netrakona district

Month	No. of fish	No. of female	% of female	No. of male	% of male	Sex ratio	χ^2	Critical value
May	6	3	50.00	3	50.00	1.0:1.0	0.00	3.84
June	9	3	33.33	6	66.67	1.0:2.0	1.00	3.84
July	8	4	50.00	4	50.00	1.0:1.0	0.00	3.84
August	8	5	62.50	3	37.50	1.7:1.0	0.50	3.84
September	7	3	42.86	4	57.14	1.0:1.3	0.14	3.84
October	6	3	50.00	3	50.00	1.0:1.0	0.00	3.84
November	6	3	50.00	3	50.00	1.0:1.0	0.00	3.84
December	7	3	42.86	4	57.14	1.0:1.3	0.14	3.84
January	5	3	60.00	2	40.00	1.5:1.0	0.20	3.84
February	6	3	50.00	3	50.00	1.0:1.0	0.00	3.84
March	5	3	60.00	2	40.00	1.5:1.0	0.20	3.84
April	6	4	66.67	2	33.33	2.0:1.0	0.66	3.84
For the year	79	40	51.52	39	48.48	1.3:0.97	0.24	3.84

Morphology of the gonads

Macroscopic examination of 79 samples of *H. menoda* was carried out during the 12 months study period. As in most bagrids, the gonads in female and male menoda catfish are bilobed, located in the dorsal portion of the body cavity, separated into two equal sizes, joined each other at the caudal region and formed a common duct at the base of uro-genital papillae. Ovaries in the developing and immature *H. menoda* are pinkish, slender, translucent and resemble immature testes in appearance. As they advance in maturity, they become pale green and greenish in colour and expand in length and diameter (Plate 15). Testes are thin, ribbon-like and somewhat segregated in developing (immature) fish but as the fish progress in maturity, they become creamier, whitish and serrated (Plate 16)

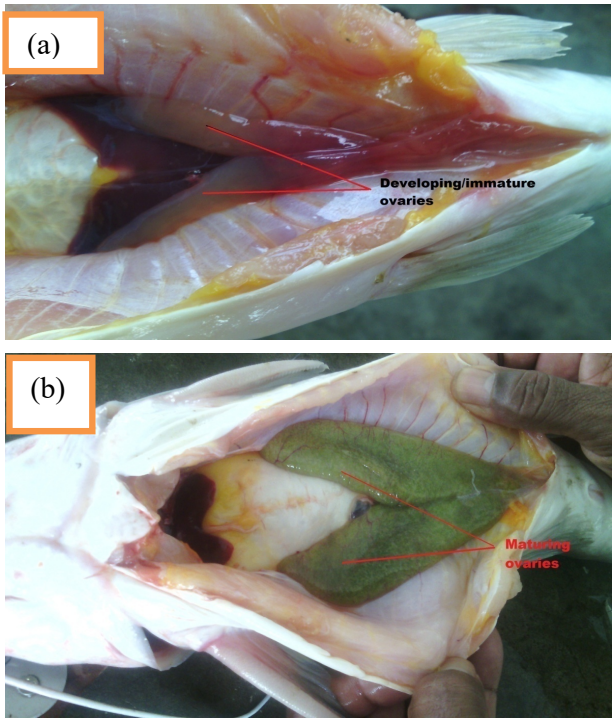


Plate 15: Immature (a) and maturing (b) stages in ovary development based on macroscopic observation for the *H. menoda*

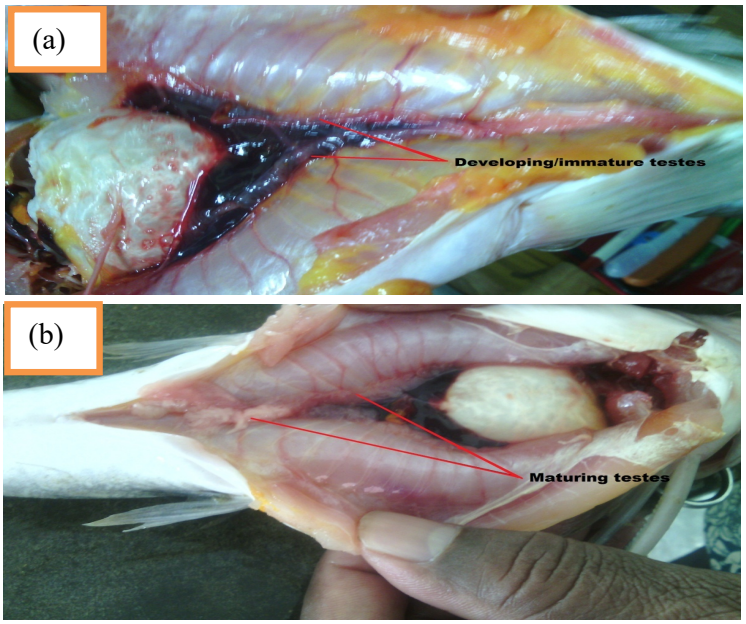


Plate 16: Immature (a) and maturing (b) stages in development of testes based on macroscopic observation for the *H. menoda*

Gonado somatic index (GSI)

The GSI is used to detect the status of gonadal development and maturity *of H. menoda* in the Kangsha River from May to November 2017. The monthly variations in the mean GSI of female *H. menoda* is shown in Table 2. The highest (12.50±4.97) mean GSI was in July while the lowest (0.00±0.00) was in December. The GSI started to increase from May to June reached the peak in July. Monthly GSI for individuals also showed that the maximum (17.96) was in July while the minimum (0.00±0.00) was in November (Table 6).

Table 6. Monthly mean ± SD and range of GSI in *H. menoda* from the Kangsha River Netrakona

Month	No. of fish examined	Minimum	Maximum	Mean GSI
May	3	1.35	3.57	2.11±1.26
June	**3**	**5.15**	**9.30**	**7.22±2.94**
July	**4**	**8.25**	**17.96**	**12.50±4.97**
August	5	0.44	0.65	0.55±0.15
September	3	0.26	0.37	0.31±0.07
October	3	0.22	0.70	0.40±0.26
November	3	0.25	0.42	0.33±0.12
December	3	0.00	0.00	0.00±0.00
January	3	0.00	0.44	0.29±0.25
February	3	0.34	1.93	1.20±0.80
March	3	0.26	0.55	0.38±0.15
April	4	0.50	0.62	0.57±0.06

Ova diameter

Ova diameter or egg size is often used to define the spawning season of a fish. From Table 7, it could be seen that in the mature ova, the smallest egg size was 0.77 mm in May for fish having 31.5 ± 2.15 cm standard length. The largest egg size was 1.66 mm in July for fish with standard length of 40.2 ± 3.54 cm. The egg diameter slightly decreased in August followed by an abrupt fall. The range in size of the mature ova is nearly one half of the total range of intra-ovarian eggs in the whole ovary. It can thus be inferred that though the fish has two modes of egg size, spawning takes place once in a year but with longer duration spanning from May to July and the eggs were completely shed in August.

Table 7. Mean (±SD) diameter of ova in mature ovaries in relation to fish length

Month	Range (mm)	Mean (±SD)	Standard length of fish (cm)
May	0.77 – 0.87	0.83 ± 0.08	31.79 ± 4.04
June	0.86 – 0.99	0.94 ± 0.04	26.91 ± 1.16
July	1.09 – 1.66	1.45 ± 0.23	36.56 ± 4.26

SD = Standard deviation

Fecundity

Fecundity refers to the number of eggs that are likely to be laid by a fish in its spawning season. It can also be referred to as the number of oocytes present in the ovary just before spawning. For the estimation of fecundity of *H, menoda*, the number of mature intra-ovarian eggs was enumerated for each individual. A total of 08 mature females found in the months of May, June and July, were examined. The fecundity estimates are presented in Table 8. The mean ovary weight was approximately 8% of the mean body weight. The mean fecundity was 77273.77±276.82 for fishes with mean length of 31.85±2.39 cm and mean body weight of 628.37±18.60g. The highest fecundity (222171.8) was in July from a fish measuring 40.20 cm and 1074.00g. The lowest (22954.99) was in May from fish that measured 25.00 cm and 389.00g.

Table 8. Egg counts of *H. menoda* collected from Kangsha River Netrakona Bangladesh (May-November 2017)

Month	Fish No.	SL (cm)	BW (g)	OW (g)	GSI	Absolute fecundity
May	1	31.50	665.00	9.00	1.35	35869.59
May	2	25.00	389.00	5.54	1.42	22954.99
May	3	33.40	478.00	17.05	3.57	39869.59
June	4	28.50	400.00	37.20	9.30	70295.76
June	5	26.70	336.00	17.30	5.15	24025.37
July	6	40.20	1074.00	88.60	8.25	222171.8
July	7	29.50	437.00	78.50	17.96	30821.26
July	8	40.00	1248.00	141.00	11.30	172181.8
Mean ± SE		31.85±2.39	628.37±18.60	49.27±0.97	7.29±2.37	77273.77±276.82
Range		25.00-40.2	336.00-1248.00	5.54-141.00	1.35-17.96	22954.99-222171.8
SL = Standard length; BW = Body weight; OW= Ovary weight						

Histological study of ovarian gametogenesis

Brief description of oocytes development stages

The eggs of an ovary do not mature at the same time but pass through developmental stages before they become fully mature. According to physiological, biochemical, morphological and histological criteria, the ovarian development is subdivided into distinct developmental stages. Brief description of the stages from microscopic observation of the ovary sections based on previously established criteria (Wallace and Selman, 1981) is given below:

Under-developed oocytes (UO): This stage is characterized by the youngest and smallest oocytes. Under-developed oocytes are often referred to as oogonia stage and are rarely seen in maturity.

Early perinucleolar stage (EPNO): Along with oocyte growth, the nucleus increases in size and multiple nucleoli become located around the periphery of the nucleus. In early perinucleolar stage oocytes were the most immature type. Oocyte cytoplasm at this stage stains deeply with haematoxylin and appear darker than the nucleus. Many nucleoli varying in size are observed within the nucleus periphery.

Late perinucleolar stage (LPNO): The late perinucleolar stage can be distinguished from the previous stage by the enlargement of the oocyte. During this period (diplotene stage of meiosis), lampbrush chromosomes are formed which disappear immediately prior to the breakdown of germinal vesicles during oocyte maturation. The cytoplasm tended to lose affinity for haematoxylin.

Cortical alveolar stage (CA): Oocytes in this stage differentiated by the appearance of cortical alveoli and the yolk envelope which seems to be empty when stained with hematoxylin.

Yolk vesicle stage (YV): This marks the beginning of the vitellogenic stage characterized by the initial formation of yolk vesicles (globules) in the periphery of the oocytes. Initially they were formed as a single row which appeared colorless when the slides were stained with haematoxylin and eosin. These yolk vesicles developed as minute bodies but gradually increased in size and number. The nucleoli were usually present at the periphery of the nucleus, but in some cases, they also appeared elsewhere in the nucleus.

Early yolk granule stage (EYG): The final stage of oocyte development was characterized by the formation of yolk granules in oocytes with fully developed yolk vesicles. They stained light pink with haematoxylin and eosin.

Late yolk granule stage (LYG): The diameter of the oocytes increased simultaneously with the advancement of yolk granule stage and oil droplets appeared within the cytoplasm. The yolk granules appeared deep pink with haematoxylin and eosin.

Pre-mature stage (PM): After the nucleus migration, nuclear membrane broke down. Yolk globules coalesced and no nucleus was observed, although the follicle layer was still visible.

Mature stage (M): This is the final stage of oogenesis characterized by complete vision of yolk globules seen together with an overall increase in oocyte translucency.

Stages of oocytes development in the menoda catfish, *Hemibagrus menoda*

May: EPNO, LPNO, YV, PM, M stages of oocytes were observed in May samples (Fig. 2 a). The sections were abundant with M and YV stages.

June: EPNO, LPNO, YV, PM, M stages of oocytes were observed in June samples (Fig. 2 b). The sections were abundant with M and YV stages.

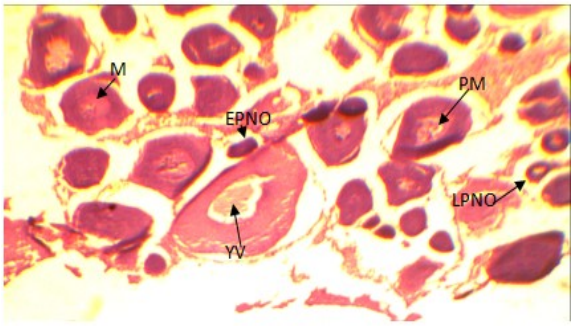
July: Mature stages (M) of oocytes were observed in July samples (Fig. 2 c). The sections were abundant with M oocytes.

August: UO, EPNO, LPNO, YV, M stages of oocytes were observed in August samples (Fig. 2 d). The sections were abundant with LPNO and YV stages.

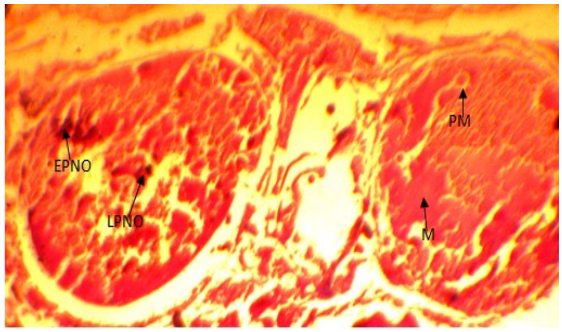
September: UO, EPNO, LPNO, YV stages of oocyte were observed in September samples (Fig. 1 e). The sections were abundant with UO and EPNO stage.

October: UO, EPNO, LPNO stages of oocytes were observed in October samples (Fig. 2 f). The sections were abundant with UO stage.

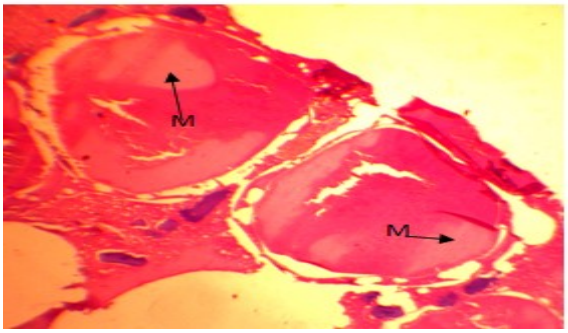
November: UO, EPNO, YV stages of oocytes were observed in November samples (Fig. 2 g). The sections were abundant with UO and EPNO stages.



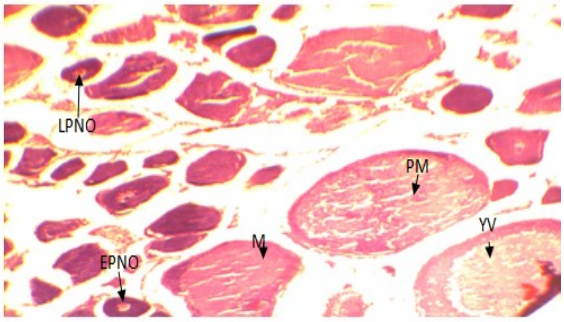
(a) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary at 10× magnification sampled in May, 2017. UO, undeveloped oocyte; EPNO, early perinucleolar oocyte; LPNO, late perinucleolar oocyte; YV, yolk vesicle stage; PM, Pre-mature stage; M, mature stage.



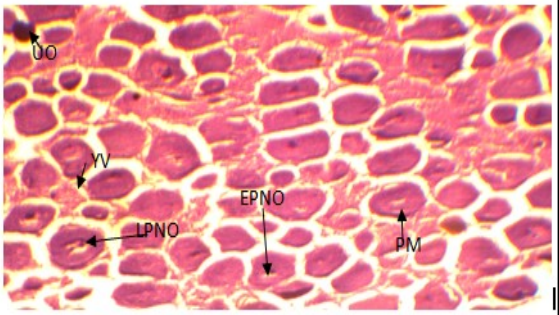
(b) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary at 10× magnification in June, 2017. EPNO, early perinucleolar stage; LPNO, late perinucleolar stage; PM, Pre-mature stage; M, mature stages.



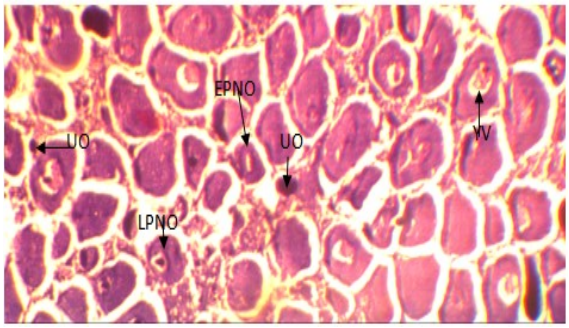
(c) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary at 10× magnification in July, 2017. M, mature stage.



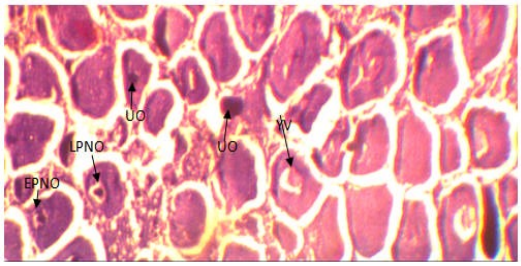
(d) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary at 10× magnification in August, 2017. EPNO, early perinucleolar stage; LPNO, late perinucleolar stage; YV, yolk vesicle stage; PM, Pre-mature stage; M, mature stage.



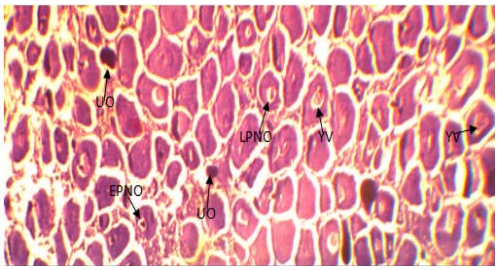
(e) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary at 10x magnification in September, 2017. UO, undeveloped ooctye stage; EPNO, early perinucleolar stage; LPNO, late perinucleolar stage; YV, yolk vesicle stage; PM, Pre-mature stage.



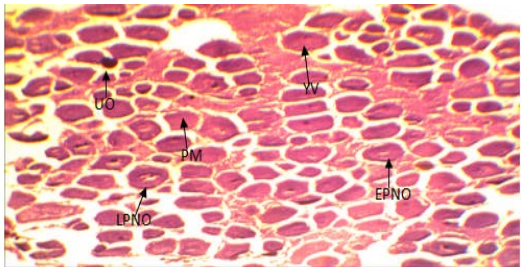
(f) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary at 10× magnification in October, 2017. UO, undeveloped ooctye stage; EPNO, early perinucleolar stage; LPNO, late perinucleolar stage; YV, yolk vesicle stage.



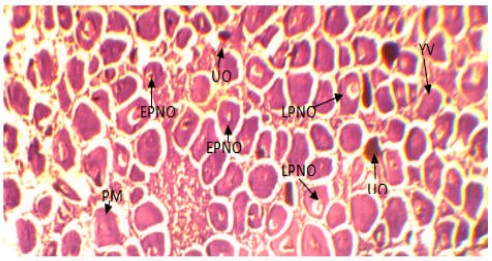
(g) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary at 10× magnifications in November, 2017. UO, undeveloped ooctye stage; EPNO, early perinucleolar stage; LPNO, late perinucleolar stage; YV, yolk vesicle stage.



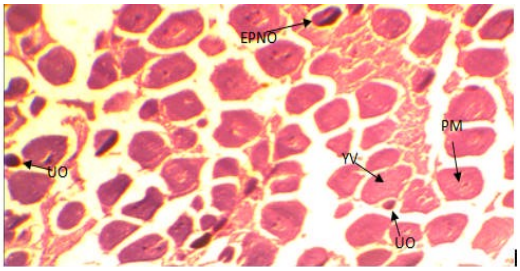
(h) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary in December, 2017. UO, undeveloped ooctye stage; EPNO, early perinucleolar stage; LPNO, late perinucleolar stage; YV, yolk vesicle stage.



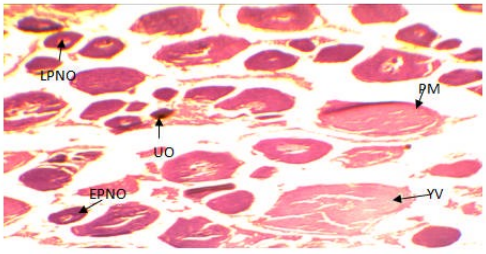
(i) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary in January, 2018. UO, undeveloped ooctye stage; EPNO, early perinucleolar stage; LPNO, late perinucleolar stage; YV, yolk vesicle stage; PM, pre-mature stage.



(j) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary in February, 2018. UO, undeveloped ooctye stage; EPNO, early perinucleolar stage; LPNO, late perinucleolar stage; YV, yolk vesicle stage; PM, pre-mature stage.



(k) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary in March, 2018. UO, undeveloped oocyte; EPNO, early perinucleolar oocyte; LPNO, late perinucleolar oocyte; YV, yolk vesicle stage; PM, Pre-mature stage.



(l) Haematoxylin-eosin stained sections of *Hemibagrus menoda* ovary sampled in April, 2018; EPNO, early perinucleolar oocyte; LPNO, late perinucleolar oocyte; YV, yolk vesicle stage; PM, Pre-mature stage.

Fig. 2: Stages in oocyte development in *H. menoda* in May (a), June (b), July (c), August (d), September (e), October (f) and November (g). December (h) 2017 and January (i), February (j), March (k) and April (l) 2018.

Experiment 2: Induced breeding trial of Menoda Catfish, *Hemibagrus menoda* through the use of different inducing hormones

Three breeding trials each of PG and HCG consisting of three treatments each were conducted by applying different doses of hormone. Each trial consisted of 3 treatments with 3 replications for each. A total of 27 brood fish and ratio of one female to two males was maintained throughout the experiment for both hormones. The breeding trials were conducted between May to July. The performance of each dosage of hormone and for each trial on the breeding of *Hemibagrus menoda* was evaluated based on the percent ovulation, fertilization, hatching and survival rate.

Breeding performance of *H. menoda* using different hormones
Spawning responses of *H. menoda* using double doses of PG extract

To find out the optimum dose of pituitary gland extract (PG) for the induced breeding of *H. menoda*, double doses of the extract were injected to the female while single doses were injected to males at the time of giving the female the 2nd dose during 3 trials. The doses of PG given, latency period, incubation temperature and spawning responses of the fish are presented in Table 7. The ovulation, fertilization, hatching and survival rates are presented in Table 8.

Latency period and incubation temperature

The time interval between the injection of female and ovulation varied between 17 to 18 hours in the 3 trials with PG. Ovulation did not occur in T_2 and T_3 (Trial-1), T_1 and T_3 (Trial-2) and T_3 in Trial-3 while the incubation temperature varied from 26 to 30 ^{0}C (Table 9).

Ovulation rate

No ovulation occurred in T_2 and T_3 (Trial-1), T_1 and T_3 (Trial-2) and T_3 (Trial-3) in females injected 35, 45, 22, 28 and 30 mg PG/kg body weight, respectively (Table 9). The percent ovulation rate was significantly ($p < 0.05$) lower in T_1 (Trial-3) than other treatments in which ovulation occurred (Table 9).

Fertilization rate

Fertilization rates indicated remarkable differences in the efficacy among the hormones in the 3 breeding trials with PG (Table 10). In Trial-1, 53.00±0.0% fertilization was observed in T_1. In Trial-2, 85.00±1.21% fertilization occurred in T_2 while in Trial-3, 85.00a±1.21 and 60.44b±0.04% fertilization occurred in T_1 and T_2, respectively. Among the 3 trials, the highest fertilization rate (85.00a±1.21%) was in T_2 (Trial-2) while the lowest (50.08c±2.12%) was in T_1 (Trial-3). Significant ($p < 0.05$) difference was observed between T_2 in Trial-2 and T_1 in Trial-3 (Table 10).

Hatching rate

In Trial-1, 75.00±0.0% hatching was found in T_1 (Table 10). In Trial-2, 90.00±1.21% hatching rate was found in T_2 and in Trial-3, 50.0±0.0 and 55.67±1.68% hatching were observed in T_1 and T_2, respectively. The highest (90.00±1.21%) hatching rate was in T_2 (Trial-2) while the lowest (50.0±0.0%) was in T_1 (Trial-3). Significant ($p < 0.05$) difference was found between T_2 (Trial-2) and T_1 (Trial-1). No significant ($p > 0.05$) difference was found between T_1 and T_2 in Trial-3 (Table 10).

Survival rate

In Trial-1, 69.00±0.0% of the hatchlings were alive 72 hours post hatching in T_1. In Trial-2, the average survival was (75.54±0.08%) in T_2 while in Trial-3, the hatching rates were 45.0±0.0 and 50.67±3.26% in T_1 and T_2, respectively (Table 10). Among the 3 trials with PG, the highest

(75.54±0.08%) survival rate was obtained in T_2 (Trial-2) while the lowest (45.0±0.0%) was in T_1 in Trial-3. There was significant ($p < 0.05$) difference between the treatments (Table 10).

Table 9. Spawning responses of *H. menoda* to different double doses of PG at 1♀:2♂ ratio

Trial	Treatment	PG dose (total)	Latency period (hrs)	Incubation temperature (^{0}C)	Remarks
Trial-1 (May 2017)	T_1	25	18	26-27	Complete ovulation, considerable number of larvae hatched and survived
	T_2	35		26-27	No ovulation
	T_3	45		26-27	No ovulation
Trial-2 (June 2017)	T_1	22		26.-28	Female brood dead after 7 hours from 2nd dose
	T_2	26	17	26.-28	Very high ovulation and fertilization observed, very high number of larvae hatched and survived
	T_3	28		26.-28	Female belly hard and vent blocked. No ovulation occurred after 21 hours from second dose
Trial-3 (July 2017)	T_1	24	17	27-30	Few eggs released, very few fertilized, none hatched
	T_2	27	17	27-30	Considerable ovulation, fertilization and hatching
	T_3	30		27-30	No ovulation

Table 10. Breeding performance of *H. menoda* in three trials with different doses of PG hormones

Trial	Treatment	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
Trial-1	T_1	100.00^a±0.0	53.00^c±0.0	75.00^b±0.0	69.00^a±0.0
	T_2	00.0^c±0.0	00.0^d±0.0	00.0^d±0.0	00.0^e±0.0
	T_3	00.0^c±0.0	00.0^d±0.0	00.0^d±0.0	00.0^e±0.0
Trial-2	T_1	00.0^c±0.0	00.0^d±0.0	00.0^d±0.0	00.0^e±0.0
	T_2	100.00^a±0.24	85.00^a±1.21	90.00^a±1.21	75.54^a±0.08
	T_3	00.0c±0.0	00.0^d±0.0	00.0^d±0.0	00.0^e±0.0
Trial-3	T_1	50.00^b±0.14	50.08^c±2.12	50.0^c±0.0	45.0^c±0.0
	T_2	100.00^a±0.0	60.44^b±0.04	55.67^c±1.68	50.67^b±3.26
	T_3	00.0^c±0.0	00.0^d±0.0	00.0^d±0.0	00.0^e±0.0

- Values in the same column with different superscripts are significantly different ($p < 0.05$)

Spawning responses of *H. menoda* using double doses of HCG extract

For the determination of optimum dose of HCG for the induced breeding of *H. menoda*, 3 trials each consisting of 3 treatments were conducted. During the breeding trials, 9 different doses of HCG extract ranging from 600 to 6500 IU/kg body weight were used. The breeding trials were conducted in the months of May, June and July. The doses used, latency period, incubation temperature and comments on the spawning responses are presented in Table 11. The estimated ovulation, fertilization, hatching and survival rates from the experiment are given in Table 12.

Latency period

The water temperature during the incubation period ranged from 26 – 28 ^{0}C while the latency period varied from 16 -18 hours (Table 11). The shortest latency period was 15 hrs in T_3 (Trial-2) while the longest (18 hrs) was in T_2 (Trial-2).

Ovulation rate

No ovulation occurred in T_1, T_2, T_3 (Trial-1) and in T_1 (Trial-2) (Table 12). There was significant ($p < 0.05$) difference between the treatments. Partial ovulation (50.00±0.14%) occurred in T_2 (Trial-2) whereas 100.00% ovulation occurred in T_3 (Trial-3) and T_1, T_2 and T_3 in Trial-3 (Table 12).

Fertilization rate

In Trial-2, no fertilization was observed in T_2 while 98.12±3.46% fertilization rate was observed in T_3. In Trial-3, highest (80.68±2.45%) fertilization rate was in T_1 followed by T_2 (65.00±0.23%) and T_3 (65.75±3.43%). Significant ($p<0.05$) difference was observed in fertilization rate between T_3 (Trial-2) and T_1, T_2 and T_3 (Trial-3). Among the 3 trials, the highest (98.12±3.46%) fertilization rate was in T_3 (Trial-2) while the lowest (65.00±0.23%) was in T_2 in Trial-2 (Table 12).

Hatching rate

In Trial-2, hatching rate of fertilized eggs was 90.23±45% in T_3. In trial-3, hatching rate was significantly ($P<0.05$) higher (88.61±2.16%) in T_1 than in T_2 (69.34±2.45%) and T_3 (62.21±5.45%). Significant difference ($p<0.05$) was observed among the trials. The highest (90.23±45%) hatching rate was in T_3 (Trial-2) while the lowest was in T_3 in Trial-3 (Table 12).

Survival rate

Significant ($p<0.05$) difference was found in the survival rates among the treatments in the 3 trials 72 hours after hatching. The survival rate was (85.00±0.45%) in T_3 (Trial-2). In Trial-3, the survival rate was higher (67.34±3.65%) in T_1 followed by T_2 (54.00±4.25%) and T_3 (42.96±3.56%). Among the 3 trials with HCG, the highest (85.00±0.45%) survival rate was in T_3 (Trial-2) while the lowest (42.96±3.56%) was in T_3 in Trial-3 (Table 12).

Table 11. Spawning responses of *H. menoda* to different double doses of HCG at 1♀:2♂ ratio

Trial	Treatment	HCG dose (IU/kg body weight (g)		Latency period (hrs)	Incubation temperature (^{0}C)	Remarks
		♀	♂			
Trial-1 (May 2017)	T_1	600	200		26-27	No ovulation
	T_2	900	300		26-27	No ovulation
	T_3	1200	400		26-27	No ovulation
Trial-2 (June 2017)	T_1	1500	500		26-27	No ovulation
	T_2	2500	1000	18	26.5-27.5	Very few eggs released but none fertilized
	T_3	3500	1500	15	26.5-27.5	Successful ovulation, fertilization and hatching
Trial-3 (July 2017)	T_1	4500	2000	16	27-28	Considerable ovulation, fertilization and hatching
	T_2	5500	2500	17	27-28	Successful ovulation, fertilization and hatching
	T_3	6500	3000	17	27-28	Successful ovulation, fertilization and hatching

Table 12. Breeding performance of *H. menoda* in three trials with different doses of HCG hormones

Trial	Treatment	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)
Trial -1	T_1	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0
	T_2	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0
	T_3	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0
Trial -2	T_1	00.0±0.0	00.0±0.0	00.0±0.0	00.0±0.0
	T_2	50.00^b±0.14	00.0±0.0	00.0±0.0	00.0±0.0
	T_3	100.00^a±00	98.12^a±3.46	90.23^a±45	85.00^a±0.45
Trial -3	T_1	100.00^a±00	80.68^b±2.45	88.61^a±2.16	67.34^b±3.65
	T_2	100.00^a±00	65.00^c±0.23	69.34^b±2.45	54.00^c±4.25
	T_3	100.00^a±00	65.75^c±3.43	62.21^b±5.45	42.96^d±3.56

➢ Values in the same column with different superscripts are significantly different ($p < 0.05$)

Breeding responses of *H. menoda* using single doses of ovupin

In order to determine the optimum dose of ovupin for the induced breeding of *H. menoda*, single doses of the hormone were injected both to the female and males at the same time for each

treatment. The doses of ovupin given, latency period, incubation temperature and spawning responses of the fish are showed in Table 13 and the ovulation, fertilization, hatching and survival rates are showed in Table 13.

Ovulation rate

From the experiment the highest average ovulation rate (100%) was recorded both in T_1 & T_2 whereas the lowest value (66.67%) was found in (T_3) (Table 14). The results from the ANOVA test indicated that there was a difference among three doses of ovupin from the perspective of ovulation rate and showed that T_3 was significantly (p<0.05) lower than T_1 & T_2. But there is no significant difference between T_1 & T_2 (Table 14).

Fertilization rate

Fertilization rates reveal mention worth differences in the effectiveness among the hormones in the 3 different treatment with ovupin (Table 14). From the experiment the fertilization rate were recorded as (71.00±3.06%) in T_1, while (84.67±3.17%) and (50.00±2.89%) fertilization occurred respectively in T_2 & T_3 (Table 14). The results from the ANOVA test indicated that there was a significant difference among three doses of ovupin treatment whereas T_2 was significantly (p<0.05) higher than T_3 but there is no significant difference between T_2 & T_1 (Table 14).

Hatching rate

The hatching rate was observed (64.00±2.08%), (83.00±1.53%) and (45.00±0.58%) in treatments of T_1, T_2 and T_3 respectively (Table 14). The highest hatching rate was recorded 83.00±1.53% in T_2 whilst the lowest hatching rate was recorded 45.00±0.58% in the treatment T_3. The result from the ANOVA test revealed that there was a significant difference among three doses of ovupin. It was found that hatching rate in T_2 was significantly (p<0.05) higher than that of T_3 but it was not significant in comparison with T_1 (Table 14).

Survival rate

In treatment T_1, (43.00±3.79%) of the hatchlings were alive 10 days after hatching while the mean survival rate were (59.33±2.33%) and (32.67±1.45%) in T_2 and T_3, respectively (Table 14). Among the 3 treatments with ovupin, the highest (59.33±2.33%) survival rate was obtained in T_2 while the lowest (32.67±1.45%) was in T_3. The results expressed that there was a difference among three doses of ovupin and a significantly (p<0.05) higher survival rate was found in treatment T_2 in comparison with T_3 but it was not significantly different from T_1 (Table 14).

Table 13. Spawning responses of *H. menoda* to different single doses of ovupin at 1♀:2♂ ratio

Treatment	Ovupin dose (ml/kg body weight)		Latency period (hrs)	Incubation temperature (^{0}C)	Remarks
	♀	♂			
T_1	4	1.5	$24\frac{1}{2}$	26-27	Complete ovulation, considerable number of larvae hatched but survival rate was poor.
T_2	6	2	24	26-27	Successful ovulation, considerable number of larvae hatched and survived.
T_3	7	3	25	27-29	Amount of ovulation was very little, hence a few number of larvae hatched and survived

Table 14. Breeding performance of *H. menoda* with different doses of Ovupin hormones

Treatment	Ovulation rate (%) M±SE	Fertilization rate (%) M±SE	Hatching rate (%) M±SE	Survival rate (%) M±SE
T_1	100.0±0.0^a	71.00±3.06^{ab}	64.00±2.08^{ab}	43.00±3.79^{ab}
T_2	100.0±0.0^a	84.67±3.17^b	83.00±1.53^b	59.33±2.33^b
T_3	66.67±0.0^b	50.00±2.89^a	45.00±0.58^a	32.67±1.45^a

(M±SE); Values of the parameter in each column with different superscripts (ab, a, & b) differs significantly ($p<0.05$

Spawning responses of *H. menoda* using Ovatide hormone

To find out the optimum dose of Ovatide for the induced breeding of *H. menoda*, single dose of the extract were injected to the female while single doses were injected to males at the same time. The doses of Ovatide given, weight of brood fishes latency period are presented in Table 15, the incubation temperature, dissolved oxygen (ppm) and P^H and spawning responses of the fish are presented in Table 17. The ovulation, fertilization, hatching and survival rates are presented in Table 16.

Latency period and incubation temperature

The time interval between the injection of female and ovulation varied between 21 to 22 hours in the 3 treatments with Ovatide. Ovulation did not occur in T_1, while the incubation temperature varied from 26 to 27 ^{0}C (Table 17).

Ovulation rate

No ovulation occurred in T_1, (Trial-1), in females injected 07 ml ovatide/kg body weight of different males and body weight, respectively (Table 16). The percent ovulation rate was lower in T_3 (Trial-1) than other treatments of T_2 in which ovulation occurred (Table 16).

Fertilization rate

The average fertilization rates were recorded as 0, 97% and 90.00% in T_1, T_2 and T_3 respectively (Table 16). The highest fertilization rate (97.0%) was recorded in 5 ml/kg body weight of Ovaptide in (T_3). For fertilization rate also revealed that T_2 was higher than T_1 and T_3.

Hatching rate

During the dose optimization of Ovatide the hatching rate were found to be 0.00%, 95.0%, 76.0% in T_1, T_2 and T_3 respectively (Table 16). The highest hatching rate was recorded as 95.0% in T_3 and lowest hatchling rate was recorded as 0.0% in T_1. Hatching rate showed that T_2 was higher than all other treatment (T_1 and T_3).

Survival rate

The survival rate of *H. menoda* with different treatment were 0.0, 85.0%, 80.0% in T_1, T_2 and T_3 respectively (Table 16) after 7 days of experiment also revealed that T_2 higher than rest of the treatment.

Table 15. Showing different doses of Ovatide with their weight and fish ratio

Treatments	Brood fish ratio	Brood fish weight (g)	Ovatide dose (ml/kg body weight)
T_1	Female	1020	7
	Male	570	3
	Male	388	3
T_2	Female	840	5
	Male	368	2.5
	Male	310	2.5
T_3	Female	475	3
	Male	295	1.5
	Male	295	1.5

Table 16. Showing breeding performances of *H. menoda* with different doses of Ovatide Hormone

Treatments	Ovulation rate (%)	Fertilization rate (%)	Hatching rate (%)	Survival rate (%)	Remarks
T_1	0	0	0	0	Female belly hard and vent blocked. No ovulation occurred after 21 hours from second dose
T_2	10	97	95	85	Complete ovulation, considerable number of larvae hatched and survived
T_3	63	90	76	80	Considerable ovulation, fertilization and hatching

Table 17. Physico-chemical parameters of different doses of Ovatide on induced breeding

Parameters	T_1	T_2	T_3
Incubation Temperature (^{0}C)	26-27	26-27	26-28
Dissolve Oxygen (ppm)	5.5	7	6.5
pH	6.8	7.2	7.5

Comparison among the better spawning successes of different doses of PG and HCG at 1♀:♂2 ratio of *H. menoda*

Based on the results obtained from the induced breeding of *H. menoda* with PG and HCG, better spawning successes were found in T_1 (Trial-1), T_2 (Trial-2) and T_1, T_2 in Trial-3 for PG. For HCG, better spawning successes were recorded in T_2 (Trial-2) and T_1, T_2, T_3 in Trial-3. Comparison of the spawning successes between the two hormones is presented in Table 18.

Incubation temperature and latency period

The latency period within which the female *H. menoda* released their eggs varied from 16 to 17 hours while the incubation temperature varied from 26 to 30 ^{0}C (Table 18). The latency period was shorter in T_2 (Trial-2) and T_1, T_2 (Trial-3) for PG and T_3 (Trial-2, T_1 (Trial-3) for HCG.

Ovulation rate

There was no significant ($p>0.05$) difference in ovulation rate between the better performing doses of PG and HCG (Table 18). Among the 3 trials each with PG and HCG, 100.00±0.0% ovulation was observed.

Fertilization rate

Amongst the hormones with better spawning successes, the highest (98.12±3.46%) fertilization rate was recorded in T_3 (Trial-3) followed by 85.00±1.21% in T_2 (Trial-2) and 80.68±2.45% in T_1 (Trial-3). There was significant ($p<0.05$) difference between T_3 (Trial-2) and T_1 (Trial-3), T_2 (Trial-2). There was no significant ($p>0.05$) difference between T_1 (Trial-3) and T_2 (Trial-2). The lowest (53.00±0.0%) fertilization rate amongst the better spawning successes was in T_1 in Trial-1 (Table 18).

Hatching rate

Comparison among the hormones with better spawning successes showed that the highest (90.23±45%) hatching rate was in T_3 (Trial-2), followed by 90.00±1.21 and 88.61±2.16% in T_2 (Trial-2) and T_1 (Trial-3), respectively, though there was no significant ($p>0.05$) difference between the treatments (Table 18).

Survival rate

The highest (85.00±0.45%) survival rate was in T_3 (Trial-2) followed by 75.54±0.08% in T_2 (Trial-2) and 69.00±0.0% in T_1 (Trial-1). Significant ($p<0.05$) difference was found between the treatments (Table 18). Amongst the hormones with better spawning successes, the lowest (42.96±3.56%) survival rate was recorded in T_3 (Trial-3). The afore mentioned results indicated that in terms of ovulation, fertilization, hatching and survival rates, the best breeding success occurred in T_3 in Trial-2 (HCG 3500 IU kg body weight injected to female), followed by T_2 in Trial-2 (26 mg PG /kg body weight).

Table 18. Comparison of spawning successes at 1♀:♂2 ratio of *H. menoda* at the best possible doses of PG and HCG in 6 trials

Parameters	**Spawning performances in 6 trials**							
Trial/treatment	**T1 (Trial-1)**	**T2 (Trial-2)**	**T1 (Trial-3)**	**T2 (Trial-3)**	**T3 (Trial-2)**	**T1 (Trial-3)**	**T2 (Trial-3)**	**T3 (Trial-3)**
Hormones doses	PG	PG	PG	PG	HCG	HCG	HCG	HCG
Doses given to female	**25**	**26**	**24**	**27**	**3500**	**4500**	**5500**	**6500**
Incubation temp. (0C)	26-27	26-28	27-30	27-30	26.5-27.5	27-28	27-28	27-28
Latency period	17	16	16	16	15	16	17	17
% of ovulation	100.00^{a}± 0.0	100.00^{a} ±0.00	50.00^{b}±0.14	100.00^{a}± 0.0	**100.00^{a} ±00**	100.00^{a} ±00	100.00^{a}±00	100.00^{a}±00
% of fertilization	53.00^{d}±0.0	85.00^{b}± 1.21	50.08^{c}±2.12	60.44^{c}±0.04	**98.12^{a}± 3.46**	80.68^{b}± 2.45	65.00^{c}±0.23	65.75^{c}±3.43
% of hatching	75.00^{b}±0.0	90.00^{a}± 1.21	50.0^{c}±0.0	55.67^{c}±1.68	**90.23^{a}± 45**	88.61^{a}± 2.16	69.34^{b}±2.45	62.21^{c}±5.45
% survival	69.00^{b}±0.0	75.54^{b}± 0.08	45.0^{c}±0.0	50.67^{c}±3.26	**85.00^{a}± 0.45**	67.34^{b}± 3.65	54.00^{c}±4.25	42.96^{d}±3.56

- Values in the same row with different superscripts are significantly different ($p < 0.05$)

12. Research highlight/findings (Bullet point – max 10 nos.):

i. The monthly mean GSI of female *H. menoda* started to increase from May to June and reached the peak (12.50±4.97) in July, indicating the peak spawning season of the fish.

ii. In the mature ova, the ova diameter ranged from 0.77mm (standard length = 31.5± 2.15 cm) in May to 1.66mm (standard length = 40.2± 3.54 cm) in July, and spawning takes place once in a year but with longer duration spawning from May to July.

iii. Oocytes in the Premature (PM) and mature (M) stages were abundant from April to August samples of ovary, indicating the spawning season.

iv. Successful ovulation occurred in the females injected 24, 25, 26 and 27 mg PG; 3500, 4500, 5500 and 6500 IU HCG; 4, 6 and 7 ml Ovupin & 7, 5 and 3 ml Ovatide/kg body weight.

v. Optimum doses of hormones were obtained from spawners injected double dose of 3500IU, 26 mg, 6 ml and 5 ml HCG, PG, ovupin and ovatide/kg body weight, respectively, in a 2♂:1♀, ratio.

vi. Among the different hormones tested, PG extract was the best. Striping method is not applicable for this fish species.

Plate 17: Larvae produced from the induced breeding of *H. menoda*

Plate 18: Instruments and chemicals used in the experiment

Plate 19: Brood fish stripping and mixing of milt with eggs

Plate 20: Experimental ponds and feeding of fish

Plate 21: Instruments, chemicals, fries etc. used for implementing the project

Plate 22: Implementation of training program under the project

B. **Implementation Position**

1. Procurement:

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment					-
i) Laptop	01	60,000	01	60,000	
ii) Laser printer	01	20,000			
iii) Digital camera	01	25,000			
(b) Lab &field equipment					-
i) Microscope with Camera	01	250,000	01	250,000	
ii) Electric balance	01	10,000	01	10,000	
iii) Hack kit	01	50,000	01	50,000	
(c) Other capital items					

2. Establishment/renovation facilities: N/A

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized:

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	32	05	37	one day	Training on breeding biology of freshwater Gangmagur, *Hemibagrus menoda*
(b) Workshop	-	-	-	-	-

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	439360	439360	439360	0	100	-
B. Field research/lab expenses and supplies	610500	565688	505688	60000	95	-
C. Operating expenses	145000	140753	128414	12339	95	-
D. Vehicle hire and fuel, oil & maintenance	100000	100000	100000	0	100	-
E. Training/workshop/seminar etc.	100000	100000	100000	0	100	-
F. Publications and printing	100000	15000	15000	0	100	-
G. Miscellaneous	45140	44247	0	44247	00	No fund
H. Capital expenses	460000	460000	435000	25000	98	-

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output (i.e. product obtained, visible, measurable)	Outcome (short term effect of the research)
To study the reproductive cycle of *H. menoda* by Fecundity, Gonado Somatic Index (GSI) and gonadal histology	Month-wise collection of fish for GSI, fecundity and histological studies	Breeding season of this fish species	Generated information on peak breeding season, fecundity, gonadosomatic index value and gonadal histology of H. menoda help to propagate and ensure the species through successful seed production
To develop induced breeding technique of *H. menoda* using inducing hormones	Domestication; and Breeding trial with different inducing agents	Development adoption of refined induced breeding technique	Species will be saved from the threat of extinction Increase fish production and higher availability of table fish of this species in the market in Bangladesh Create employment generation in the field of brood rearing, seed production and hatchery operation, rearing and culture of the species and trading

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet /leaflet/flyer etc.			
Journal publication	-	**03**	1. Jega IS, Miah MI, Fatema MK, **Shahjahan M**. Food and feeding habits of *Hemibagrus menoda* (Hamilton, 1822) (Siluriformes, Bagridae) in Kangsha River, Bangladesh. ***Bangladesh Journal of Fisheries***, 30(1), 47-59; 2018. 2. Jega IS, Miah MI, Haque MM, **Shahjahan M**, Fatema MK, Ahmed ZF. Sex ratio, length-weight relationships and seasonal variations in condition factor of menoda catfish *Hemibagrus menoda* (Hamilton,

			1822) of the Kangsha River in Bangladesh. ***International Journal of Fisheries and Aquatic Studies*****,** 5(5): 49-54; 2017. 3. Jega IS, Omar A, Miah MI, Haque MM, **Shahjahan M.** Embryology and early ontogenesis of the threatened menoda catfish, *Hemibagrus menoda* (Hamilton, 1822). ***International Journal of Fisheries and Aquatic studies***, 6(5): 225-230; 2018.
Information development			
MS thesis	**-**	**02**	1. Dose Optimization of Ovaprim Super Hormone for Induced Breeding of Freshwater Gang Magur *Hemibagrus menda* (Hamilton 1822) 2. Dose optimization of Ovatide for induced breeding of freshwater Gangmagur *Hemibagrus menoda* (Hamilton 1822)

F. Technology/Knowledge generation/Policy Support (as applied):

- **i.** Generation of technology (Commodity & Non-commodity)
- **ii.** Generation of new knowledge that help in developing more technology in future
- **iii.** Technology transferred that help increased agricultural productivity and farmers' income

Transfer of technology on induced breeding of freshwater Gangmagur to the hatchery owners and technician will help to increase productivity and farmers' income

- **iv. Policy Support (n/a)**

G. Information regarding Desk and Field Monitoring

i) Desk Monitoring [description & output of consultation meeting, monitoring workshops/ seminars etc.):

1. Workshop on mid-term review of research progress under CRG support, Fisheries division, BARC, 10-11 April 2018.
2. Annual review workshop on CRG sub-projects of fisheries division, BARC, 19-20 September 2018.

3. Field Monitoring (time & No. of visit, Team visit and output): N/A

H. Lesson Learned/Challenges (if any)

N/A

I. Challenges (if any)

N/A

Signature of the Principal Investigator
Date …….
Seal

Counter signature of the Head of the organization/authorized representative
Date ……..
Seal

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