Final Report
on

Molecular Approach for Giant Snakehead (*Channa marulius*) Breeding & Development of Its Culture Techniques for the Fish Farmer

Prepared by

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IDRS-BFRI Project

Shrimp Research Station, Bagerhat-9300

Final Report

A. Title of the project: Molecular approach for giant snakehead (*Channa marulius*) breeding and development of its culture techniques for the fish farmer

Type of project		On going		√ Complete

Date:	Starting	01.07.2013	Completion	30.06.2014

Where research is performed: Department of Fisheries Management, BAU, Mymensingh

B. Address of Coordinator or Principle Investigator:

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C. Summary of Research Project:

1. Introduction

Induced fish breeding has been practiced in Bangladesh for the last three decades. Reproduction of fish in captivity can be controlled by environmental manipulations, such as photoperiod, water temperature or spawning substrate. However, the eco-biology of some fishes is not well known, or it is impractical or even impossible to simulate the required environmental parameters for natural reproductive performance (i.e., spawning migration, depth, riverine hydraulics, etc.). In these instances, use of exogenous hormones is an effective way to induce reproductive maturation and produce fertilized eggs. Furthermore, in all cultured fishes, hormonal manipulations may be used as management tools to enhance the efficiency of egg production, increase spermiation and facilitate hatchery operations. Finally, hormonal therapies may be employed to induce gamete maturation. In our country the hormones used for manipulating fish maturation and ovulation include pituitary extract and HCG (Human Chorionic Gonadotropin). However, this traditional technique has become routine practice in every fish hatcheries of our country.

Great snakehead murrel (locally called, gajar) *Channa marulius* is one among the highly priced freshwater air breathing fish species in Bangladesh. The fish is well known for its taste, high nutritive value, recuperative and medicinal qualities, is sometimes recommended as a diet during convalescence. However, only about two decades ago *C. marulius* were available in many water bodies in the haors, baors, beels, rivers, ponds, ditches and even in irrigation canals of Bangladesh. The main reasons are the destruction of their breeding grounds, catching of young juveniles and the outbreaks of ulcerative syndrome disease, use of agro-chemicals and pesticides. In Bangladesh, the snakehead murrel is of economic importance as food fish in freshwater capture fisheries and has great potential for aquaculture. But fish farmers in Bangladesh are not familiar with murrel

culture due to want of breeding, feeding and culture techniques. Therefore, it is imperative to make murrel culture popular among fish farmers and unemployed youths for income generation. This project aims at application of molecular techniques (application of specific GnRHs) for induced breeding of this fish over the traditional hypophysation technique to determine the most effective dose of these specific peptides.

Prior to developing molecular techniques of induced breeding, the reproductive biology of this fish will be studied first. The annual gonadal cycles, particularly the ovarian cycles will be studied to understand the path of oocyte development and maturation of this fish. It is expected that study of reproductive biology of this fish will help identify the breeding season, peak breeding season of this fish. The information on basic biology data will be utilized for induced breeding of this fish in captivity and ultimately making steady seed supply a possibility.

2. Objectives

Considering the importance of reproductive biology of the great snakehead, the current study was designed with the following specific objectives:

1. To determine the gametogenesis of *C. marulius*;
2. To know the location of GnRH genes in the brain and pituitary using immunohistochemistry (IHC) and selection of proper inducing agents;
3. To standardize the dose of GnRHa/dopamine antagonists for the successful induced breeding of *C. marulius*; and
4. To know the fry rearing and culture techniques of giant snakehead.

3. Methodology

Sampling of Gonad

During the study period sampling of gonad form mature male and female were done monthly from the fishermen catches in the haor area of Sylhet and Mymensingh region. After dissecting the gonads form the fish body, they were fixed into 10% formalin on the spot of sampling.

Dehydration and Preservation of Gonad

The fixed gonads were passed through graded alcohol series to dehydrate them. Finally the gonads in 100% alcohol were preserves at –20°C until they will be embedded into paraffin.

Embedding, sectioning and staining

The dehydrated gonad samples were embedded into paraffin. Sectioning was done using microtome machine. The gonads sectioned were then stained with hematoxylene-eosin stain.

Microscopic observation and identification of gonadal stages

Finally the gonad sections were observed under microscope. These microscopic observations were helped to identify the gonadal stages of male and female and the course of gonadal maturation.

4. Results

4.1 Histological study of gametogenesis

4.1.1 Oocytes stages in female *C. marulius*

The oocytes stages were observed and recorded month-wise as follows:

July: M, mature and UO, undeveloped oocytes were observed in July sample. M stages were highest in number [Plate 4.1 (a-b)].

August: LYG, late yolk granule and LPNO, late perinuclear oocyte stages were observed in August sample [Plate 4.2 (a-b)].

October: UO, undeveloped oocyte; YV, yolk vesicle EPNO, early perinuclear oocyte stages were observed in October sample [Plate 4.3 (a-b)].

November: UO, undeveloped oocyte; EPNO, early perinuclear oocyte; LPNO, late perinuclear oocyte and YG, yolk granule stages were observed in November sample [Plate 4.4 (a-b)].

January: UO, Undeveloped Oocyte; EPNO, Early Perinuclear Oocyte and EYG, Early Yolk Granule stages were observed in January sample [Plate 4.5 (a-b)].

February: UO, undeveloped oocyte; EPNO, early perinuclear oocyte; LPNO, late perinuclear oocyte and YV, Yolk Vesicle stages were observed in February sample [Plate 4.6 (a-b)].

March: UO, undeveloped oocyte; EYG, early yolk granule and EPNO, early perinuclear oocyte stages were observed in March sample [Plate 4.7 (a-b)].

May: UO, undeveloped oocyte; EPNO, early perinuclear oocyte; EYG, early yolk granule and YV, yolk vesicle stages were observed in May sample [Plate 4.8 (a-b)].

June: UO, undeveloped oocyte; EPNO, early perinuclear oocyte; LPNO, late perinuclear oocyte; EYG, early yolk granule; YV, yolk vesicle; M, mature and PM, pre-mature stages were observed in June sample [Plate 4.9 (a-b)].

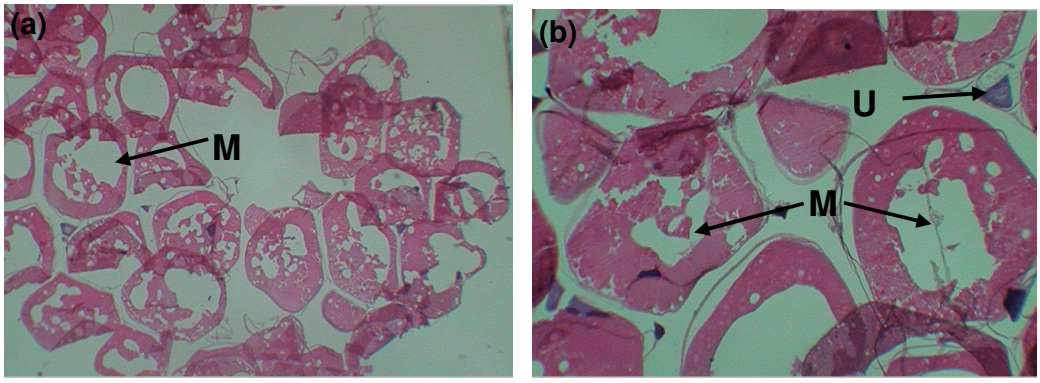

Plate 4.1. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 4X (b) 10X magnification in July, 2013. M, Mature stage; UO, Undevelop Oocyte.

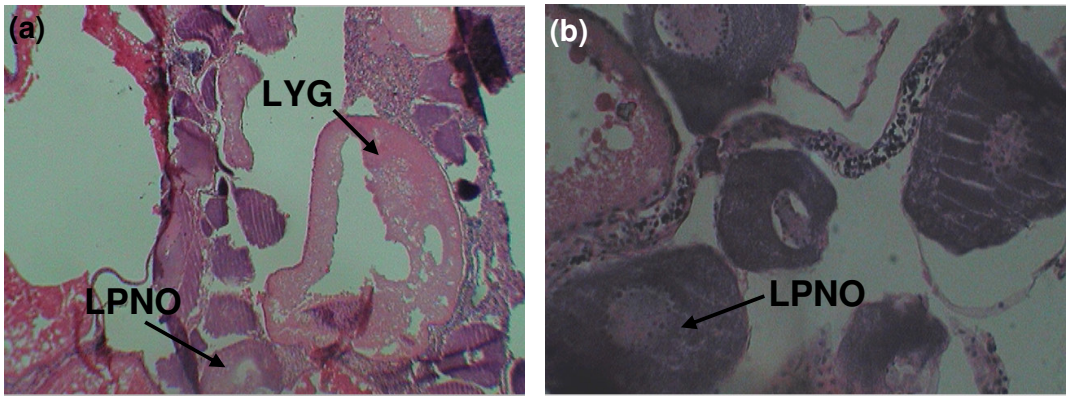

Plate 4.2. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 10X (b) 40X magnification in August, 2013. LYG, Late Yolk Granule; LPNO, Late

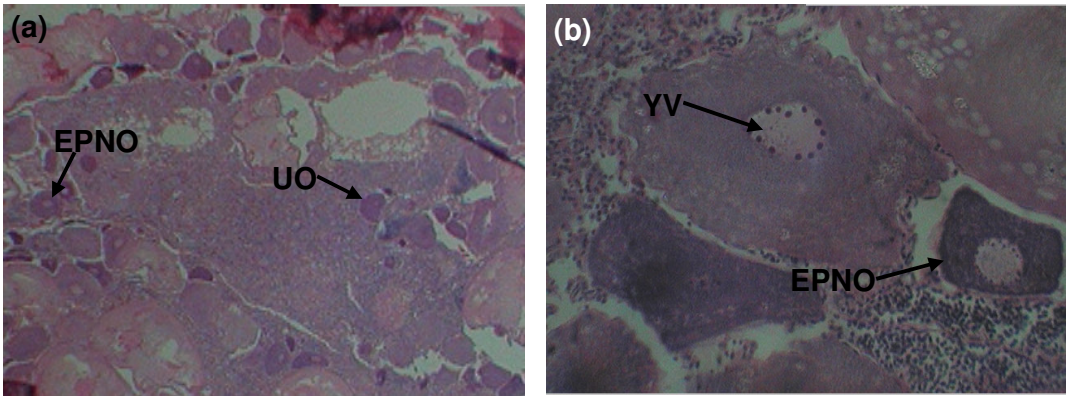

Plate 4.3. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 10X (b) 40X magnification in October, 2013. UO, Undeveloped Oocyte; EPNO, Early Perinuclear Oocyte; YV, Yolk Vesicle stage.

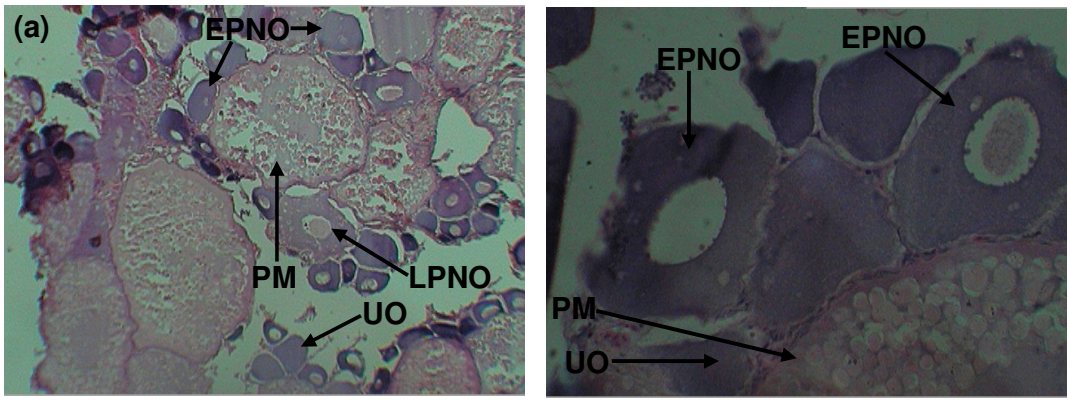

Plate 4.4. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 10X (b) 40X magnification in November, 2013. UO, Undeveloped Oocyte; EPNO, Early Perinuclear Oocyte; LPNO, Late Perinuclear Oocyte; YG, Yolk Granule stage.

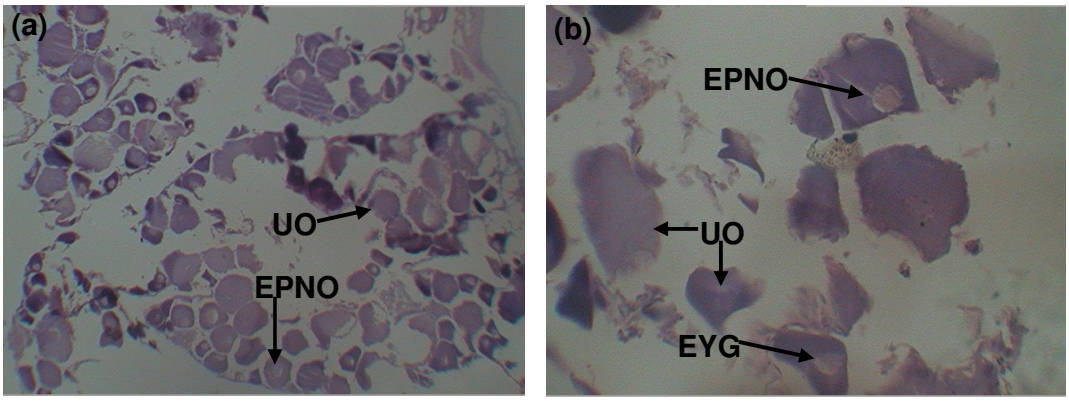

Plate 4.5. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 10X (b) 40X magnification in January, 2012. UO, Undeveloped Oocyte; EPNO, Early Perinuclear Oocyte; EYG, Early Yolk Granule stage.

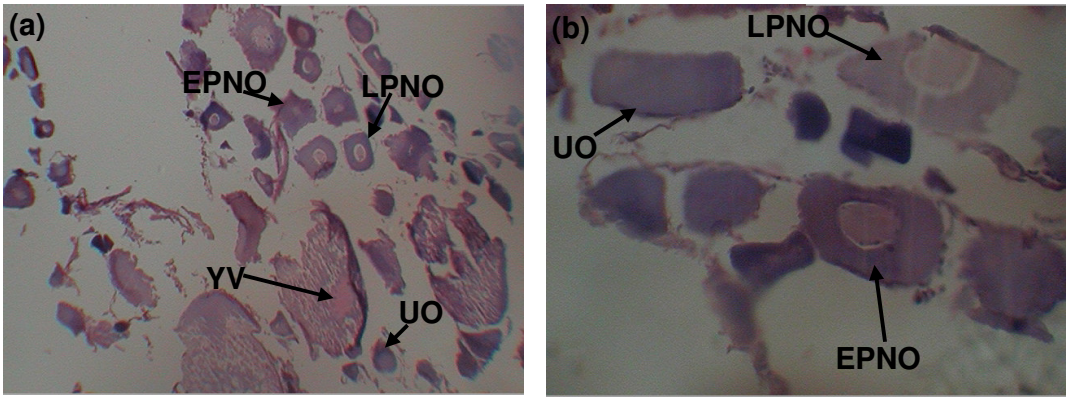

Plate 4.6. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 10X (b) 40X magnification in February, 2012. UO, Undeveloped Oocyte; EPNO, Early Perinuclear Oocyte; LPNO, Late Perinuclear Oocyte; YV, Yolk Vesicle stage.

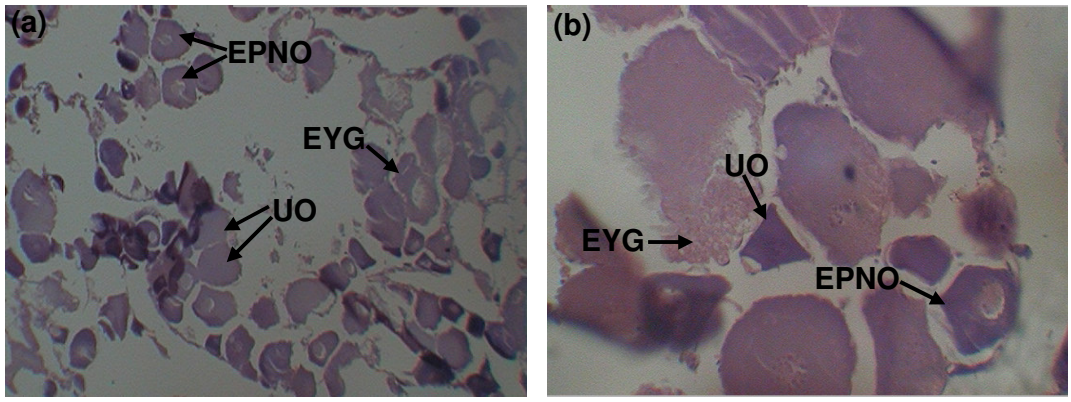

Plate 4.7. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 10X (b) 40X magnification in March, 2012. UO, Undeveloped Oocyte; EPNO,Early Perinuclear Oocyte.

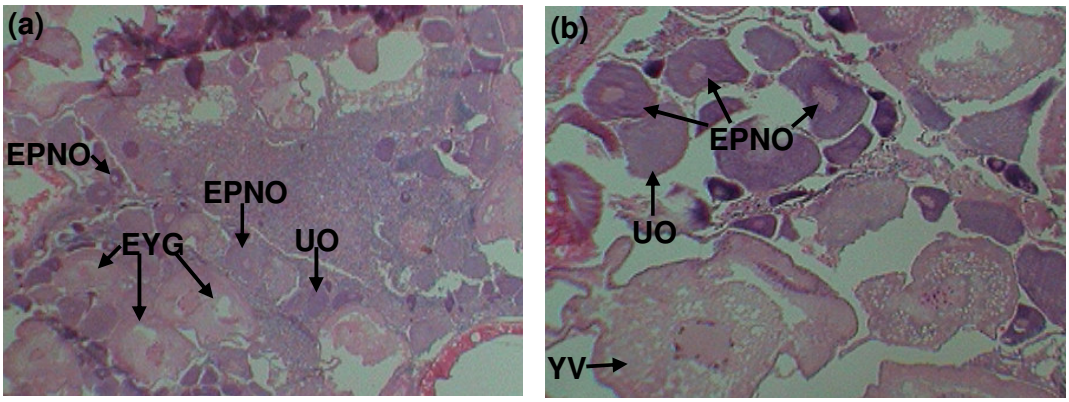

Plate 4.8. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 4X (b) 10X magnification in May, 2012. UO, Undeveloped Oocyte; EPNO, Early Perinuclear Oocyte; EYG, Early Yolk Granule; YV, Yolk Vesicle stage.

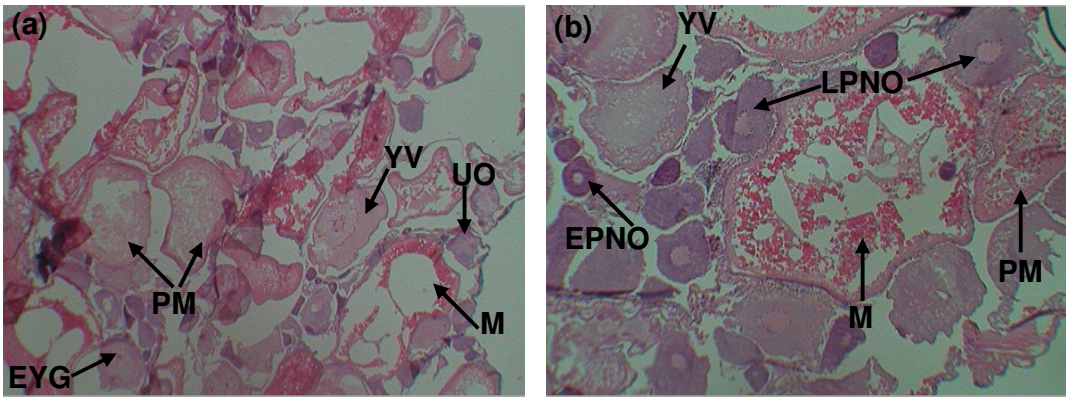

Plate 4.9. Haematoxylin-eosin stained sections of *C. marulius* ovary at (a) 4X (b) 10X magnification in June, 2012. UO, Undeveloped Oocyte; EPNO, Early Perinuclear Oocyte; LPNO, Late Perinuclear Oocyte; EYG, Early Yolk Granule; YV, Yolk Vesicle; M, Mature; PM, Pre-mature.

4.1.2 Stages of testicular germ cells

The testicular germ cell stages of *C. marulius* were observed and recorded in the month of July as follows:

July: SPC, spermatocytes; SPT, spermatids; SPZ, spermamatozoa and LU, lumen were observed in July sample. SPZ were highest in number [Plate 4.10 (a-b)].

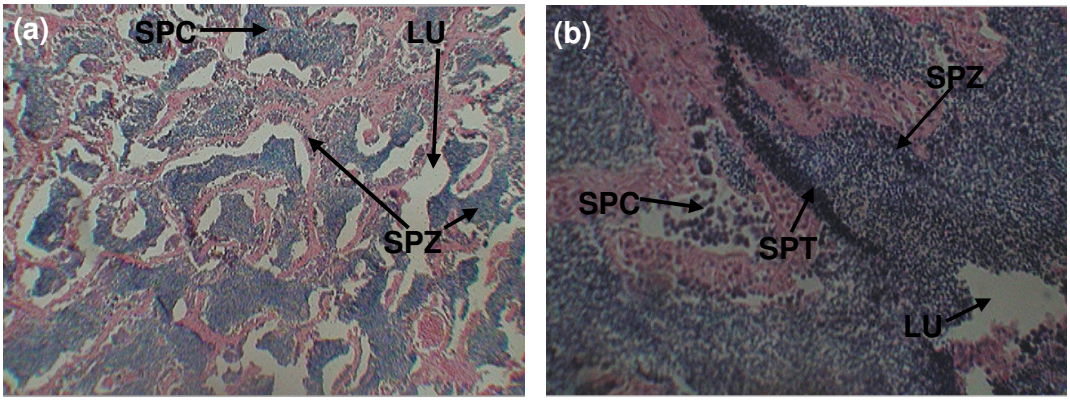

Plate 4.10. Haematoxylin-eosin (H-E) stained sections of *C. marulius* testes at (a) 10X (b) 40X magnification in July, 2011. SPC, spermatocytes; SPT, spermatids; SPZ, spermamatozoa; LU, lumen.

4.2 Gonado-somatic Index (GSI)

4.2.1 GSI of female *C. marulius*

GSI of female *C. marulius* was calculated during July 2013 to June 2014. GSI values from the available samples ranged from 0.018 to 0.42 during the study period. The highest and the lowest GSI values were 0.42 and 0.018 observed in July and in January, respectively. Monthly mean GSI values of female *C. marulius* are presented in Figure 4.11.

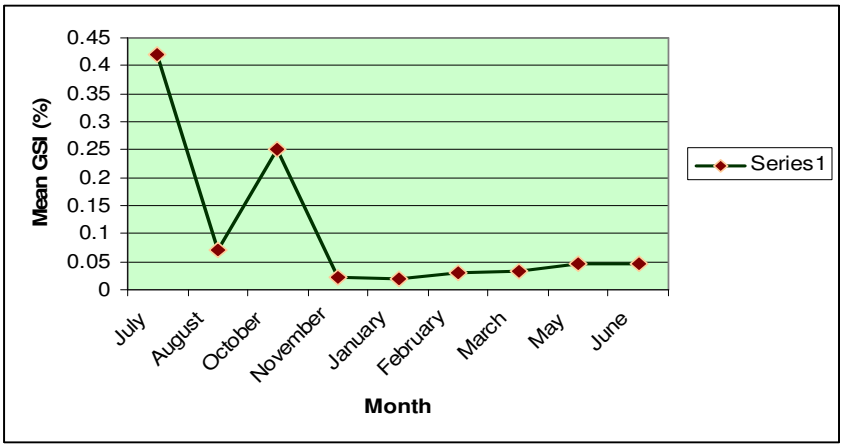

Figure 4.11 Monthly mean GSI values of female *C. marulius.*

4.2.2 GSI of male *C. marulius*

GSI of male *C. marulius* was calculated during July 2013 to June 2014. GSI values from the available samples ranged from 0.018 to 0.056 during the study period. The highest GSI value 0.056 was observed in July. Monthly mean GSI values of male *C. marulius* are presented in Figure 4.12.

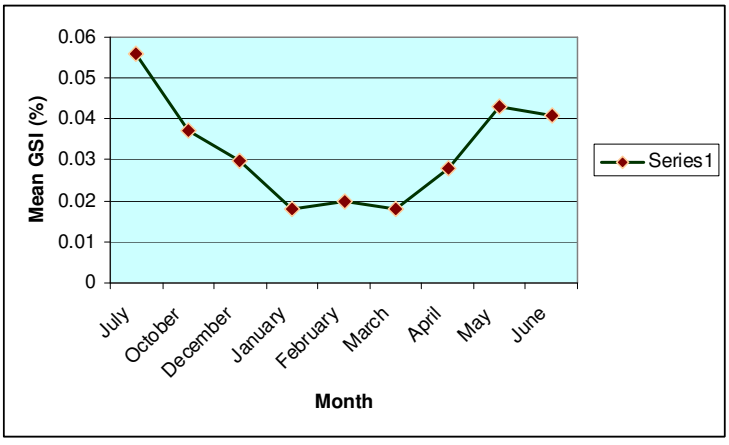

Figure 4.12 Monthly mean GSI values of male *C. marulius.*

D. New technology development (if any): Breeding season & stages of oocytes of endangered *C. marulius* identified.

E. Outcomes of the research project:

From the histological study of testis of *C. marulius* in the July sample, the following germ cell stages were identified; spermatids (SPT), spermatozoa (SPZ), spermatocytes (SPC) and also empty lumen of tubules (LU). High proportions of mature germ cells (SPT and SPZ) were observed in the July samples of testis indicating peak breeding season of the fish; although there were evidences of SPC, but were very few. The findings from GSI value and ovarian and testicular stages concludes that *C. marulius* from Sylhet basin attains peak reproductive maturity in the month of July. Both the ovary and testis develop and mature synchronously.

F. Total budget: Tk. 5,00,000/-

G. Budget status:

Line Item	Budget allocated	Budget incurred	Balance
1. Personnel	1,14,000	1,14,000	Nil
2. Travel	30,000	30,000	Nil
3. Operational Cost	3,16,000	3,16,000	Nil
4. Workshop/seminar	20,000	20,000	Nil
5. Others	15,000	15,000	Nil
Sub total	**4,95,000**	**4,95,000**	Nil
Overhead	5,000	5,000	Nil
Grand Total	**5,00,000**	**5,00,000**	Nil

Acknowledgements

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(**Dr. Md. Shahjahan**)