# REPRODUCTIVE BIOLOGY OF INDIAN MAJOR CARP (BLACK ROHU, *Labeo calbasu*) FROM THE KAPTAI LAKE OF BANGLADESH : A STUDY TO BROODSTOCK MANAGEMENT

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**Department of Fish Biology and Biotechnology**

**Faculty of Fisheries**

**Chattogram Veterinary and Animal Sciences University**

**Chattogram-4225, Bangladesh**

**JUNE 2022**

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**It is to certify that we have examined the above Master's thesis and have found that it is complete and satisfactory in all respects and that all revisions required by the thesis examination committee have been made**

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**The Author**

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# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

# LIST OF IMAGES

# LIST OF APPENDICES

# LIST OF ABBREVIATIONS

| | |
|---|---|
| % | Percent |
| µm | Micrometer |
| BW | Body Weight |
| cm | Centimetre |
| DoF | Department of Fisheries |
| DI | Dobriyal Index |
| DPX | Dibutylphthalate Polystyrene Xylene (Resin-based slide mountant) |
| FAO | Food and Agriculture Organization |
| g | Gram |
| GDP | Gross Domestic Product |
| GSI | Gonado-somatic Index |
| ha | Hectare |
| HSI | Hepato-Somatic Index |
| $L_m$ | Length at First Maturity |
| LWR | Length-Weight Relationship |
| MT | Metric Ton |
| $^oC$ | Degree Celsius |
| $R^2$ | Coefficient of Determination |
| SD | Standard Deviation |
| SL | Standard Length |
| TL | Total Length |
| UAE | United Arab Emirates |
| W | Weight |
| WGS84 | World Geodetic System 1984 |

# ABSTRACT

Life-history knowledge is significant for dynamic conservation, population growth, and broodstock management of fish populations, particularly for fish with high monetary worth. However, to date, very little is known about the life-history of commercially important carp fish species; therefore, this study aimed to investigate the reproductive biology of the Black Rohu, *Labeo calbasu,* in the Kaptai lake of Bangladesh from November 2020 to October 2021. Analyses of morphometric parameters revealed the exponent 'b' value of the standard length-weight relationship of *L. calbasu* was 2.9035 for both sexes; 2.4481 for males, and 3.0077 for females, indicating negative allometric growth ($b < 3$) for both sexes combined and for males, whereas isometric growth for females ($b = 3$). The average mean condition factor (K) and average mean relative condition factor (Kn) values were 1.453 ± 0.077 and 1.014 ± 0.055, respectively, indicating the fish's good health. The maximum mean HSI value was 1.19 ± 0.10 in July for males, and 1.25 ± 0.36 was found in February for females. The mean GSI value ranged from 0.05 ± 0.01 (December) to 2.14 ± 0.56 (June) in males and 0.23 ± 0.10 (December) to 19.5 ± 2.42 (June) in females. The oocyte diameter was found to be maximum in June (1152.9 ± 115.2 µm), and that was minimum in December (65.9 ± 49.9 µm). The oocyte diameter showed a positive relationship with GSI value, with the largest oocyte size and the greatest GSI value in June. Based on the gonado-somatic index, modified gonado-somatic index, and Dobriyal index, the length at first sexual maturity ($L_m$) was calculated as 40.0 cm TL for males and 41.5 cm TL for females. The fecundity was determined from randomly collected gravid female fish samples and varied from 446,264 to 1,063,644 eggs/individual with an average value of 828,198 eggs/individual. The GSI, oocyte diameter and gonadal maturity in males and females were synchronized in this species, indicating a single spawning period annually and its ranges from April to June. The study findings will be helpful in the management of *L. calbasu* and related species in the Kaptai lake, Bangladesh.



**Key Words:** Fecundity, GSI, HSI, Histology of gonad, *Labeo calbasu*, Length at first sexual maturity

# CHAPTER 01

# INTRODUCTION

Conservation and management of fisheries stocks require a basic understanding of the life cycle patterns, habitat, and exploitation status. It is also crucial to have precise information and accurate data on reproductive physiology, length at first maturity, fecundity, and population structure depending on age and growth, spawning seasons and grounds for proper management of the fish stock when it comes to species with significant economic importance. Studies focusing on acquiring such information are vital to accurately undertaking a quantitative analysis of fisheries stocks. In addition, understanding the reproductive biology of a species is essential for developing effective management plans and determining the level of exploitation that may be considered sustainable (Hunter, 1992; Murua et al., 2003). Reproduction of fish in the natural environment is the fundamental way of replenishing fish populations that have been depleted in the wild (Olusegun, 2011). The ability of given species to recruit in a challenging environment due to climate-induced issues determines the effectiveness of its replenishment (Barange et al., 2018). The seasonality of the reproductive events may be disrupted by climate-related issues, which could further terminate the spawning season of any species (Zahangir et al., 2022). Therefore, it is necessary to endeavor to comprehend the mechanism that governs and drives the population biology of commercially important fish species for better aquaculture outputs and broodstock management.

In Bangladesh, fish is the second most valuable agricultural product, contributing 3.52% to the national GDP, and more than 12% of the 170 million people of the country depend on it for their jobs and means of subsistence (DoF, 2020). This country, with extensive water resources, is one of the world's major fish producers, producing 45.03 lakh metric tons in 2019–20; inland open water (catch) contributes 27.72% (more than one-fourth) to the total production (12.48 lakh MT) (DoF, 2020). Additionally, it contains 3,866,091 hectares of inland water, with 97.8% of the total catch coming from the open water fishery. Bangladesh ranks third ($3^{rd}$) in inland open water capture production and fifth ($5^{th}$) in aquaculture production (FAO, 2020). The open water fisheries resources consist of floodplains (2,651,567 hectares), rivers and estuaries (853,863 hectares), the Beels (114,161 hectares), mangrove ecosystems, e.g., the

Sundarbans (177,700 hectares), and the Kaptai lake (68,800 ha) (DoF, 2020). Despite having such a potential ecosystem in Bangladesh, the primary inland water fisheries resources from the wild are declining continuously (Shamsuzzaman et al., 2017). Therefore, it is necessary to investigate the life-history pattern of major fisheries populations, especially those that are commercially important, to increase the production of those species from the open water bodies in Bangladesh.

Wetlands, one of the nation's natural treasures, play a crucial role in reducing poverty and improving the lives of underprivileged communities in Bangladesh. The Kaptai lake is one of Southeast Asia's largest artificial freshwater lakes and an important inland water fisheries (capture) resource in Bangladesh, covering roughly 58,300 hectares (68,800 hectares in total supply). It makes up 46.8% of Bangladesh's total pond area and is crucial to the country's inland water resources (Ahmed et al., 1999). The Karnaphuli River in Kaptai was dammed in 1961 primarily to generate electricity via hydropower (Fernando, 1980), with the possible alternatives being navigation, drainage and irrigation, fisheries, and flood control. Thus, the Karnaphuli river, which springs in the highlands of India, runs through this area and causes inundation of the river basin and low-lying areas, resulting from the dam's restriction of river flow. Additionally, water for the lake is provided by the rivers Chengi, Mayani, Kasalong, and Riankhiang (Ahmed et al., 2005). The Kaptai lake contributed 0.28% of overall fisheries production in 2019–20 (DoF, 2020), presenting an enormous opportunity for fish production, which is an essential protein source in the Bangladeshi people's daily intake. In 2019−20, the Kaptai lake contributed approximately 12,696 metric tons, with a significant variation in previous years (DoF, 2020). The Kaptai lake is home to 66 native species across 17 taxonomic families, as well as two introduced species and two species of shrimp (Rayhan et al., 2021). The most widely caught fish include the black rohu (*Labeo calbasu*), rohu carp (*L. rohita*), catla (*Gibelion catla*), mrigal (*Cirrhinus mrigala*), bata (*L. bata*), chapila (*Gudusia chapra*), kechki (*Corica soborna*), ayre (*Mystus aor*), kuncho chingri (*Macrobrachium lamarrei*), kajoli (*Ailia coila*), mola (*Amblypharyngodon mola*), tilapia (*Oreochromis mossambicus*) and nile tilapia (*O. niloticus*) (Suman et al., 2021).

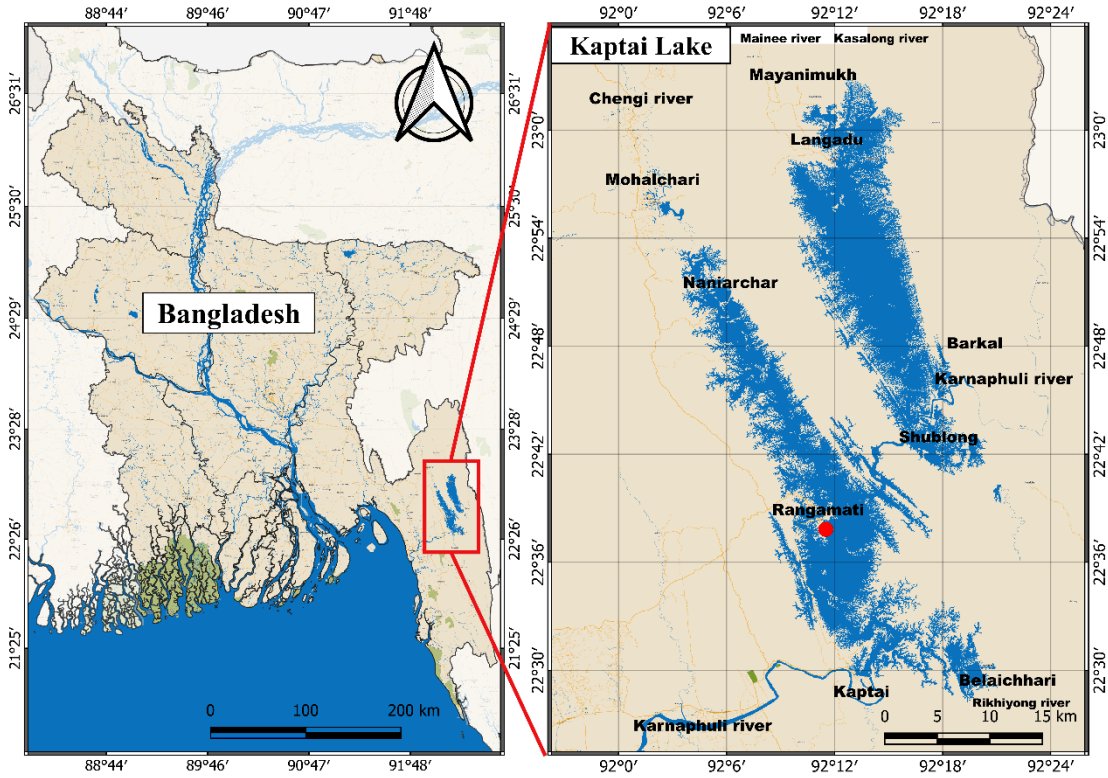



Image 1: Map of the Kaptai lake (map was made with QGIS 3.22 software, and WGS84 coordinate systems were implemented)

Indian major carps were the most prevalent fish species at the beginning of the commercial exploitation of fish from the Kaptai lake, making up more than 60% of the total production (Patwary et al., 2016). The Kaptai lake is also well-known for having spawning grounds for Indian major carps (IMCs). Carp species in the Kaptai lake accounted for more than 80% of total production in 1965–1966, 6.42% in 1998–1999, 5.1% in 2000–2001, 4% in 2007–2008, and 1.56% in 2018–2019 (Suman et al., 2021). Thus, the productivity of major carp species in the Kaptai lake has decreased dramatically (Patwary et al., 2016). Currently, two significant clupeid species, the chapila (*Gudusia chapra*) and the kechki (*Corica soborna*), account for more than 60% of the entire fish output in the Kaptai lake, whereas previously, this percentage was mostly dependent on carp species (Hussain, 2020). Due to the sharp rise in unwelcome clupeid and other small fish species, the Kaptai lake is in danger of losing its fish biodiversity (Suman et al., 2021). The outcome has been huge losses for native carp and other large-sized commercial fish species.

The reasons for diminishing carp species productivity in the Kaptai lake have been identified by researchers. The loss of aquatic ecosystems and inadequate or poor spawning stock are two major contributing factors. Because of anthropogenic effects and climate change, the number of high-valued fish in Kaptai lake has decreased quickly over time. Furthermore, a declining trend in the production of carp species in the Kaptai lake is observed due to habitat deterioration and a variety of human influences (Rayhan et al., 2021). The Kaptai lake used to be a spawning area for many carp species, and local fish farmers still collect eggs from several breeding grounds of this reservoir, including Kachalong, Karnafuli, Chengi, and Rengkhong channels (Eagle and Chakma, 2017). It is recommended that a comprehensive reservoir management strategy should be obtained to safeguard spawning areas from fish exploitation during the appropriate season. Though Bangladesh fisheries development corporation (BFDC) postulated a 90-day ban period to protect the fish from being caught during the spawning season based on previous studies for some other species from different habitats in other locations (Shalehin et al., 2022), the result is still unsatisfactory because the Kaptai lake does not yet have any species-specific or habitat-specific data. Thus, a specific conservation and management approach for this economically important fish species from the Kaptai lake is needed in order to ensure sustainable harvest and conservation.

The Indian major carp, Black Rohu, *Labeo calbasu* belongs to the Cyprinidae family of the Cypriniformes order, and it is one of Bangladesh's most important carp species, alongside *L. rohita*, *Cirrhinus mrigala,* and *Gibelion catla*. It is an important carp fish in the Kaptai lake (Ahmed et al., 2006), accounted for a yearly production of 36 metric tons in 2017–18 (DoF, 2018), and reduced to 14 metric tons in 2019 – 20 (DoF, 2020). Knowledge of reproductive biology, precise spawning season and ground may therefore help to increase the production of this high-valued fish species from the Kaptai lake. However, to date, there is no information regarding the reproductive biology, length at first sexual maturity, fecundity, and spawning season of the *L. calbasu* from the Kaptai lake.

Image 2: The Black Rohu, *Labeo calbasu* (Hamilton, 1822)

When attempting to pinpoint the precise timing of spawning and the number of times it takes place throughout a breeding season, it is crucial to check the gonadal development cycle and histological inspection of the gonad. The various phases of the ovary and testis can be easily identified by histological examination of the gonads. It offers a general concept of the fishes' ovum diameter, fertility, and peak spawning season, which helps to identify the breeding season further and is also useful for effective conservation and broodstock management.

This research will help us to understand the spawning season of the *L. calbasu* broodstock in the Kaptai lake and compare it to the current ban period in the reservoir to ensure that young *L. calbasu* are efficiently recruited. Furthermore, studying the various stages of fish gonadal maturation in the Kaptai lake helps restrict its fishing during the spawning season, allowing the fish stock to recover (Noble and Jones, 1993).

### 1.1. Aims and objective of the research

- To understand the primary biological indices of *L. calbasu* from the Kaptai lake; and

- To assess the habitat-specific spawning season by studying the gonadal maturation cycle of *L. calbasu* in the Kaptai lake for broodstock management.

# CHAPTER 02

# REVIEW OF LITERATURE

Previous studies on the length-weight relationship, GSI, length at first sexual maturity, oocyte diameter, cyclic variations in gonadal development, and fertility of *L. calbasu* fish species are all discussed in this chapter. In addition, the following information was obtained to design the current study and validate the new findings.

## 2.1. Length-weight relationship and growth pattern

Growth characteristics, such as weight and length, and species health, which are affected by a wide range of biological and environmental factors, must be evaluated as part of any biometric study of fish (Morato et al., 2001). Some researchers suggest that establishing length-weight relations in fish is crucial for comprehending development patterns, general health, habitat conditions, life history, fatness, condition factor, and other observable traits (Schneider et al., 2000; Froese, 2006).

The study of fish length-weight relationships is vital for understanding their morphology, biology, growth, and overall well-being and differentiating unit populations, which is useful in fisheries management. The growth of fish, as well as other animals, is often proportional to their body length. We can therefore conclude that length and growth are correlated. The "cube law" expresses the length-weight relationship, i.e., the weight of the fish will be proportional to the cube of their length, based on its dimensional equality, which may be mathematically expressed as $W = qL^b$ (Allen, 1938; Brody and Lardy, 1946; Roy, 1987). The majority of the fish, however, do not exhibit the cube rule in their natural habitat; hence, the relationship between length to weight may depart from the ideal values (3.0).

Furthermore, the body shape of fish is changed in association with their age and season, which might be due to the availability of foods and other environmental parameters. Because of this, the b-value for each fish species may be much more or lower than the optimal value (3.0), suggesting an allometric growth pattern (Gayanilo and Pauly, 1997). Growth is negatively allometric if it is less than 3.0 since fish become slenderer as they grow in length, which may indicate that the habitat is unsuitable for growth. On the other hand, fish become heavier and exhibit a positive allometric growth pattern for

their particular lengths if the b-value exceeds 3.0, which may result from ideal conditions (Abowei, 2010).

Basak and Hadiuzzaman (2019) studied the length-weight relationship of the *L. calbasu* from the specimen collected from the Tabalchari area of the Kaptai lake. They reported Ln W = −4.19 + 2.97 Ln TL being 'b' value 2.97 for the weight ranges from 52 gm to 1148 gm and length ranges from 15 cm to 42 cm.

Vahneichong et al. (2018) investigated the length-weight relationships of *L. calbasu* from the marshes of the South 24 Parganas region of West Bengal, India. A nine-month analysis from December 2006 to August 2007 concluded the length-weight relationship as following; for the size class < 26 cm, $W = 0.007367TL^{3.031}$ ; and for ≥26 cm, $W = 0.007269TL^{3.181}$ and found the isometric growth pattern for both size classes.

In a study of the length-weight relationship of two important carps from the Kaptai lake, Ahmed and Saha (1996) reported the 'b' values of 3.13 and 3.15 for *L. calbasu* and *L. rohita*, respectively.

Choudhary et al. (1991) estimated the length-weight relationship of different size groups of *L. calbasu* from the Rana Pratap Sagar reservoir, Rajasthan, India, and reported (i) the 'b' value of 3.29 for the length class 31 – 40 cm; positive allometric (ii) 'b' value 2.71 for the length class of 41 – 50 cm, negative allometric; and (iii) 'b' value 2.42 for length class of 51 – 60 cm, negative allometric. They found the highest growth exponent in the lowest size group.

There have been many previous studies that have documented allometric growth in *L. calbasu* (Natarajan, 1971; Chatterji et al., 1980; Vinci and Sugunan, 1981; Alam et al., 2000; Haroon et al., 2002; Naeem et al., 2012; Rizvi et al., 2012) yet some studies have shown isometric growth pattern in this fish species (Natarajan, 1972; Pathak, 1976; Khan, 1988).

### 2.2. Condition factor (K) and relative condition factor (Kn)

Given the theory that individuals of a given length exhibiting a greater weight are in better condition, the condition factor has become a standard indicator in fish biology. This notion has been employed as a new datum for studying reproduction and seasonal cycles of feeding processes, resulting in a year-to-year fluctuation in the index. In addition, comparing different populations allows us to assess the quality of the habitats in which these animals dwell (Lima-Junior et al., 2002). This index is at its highest

during the spawning season. However, to compare fish health, the relative condition factor (Kn) is also used (Rodriguez et al., 2017).

The relative condition factor (Kn) can be used to measure fish roundness, so aquaculturists can determine whether their fish are healthier than average. In addition, this component may also be used to measure the individuals' overall health, fatness or gonad development status (Kurup and Samuel, 1987). Thus, Kn more than one (> 1) indicates the fish is in good health, and Kn less than one (< 1) denotes the deteriorated health status of the fish (Le Cren, 1951).

Basak and Hadiuzzaman (2019) documented the mean condition factor and the mean relative condition factor for *L. calbasu* fish collected from three (03) different locations a) Tabalchhari, b) Mohalchhari, and c) Langadu area of the Kaptai lake, Bangladesh. They found the value of mean condition factors 1.396, 1.310, and 1.367 for three locations, and the mean relative condition factor was 1.02, 1.00, and 1.03, respectively.

Choudhary et al. (1991) documented the condition factor (K) and relative condition factor of *L. calbasu* fish collected from the Rana Pratap Sagar reservoir, Rajasthan, India. The condition factor (K) ranged from 1.15 − 1.25, and the relative condition factor (Kn) ranged from 0.995 − 1.029 for the length class 41 cm to 60 cm.

### 2.3. Hepato-somatic index (HSI)

The hepato-somatic index (HSI) in fisheries research is vital for indicating liver energy stores (Cerdá et al., 1996). The HSI is also a measure of fish energy reserves, especially for non-fatty fish (Chellappa et al., 1995), and *L. calbasu* is a non-fatty fish having only 0.98% of lipid content by weight (Memon et al., 2010). Polluted water has been demonstrated to alter the mean HSI of fish (Figueiredo-Fernandes et al., 2007). However, no data was found on the hepatosomatic index of *L. calbasu*.

### 2.4. Gonado-somatic index (GSI) and oocyte diameter

The gonado-somatic index, often known as the GSI, is one of the characteristics considered in fish reproductive research. GSI can be used to determine whether gonads are hydrated, and, as a result, it is easier to determine the starting point of the breeding season by observing the increased gonad weight (Shinkafi and Ipinjolu, 2012). To determine the spawning period, GSI plays a significant role as there is a cyclic change in gonad weight concerning total body weight. A rising GSI indicates that the spawning

season is approaching, while a decreasing GSI indicates that the spawning season has already taken place (Nieland and Wilson, 1993; Jons and Miranda, 1997; Smith, 2008).

In a hatchery near Faridpur, Bangladesh, Kabir and Quddus (2013) determined the GSI from male and female *L. calbasu*. They documented the GSI value for males and females ranging from 0.12 ± 0.06 − 1.68 ± 0.11 and 0.37 ± 0.06 − 18.58 ± 0.06, respectively, with the peak spawning month as July.

Kabir and Quddus (2013) reported that the peak oocyte diameter of a gravid *L. calbasu* in a fish hatchery in Faridpur, Bangladesh, was 1300 ± 40 micrometers (µm) in July.

### 2.5. Length at first sexual maturity ($L_m$)

The length at first sexual maturity is a subject that attracts much interest in the fishing industry due to its frequent use as an indicator of the tiniest size at which a fish may be legally caught (Lucifora et al., 1999).

The length at first maturity ($L_m$) for *L. calbasu* was found to be 30.6 – 40 cm for males and 33.6 – 45 cm for females at different waterbodies (Natarajan, 1971; Rao and Rao, 1972; Pathak and Jhingran, 1977; Vinci and Sugunan, 1981).

### 2.6. Fecundity

Fecundity is the maximum reproductive production of an individual (typically a female) during its reproductive season, and it is one of the most fundamental concepts in population biology (Bradshaw and McMahon, 2008). The quantity of oocytes present in the ovaries before the onset of the mating season is used to estimate the fecundity of a species (Bagenal and Braum, 1978).

Kabir and Quddus (2013) researched the fecundity of *L. calbasu* in a hatchery in the Faridpur area of Bangladesh. The fecundity ranged from 37,454 − 427,030 eggs/individual with an average fecundity of 230,242 eggs/individual. The fish that measured 51.3 centimeters in total length and weighed 1,785 grams had the highest fertility, whereas the fish that measured 32.5 centimeters in total length and weighed 720 grams had the lowest fecundity and 1,359 ova and 213 ova were present per gram of ovarian weight and body weight, respectively. Some other works on the fecundity count of *L. calbasu* by researchers from different locations are given in the table below (Table 1).

Table 1: Fecundity of *L. calbasu* in different locations

| Fecundity | Location | Author |
|---|---|---|
| 37,454 – 427,030 | Faridpur, Bangladesh | Kabir and Quddus, 2013 |
| 40,200 – 517,500 | Godavari river, India | Rao and Rao, 1972 |
| 67,500 – 572,460 | Nagarjunasagar Reservoir, India | Vinci and Sugunan, 1981 |
| 93,972 – 466,400 | Loni reservoir, Madhya Pradesh, India | Pathak and Jhingran, 1977 |
| 288,000 – 438,000 | Barrackpore, India. | Sukumaran, 1969 |
| 109,700 – 980,700 | Bhavanisagar Reservoir, India | Natarajan, 1971 |
| 312,100 – 657,600 | Gohad reservoir, India | Mishra and Saksena, 2012 |
| 739,440 | Punjab, India | Khan, 1934 |

### 2.7. Reproductive biology and spawning season

Knowledge of fish reproductive biology is crucial for effective management and the long-term viability of fish stocks (Temesgen, 2017). Furthermore, it is also vital to have a solid understanding of the reproductive features of fish in order to provide appropriate scientific recommendations for fisheries management (Hossain et al., 2017). The *L. calbasu* seems to be a seasonal breeder; it only reproduces during the rainy season (Qasim and Qayyum, 1962; Bhuiyan, 1964; Natarajan, 1971; Rao and Rao, 1972).

After reviewing some earlier research and documenting the reproductive seasonality of the *L. calbasu*, Gupta and Banerjee (2015) came to the conclusion that the breeding seasons of the *L. calbasu* used to fluctuate depending on the monsoon floods in various parts of the world. It used to be the case that the breeding season would change in various places to coincide with the monsoon floods that occurred in various locations. *L. calbasu* is most likely to be seen spawning in July and August in Punjab (Khan, 1924; Qasim and Qayyum, 1962). Breeding occurs from June to September in the Loni reservoir in Madhya Pradesh, with the most spawning activity in July (Pathak, 1976; Pathak and Jhingran, 1977). According to Mishra and Saksena (2012), the breeding season for this species is from May to August at the Gohad reservoir in Madhya Pradesh. It used to be the case that the mating season in southern India began at the end of May, coinciding with the beginning of the southwest monsoon, and lasted until the end of October (Chacko and Kurian, 1949). June is the most productive month for breeding in the Godavari River system (Rao and Rao, 1972). According to Vinci and Sugunan (1981), the breeding season runs from July to September, and August is when

most eggs are laid in the Nagarjunasagar reservoir in Telangana. According to Bhuiyan et al. (2013), the mating season for this species runs from April to August, while Kabir and Quddus (2013) found that July was the peak month of breeding for *L. calbasu* in a stocking pond in Faridpur, Bangladesh.

According to the aforementioned data, no significant information was found regarding the reproductive biology of the commercially significant carp species, *L. calbasu*, in the Kaptai lake. Therefore, this study was undertaken in order to determine the precise time when this fish species spawns in this reservoir.

# CHAPTER 03

# MATERIALS AND METHODS

## 3.1. Sampling site and collection of samples

Fish samples (both male and female *L. calbasu*) were obtained from the local Banarupa Bazar fish market and fish landing site of the Bangladesh Fisheries Development Corporation (BFDC), Rangamati, Bangladesh (Image 3). During the study period, 136 fish (average weight 1151 ± 299.8 gm, and length 42.8 ± 2.99 cm) were collected monthly (November 2020 to October 2021). The collected fish were kept fresh in an insulated iced box during the transport and brought to the Faculty of Fisheries of the Chattogram Veterinary and Animal Sciences University, Chattogram, for further analysis.

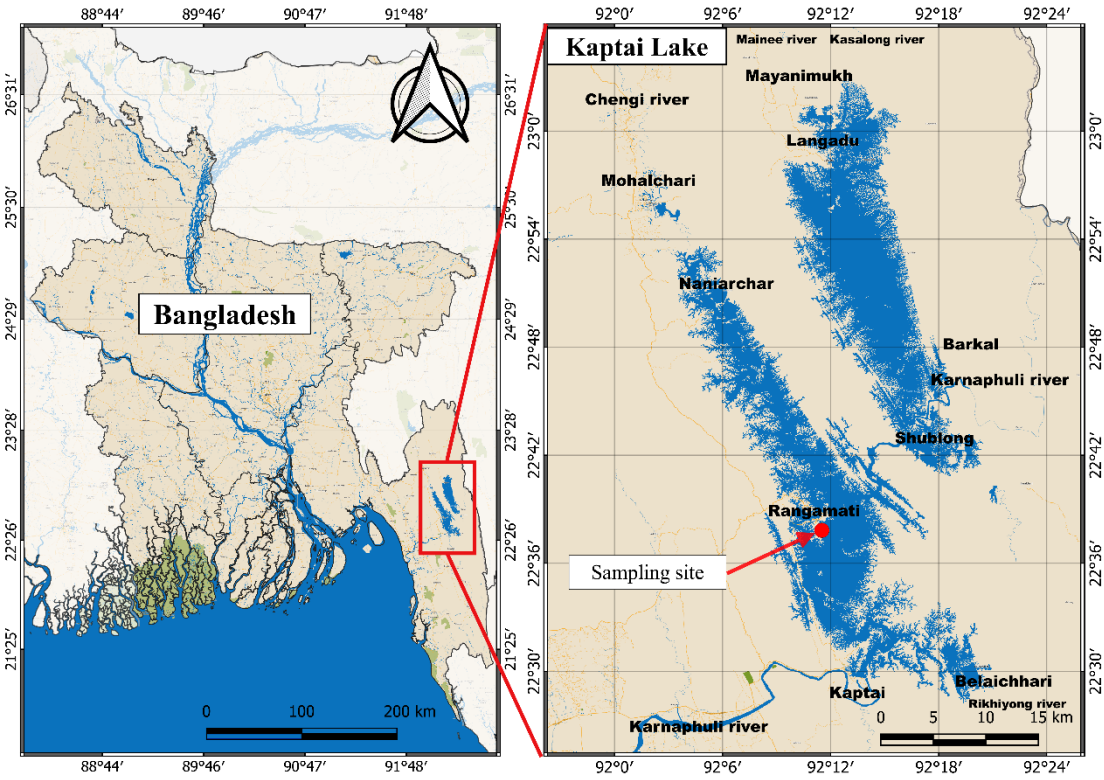



Image 3: Map of the Kaptai lake, the arrow indicating the sampling site

## 3.2. Determination of the length-weight relationship

Total length (TL, from the tip of the snout to caudal fin) and standard lengths (SL, from the tip of the snout to the last vertebrae of individual fish samples were measured by a measuring scale and recorded as centimeters (cm). The body weight of individual fish was measured in grams (g) using an electric balance (Redwag WPT1211NV, Poland).

Image 4: Recording of length-weight data of *L. calbasu*

The length-weight relationship was determined by plotting the data into the following exponential equation according to Le Cren (1951).

$$TW = aTL^b$$

Where,

TW is the total weight (taken in gram (g)),

TL is the total length (taken in centimeter units),

"a" is the body form-related co-efficient and

"b" is an exponent

The estimated values for the constant 'a' and 'b' for the equations were derived from the log-transformed length and weight (Le Cren, 1951).

$$\text{Log } TW = \text{Log } a + b \text{ Log } TL$$

**3.3. Determination of condition factor (K) and relative condition factor (Kn)**

The monthly samples were employed to evaluate the condition factor (K) and the relative condition factor (Kn), which were used to assess the seasonal fluctuations in fish conditions. The following equation was used to compute the condition factor (Lima-Junior et al., 2002).

$$K = W/L^b$$

Where,

K = condition factor;

W = weight calculated from length-weight relationship;

L = total length of fish

b = is an exponent calculated from the length-weight relationship

The relative condition factor "Kn" (Le Cren, 1951) was estimated by using the following formula,

$$Kn = W_0/W_c$$

Where,

$W_0$ = Actual weight of fish (g),

Wc = Expected weight,

Wc = Log W* = log a + b log L

W* = Average weight of the collected fish

**3.4. Determination of gonado-somatic index (GSI) and hepato-somatic index (HSI)**

At first, the gonad and liver of individual fish were collected and weighed to determine the GSI and HSI. For that, the fish was initially laid out on a tray; then, it was dissected by cutting it along its ventral side from its anus to its lower jaw. After removal of the excess fat, blood vessels, and gut, the gonads and liver were gently collected from the body cavity with forceps. Next, the acetocarmine gonad squashing procedure was used to determine the sex of the individual fish from the collected gonads (Guerrero and Shelton, 1974). Finally, the weight of each fish gonad and liver was precisely measured using an electrical balance (EK600 Dual, U.A.E.), and the data were recorded for further calculation of GSI and HSI by the following equation (Maddock and Burton, 1998).

$$\text{GSI (\%)} = \frac{\text{Weight of gonad (g)}}{\text{Total body weight of fish (g)}} \times 100$$

$$\text{HSI (\%)} = \frac{\text{Weight of liver (g)}}{\text{Total body weight of fish (g)}} \times 100$$

The collected gonad samples were stored in Bouin's fixative (Ortiz-Hidalgo, 1992) at room temperature (25° C) for 24 hours. The next day, samples were transferred to 70% ethanol until histological analysis (Culling, 1974) (Image 5).

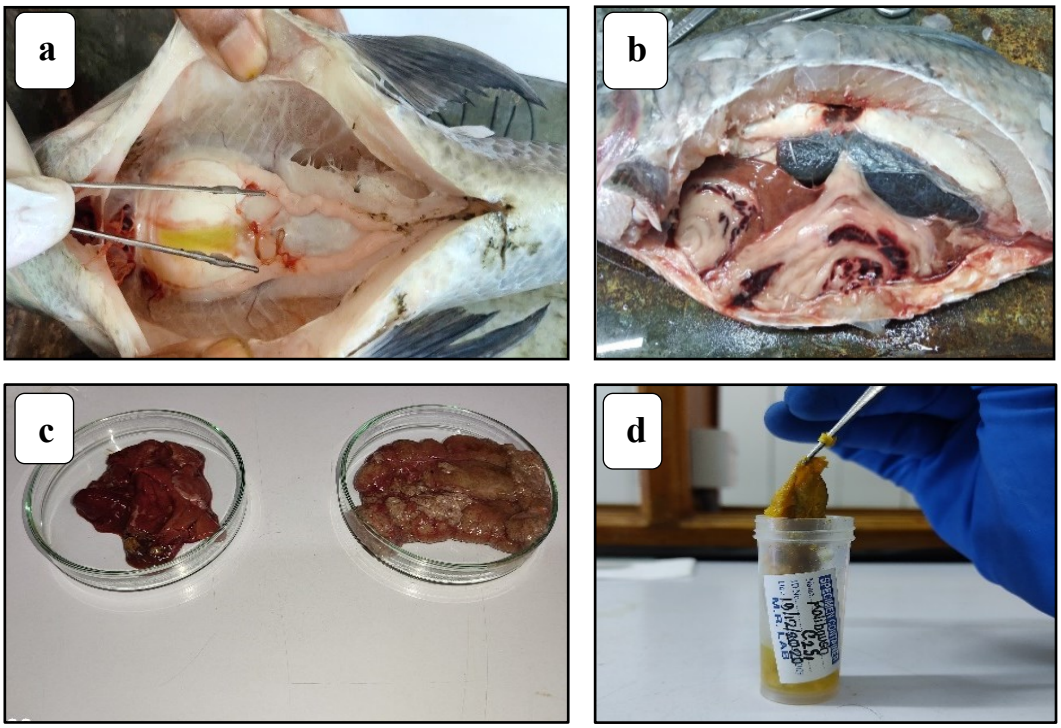



Image 5: Testes, ovary, and liver of *L. calbasu* after dissecting the abdominal cavity (a, b); collecting those into Petri dishes for weighing (c); samples into the vial for fixing with Buin's fixative (d)

### 3.5. Determination of length at first sexual maturity ($L_m$)

Multiple functional relationships were used to estimate the length at first sexual maturity size ($L_m$), including (i) the relationship of the gonado-somatic index (GSI) to total length (TL) (Nikolsky and Birkett, 1963), (ii) the relationship between modified gonado-somatic index (MGSI) to TL (Nikolsky and Birkett, 1963), and (iii) the relationship between Dobriyal index (DI) to TL (Dobriyal et al., 1999).

These were calculated as,

$$\text{Modified gonado somatic index, MGSI (\%)} = \frac{\text{gonad weight}}{\text{body weight - gonad weight}} \times 100$$

$$\text{Dobriyal index, DI} = \sqrt[3]{\text{gonad weight}}$$

### 3.6. Determination of fecundity and oocyte diameter

The gravimetric method was used to determine the fecundity of *L. calbasu*. In the first step, three to five sub-samples from different ovarian areas were taken and weighed. After weighing each sub-sample, eggs were counted from each sub-sample, and the following formula was used to calculate the absolute fecundity (Murua et al., 2003).

$$\text{Fecundity} = \frac{\left[\sum_i \frac{O_i}{W_i}\right]}{n} \times W_{ovary}$$

Where,

i = Corresponding sample number (1, 2, 3, … ),

$O_i$ = Number of oocytes in a weighted sample of ovarian tissue,

$w_i$ = Weight of sample of ovarian tissue,

$W_{ovary}$ = Total weight of the ovary.

An ocular microscope (Carl ZEISS Primostar 41550-0057-000, Germany) equipped with a digital camera (TUCSEN ISH500, H – series) and TCapture software (Tucsen Photonics Co., Ltd.) was used to measure the oocyte diameter and recorded in micrometers (µm) (1µm = $10^{-6}$ meter = 1/1000 cm) (Image 6). Oocyte diameter was measured for the individual female and expressed as monthly variations.

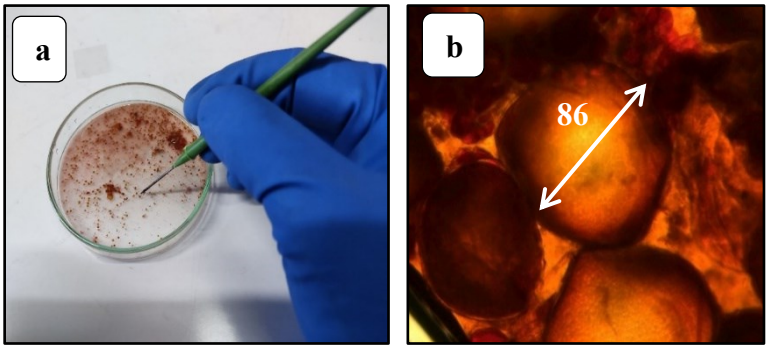



Image 6: Counting of eggs in order to determine fecundity (a) and measurement of oocyte diameter (b)

### 3.7. Histological analysis of gonad

Previously preserved samples in 70% ethanol were used for histological investigation following standard histological procedures used in earlier studies (Akhter et al., 2020; Islam et al., 2021). At first, the samples were dehydrated by different grades of ethanol (70, 80, 90, 95, 100, and 100%). After dehydration, samples were embedded with paraffin wax. Then, the embedded wax block was sectioned using a microtome machine at five (5) µm thickness (KD 2258 rotary microtome, China). Next, the sections were stained by standard staining procedure using hematoxylin and eosin (H & E) and then subjected to a histological examination under a microscope (Carl ZEISS Primostar

41550-0057-000, Germany). The description of the detailed histological analysis is as follows.

### 3.7.1. Preparation for the histological procedure

Different kinds of chemicals and solutions are needed for histological operations. Therefore, it ensured that all chemicals and solutions were available, at the right concentration and dilution, before carrying out the procedure. Then, plastic cassettes were positioned where the tissue would be placed for the following process and sequentially identified with a pencil.

### 3.7.2. Washing of fixed tissue

Excess fixatives were drained from the vials to avoid interfering with subsequent histology procedures, and tissues were rinsed for 5–10 minutes under running water.

### 3.7.3. Dehydration

The gonads were rinsed, and the gonads were sliced into small (≥1 cm) pieces before being placed individually in pre-labelled cassettes. The tissue pieces in the cassette were then dehydrated by passing them through progressively graded ethanol concentrations (Table-1).

Table 2: Dehydration schedule

| SI. No. | Solution | Time |
|---|---|---|
| **1** | 50% ethanol | 2 hours |
| **2** | 70% ethanol | 2 hours |
| **3** | 80% ethanol | 2 hours |
| **4** | 90% ethanol | 2 hours |
| **5** | 95% ethanol | 2 hours |
| **6** | 100% ethanol (1) | 2 hours |
| **7** | 100% ethanol (2) | 2 hours |

### 3.7.4. Cleaning

Following dehydration, the tissue pieces in the cassette were washed many times in xylene to eliminate any traces of ethanol and replace any remaining lipid content from the cell with xylene (Table-2).

Table 3: Cleaning schedule

| SI. No. | Solution | Time |
|---|---|---|
| **1** | Ethanol + Xylene (1:1) | 2 hours |
| **2** | Xylene (1) | 2 hours |
| **3** | Xylene (2) | 2 hours |

### 3.7.5. Infiltration

The tissue blocks were immediately taken from xylene after cleaning, then covered in molten paraffin and kept in a hot air oven to a temperature of usually 60°C, as shown in the following chart. Xylene evaporates when heated, leaving a space in the tissue filled with paraffin.

Table 4: Infiltration schedule

| SI. No. | Solution | Time |
|---|---|---|
| **1** | Xylene + Paraffin (1:1) | 2 hours |
| **2** | Paraffin | 2 hours |
| **3** | Paraffin | 2 hours |
| **4** | Paraffin | 2 hours |

### 3.7.6. Embedding

Following removal from the paraffin, each tissue sample was placed in a prefabricated paper or aluminum block and labelled appropriately before refilling with the molten paraffin. The inserted blocks were then left out overnight to cool at ambient temperature.

### 3.7.7. Trimming

During trimming, the excess wax layers of the implanted blocks are cut away by a knife to reveal more functional blocks below. The trimming made it simple to section the piece into smaller slices. This process included both lateral and surface trimming.

### 3.7.8. Sectioning

Tissue is cut into 5µm thin sections from tiny paraffin blocks using a hand-held rotatory microtome (KD-2258 Rotary Microtome, China) (1µm = $10^{-6}$ meter = 1/1000 cm). After being sectioned, the ribbon-like pieces were floated in a 42° C water bath for three to four minutes.

### 3.7.9. Attachment of section on glass slide and drying

After carefully dispersing in a water bath, the sections were transferred to adhesive-coated glass slides (gelatin, egg albumin). The glass slides containing the section(s) were dried in a slide warmer at 37°C overnight.

### 3.7.10. Staining

When the tissue section containing the slide was dry, staining could begin. The tissue was stained with hematoxylin and eosin using the protocols outlined in Table 4.

Table 5: Staining schedule

| Sl. No. | Treatment | Time | Stages |
|---|---|---|---|
| **1** | Xylene – 1 | 10 min | Deparaffinization |
| **2** | Xylene – 2 | 10 min | |
| **3** | Xylene – 3 | 10 min | |
| **4** | 100% Ethanol-1 | 5 min | Rehydration |
| **5** | 100% Ethanol- 2 | 5 min | |
| **6** | 95% Ethanol | 3 min | |
| **7** | 70% Ethanol | 3 min | |
| **8** | 50% Ethanol | 2 min | |
| **9** | Distilled water | 10 – 15 dips | |
| **10** | Hematoxylin | 1 – 1.5 min | Staining |
| **11** | Tap water wash | 15 min | |
| **12** | Acid alcohol 1% | 2 – 4 dips | |
| **13** | Tap water wash | 5 min | |
| **14** | 50% Ethanol | 10 – 15 dips | |
| **15** | 95% Ethanol | 30 sec | |
| **16** | Eosin Y | 3 – 5 min | |
| **17** | 95% Ethanol– 2 | 2 min | Removal of extra dye and Dehydration |
| **18** | 100% Ethanol– 3 | 2 min | |
| **19** | 100% Ethanol – 4 | 2 min | |
| **20** | 100% Ethanol + pure xylene (50% + 50%) | 2 min | Cleaning and removal of ethanol |
| **21** | Xylene- 1 | 20 min | |
| **22** | Xylene- 2 | 20 min | |

### 3.6.11. Mounting

After staining, a glass slide containing a tissue segment was covered by a thin cover slip. Next, each slide was coated with the required amount of DPX (Qualikems Fine Chemical Pvt. Ltd., India), followed by the attachment of a cover slip, and then allowed the DPX to solidify at room temperature overnight.

### 3.7.12. Microscopic examination and identification of maturation stages

The slides were mounted and examined using a microscope (Carl ZEISS Primostar 41550-0057-000, Germany) connected to a computer equipped with a digital camera (TUCSEN ISH500, H – series) and TCapture software (Tucsen Photonics Co., Ltd.). This system was used to capture several images at varying magnifications.

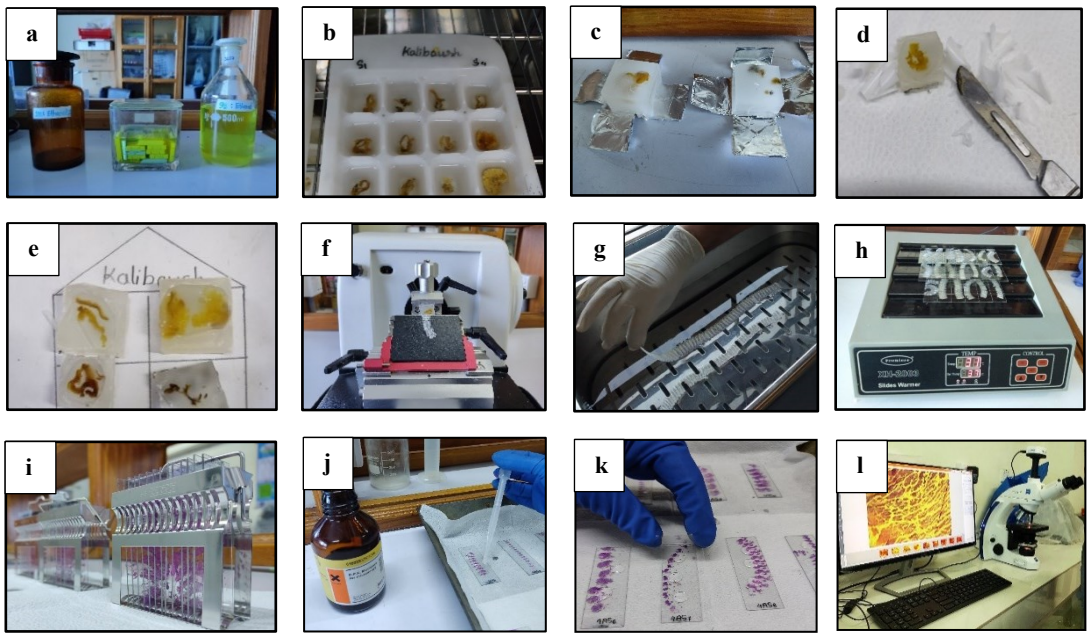



Image 7: Histological procedure; Dehydration and cleaning (a), Infiltration (b), Embedding (c), Trimming and trimmed blocks (d, e), Sectioning (f), Sample ribbon into the water bath and attaching into a glass slide (g), Drying (h), Stained slides (i), Applying DPX (j), Mounting with cover glasses (k), Microscopic observation (l).

### 3.8. Statistical analysis

Data were presented as mean ± standard deviation (SD). In addition, both the homogeneity and dispersion of the data were assessed. Finally, the Pearson regression (r) analysis was conducted to investigate the connection between the GSI and HSI. In

order to carry out the statistical analysis, Microsoft Excel 2016 was utilized. The data gathered in various months were compared for significant differences using the one-way analysis of variance (ANOVA). In addition, student t-tests were used to determine whether there were any significant differences between male and female *L. calbasu*.

# CHAPTER 04

# RESULTS

## 4.1. Length-weight relationship and growth pattern

In the present study, the intercept (a) and slope (b) of the regression analysis was found to be –1.6862 and 2.9035, respectively, for the *L. calbasu*. The total-length weight relationship based on pooled data was $W = 0.0206 \times TL^{2.9035}$ ($R^2 = 0.8479$), the logarithmic equation being log W = −1.6862 + 2.9035 log TL. The value of 'b' in the length-weight relationship reveals that both male and female *L. calbasu* exhibit negative allometric growth (b < 3) pattern (Figure 1A).

In the case of males, the length-weight relationship was established as $W = 0.1117 \times TL^{2.4481}$ , where the $R^2$ was 0.7609. The logarithmic equation being Log W = − 0.9518 + 2.4481 Log TL for males and the 'b' value (2.4481) for males indicates the negative allometric growth (b < 3) (Figure 1B).

The following exponential equations of length-weight relationship for female was found to be $W = 0.0141 \times TL^{3.0077}$ ($R^2 = 0.8737$). The logarithmic equation being Log W = − 1.8518 + 3.0077 Log TL. The 'b' value (3.0077) of the length-weight relationship of females indicates the isometric growth pattern (Figure 1C).

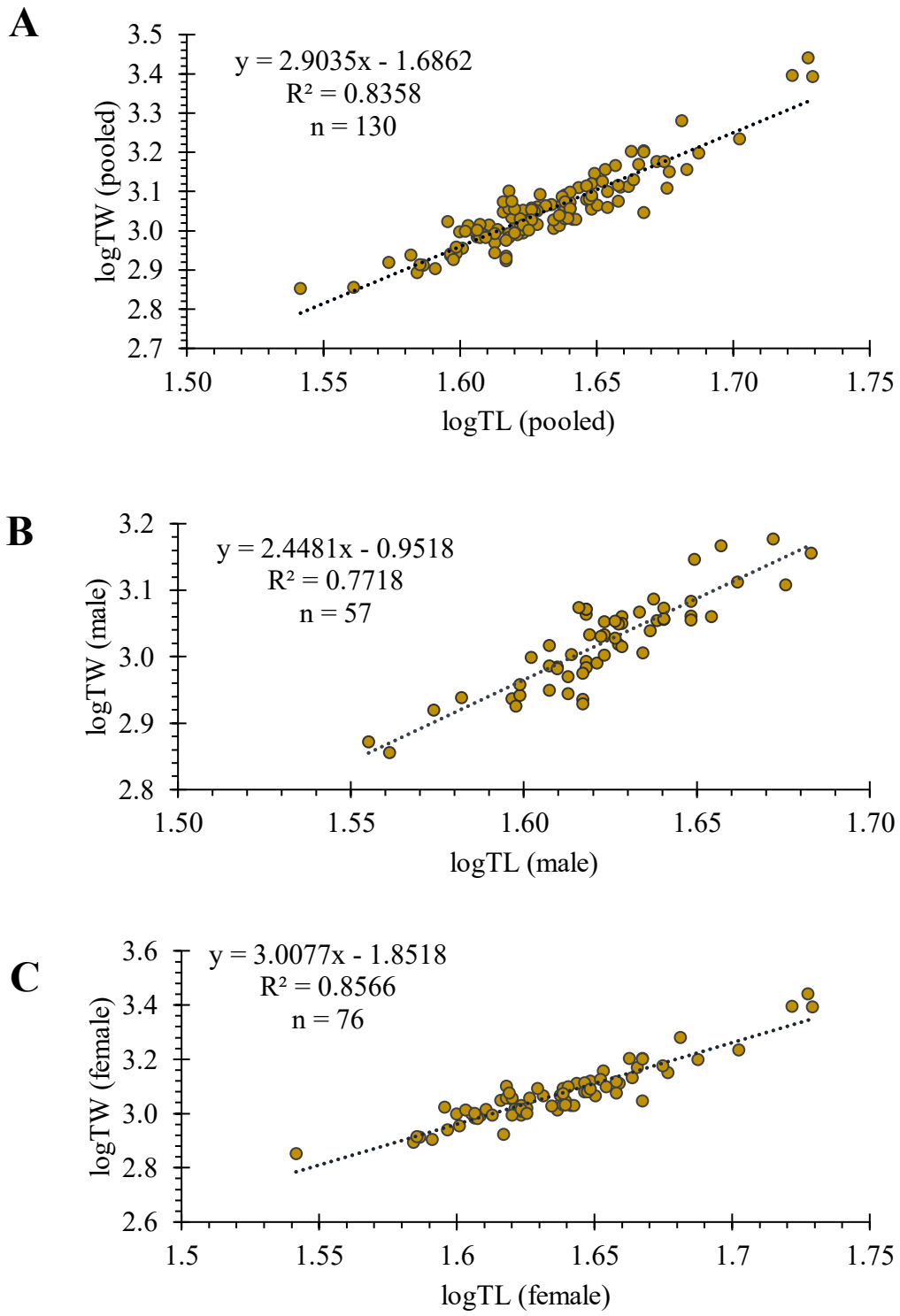
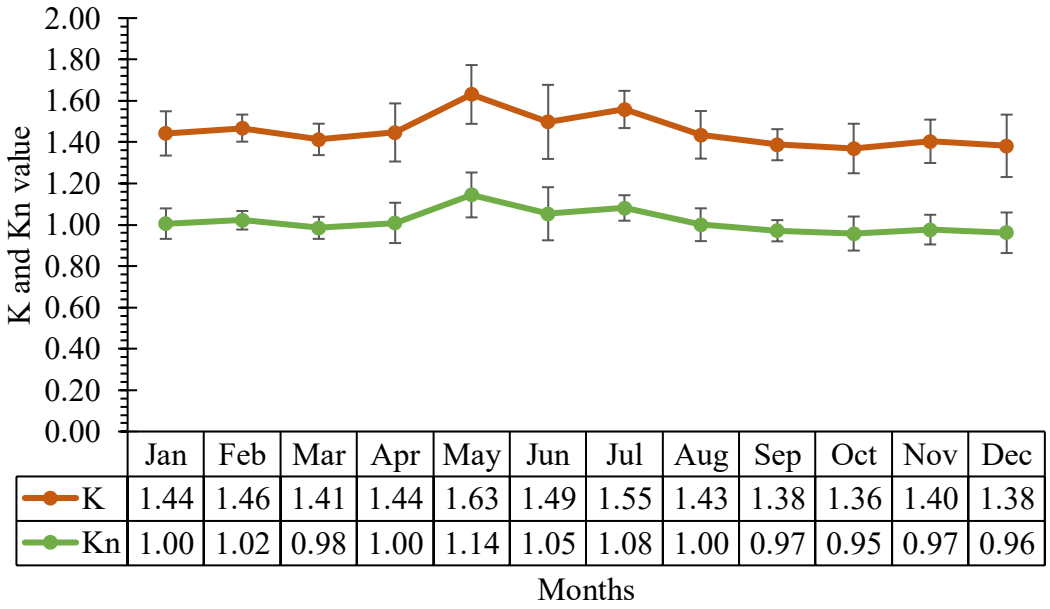



Figure 1: Logarithmic relationship between the total length and weight of *L. calbasu* of pooled data (A), male (B), female (C) (n = 130 for the pooled group, n = 57 for male and, n = 76 for female) collected from the Kaptai lake, Bangladesh. The X-axis indicates the common logarithm (log10) of total length, whereas Y-axis indicates the common logarithm (log10) of total weight; the $R^2$ value indicates the coefficient of determination.

### 4.2. Condition factor (K) and relative condition factor (Kn)

The condition factor (K) and relative condition factor (Kn) are calculated from the monthly collected fish samples to evaluate the seasonal changes in the general health of fish. The monthly mean K values ranged from 1.369 ± 0.119 to 1.631 ± 0.142 with an average of 1.453 ± 0.077. The monthly mean Kn values ranged from 0.962 ± 0.082 to 1.144 ± 0.109, with an average value of 1.014 ± 0.055 (Table 5). The average K value was found to be minimum (1.369 ± 0.119) in October and maximum (1.631 ± 0.142) in May, whereas the mean Kn was minimum (0.962 ± 0.082) during December and maximum (1.144 ± 0.109) during May (Figure 2).




| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| K | 1.44 | 1.46 | 1.41 | 1.44 | 1.63 | 1.49 | 1.55 | 1.43 | 1.38 | 1.36 | 1.40 | 1.38 |
| Kn | 1.00 | 1.02 | 0.98 | 1.00 | 1.14 | 1.05 | 1.08 | 1.00 | 0.97 | 0.95 | 0.97 | 0.96 |

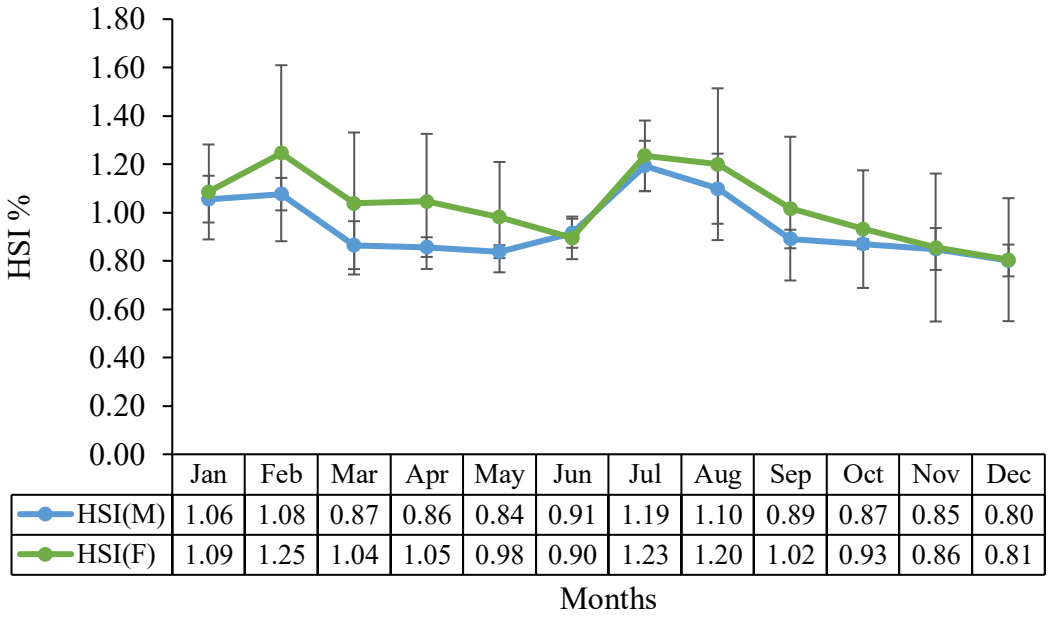
Figure 2: Condition factor and relative condition factor of *L. calbasu* collected monthly (November 2020 to October 2021) from the Kaptai lake, Bangladesh. Values are presented as 'mean ± SD' (n = 10−15 per month of both sexes). The Red line indicates the condition factor (K), and the green line indicates the relative condition factor (Kn).

Table 6: Condition factor and relative condition factor of male and female of *L. calbasu* collected monthly (November 2020 to October 2021) from the Kaptai lake, Bangladesh. Values are presented as 'mean ± SD' (n = 10−15 per month of both sexes).

| Months | | Mean 'K' male | Mean 'K' female | Mean 'Kn' male | Mean 'Kn' female |
|---|---|---|---|---|---|
| January | | 1.553 ± 0.077 | 1.395 ± 0.081 | 1.084 ± 0.053 | 0.972 ± 0.044 |
| February | | 1.440 ± 0.019 | 1.484 ± 0.078 | 1.001 ± 0.010 | 1.034 ± 0.051 |
| March | | 1.419 ± 0.072 | 1.407 ± 0.086 | 0.989 ± 0.052 | 0.981 ± 0.057 |
| April | | 1.391 ± 0.133 | 1.484 ± 0.144 | 0.972 ± 0.095 | 1.034 ± 0.102 |
| May | | 1.467 ± 0.110 | 1.722 ± 0.121 | 1.017 ± 0.071 | 1.208 ± 0.097 |
| June | | 1.351 ± 0.103 | 1.643 ± 0.072 | 0.947 ± 0.067 | 1.159 ± 0.053 |
| July | | 1.429 ± 0.129 | 1.525 ± 0.068 | 1.001 ± 0.090 | 1.063 ± 0.041 |
| August | | 1.436 ± 0.129 | 1.435 ± 0.118 | 1.001 ± 0.087 | 1.001 ± 0.083 |
| September | | 1.410 ± 0.077 | 1.375 ± 0.077 | 0.985 ± 0.052 | 0.963 ± 0.053 |
| October | | 1.378 ± 0.102 | 1.363 ± 0.140 | 0.962 ± 0.069 | 0.955 ± 0.097 |
| November | | 1.402 ± 0.081 | 1.406 ± 0.132 | 0.974 ± 0.052 | 0.979 ± 0.092 |
| December | | 1.355 ± 0.177 | 1.400 ± 0.146 | 0.939 ± 0.119 | 0.976 ± 0.090 |
| Average | | 1.419 ± 0.101 | 1.470 ± 0.105 | 0.989 ± 0.068 | 1.027 ± 0.072 |
| Range | Min | 1.351 ± 0.103 (Jun) | 1.363 ± 0.140 (Oct) | 0.939 ± 0.119 (Dec) | 0.963 ± 0.053 (Sep) |
| | Max | 1.553 ± 0.077 (Jan) | 1.722 ± 0.121 (May) | 1.084 ± 0.053 (Jan) | 1.208 ± 0.097 (May) |

### 4.3. Hepato somatic index (HSI)

Monthly changes in HSI values for male and female *L. calbasu* were calculated, and the results are presented in (Figure 3). In males, the minimum mean value of HSI was 0.80 ± 0.07 in males found in December, and the maximum mean value of HSI (1.19 ± 0.10) was found in July. In females, the maximum (1.25 ± 0.36) and minimum (0.81 ± 0.10) HSI values were found in February and December, respectively. No apparent differences were observed in the HSI values between males and females in any particular month.




| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| HSI(M) | 1.06 | 1.08 | 0.87 | 0.86 | 0.84 | 0.91 | 1.19 | 1.10 | 0.89 | 0.87 | 0.85 | 0.80 |
| HSI(F) | 1.09 | 1.25 | 1.04 | 1.05 | 0.98 | 0.90 | 1.23 | 1.20 | 1.02 | 0.93 | 0.86 | 0.81 |

Figure 3: Hepato-somatic index (HSI) of *L. calbasu* collected monthly (November 2020 to October 2021) from the Kaptai lake, Bangladesh. Values are presented as 'mean ± SD' (n = 10−15 per month of both sexes). The blue line indicates the HSI value for males, and the green line indicates the HSI value for females.

### 4.4. Gonado somatic index (GSI)

This study considered males and females for calculating GSI throughout the year. The highest GSI value (19.5 ± 2.42) for females was found in June. The mean GSI value of female *L. calbasu* increased from March onwards and peaked significantly in June ($p < 0.005$). For females, the GSI value sharply declined in July and remained low in the months thereafter. In males, the mean GSI value ranged from 0.05 ± 0.01 to 2.14 ± 0.56. The highest GSI (2.14 ± 0.56) was also found significantly in June ($p < 0.005$). After that GSI value decreased drastically in July, revealing a similar trend in the monthly GSI pattern with the female (Figure 4). GSI value began to increase from March onwards and reached a peak in June in both sexes.

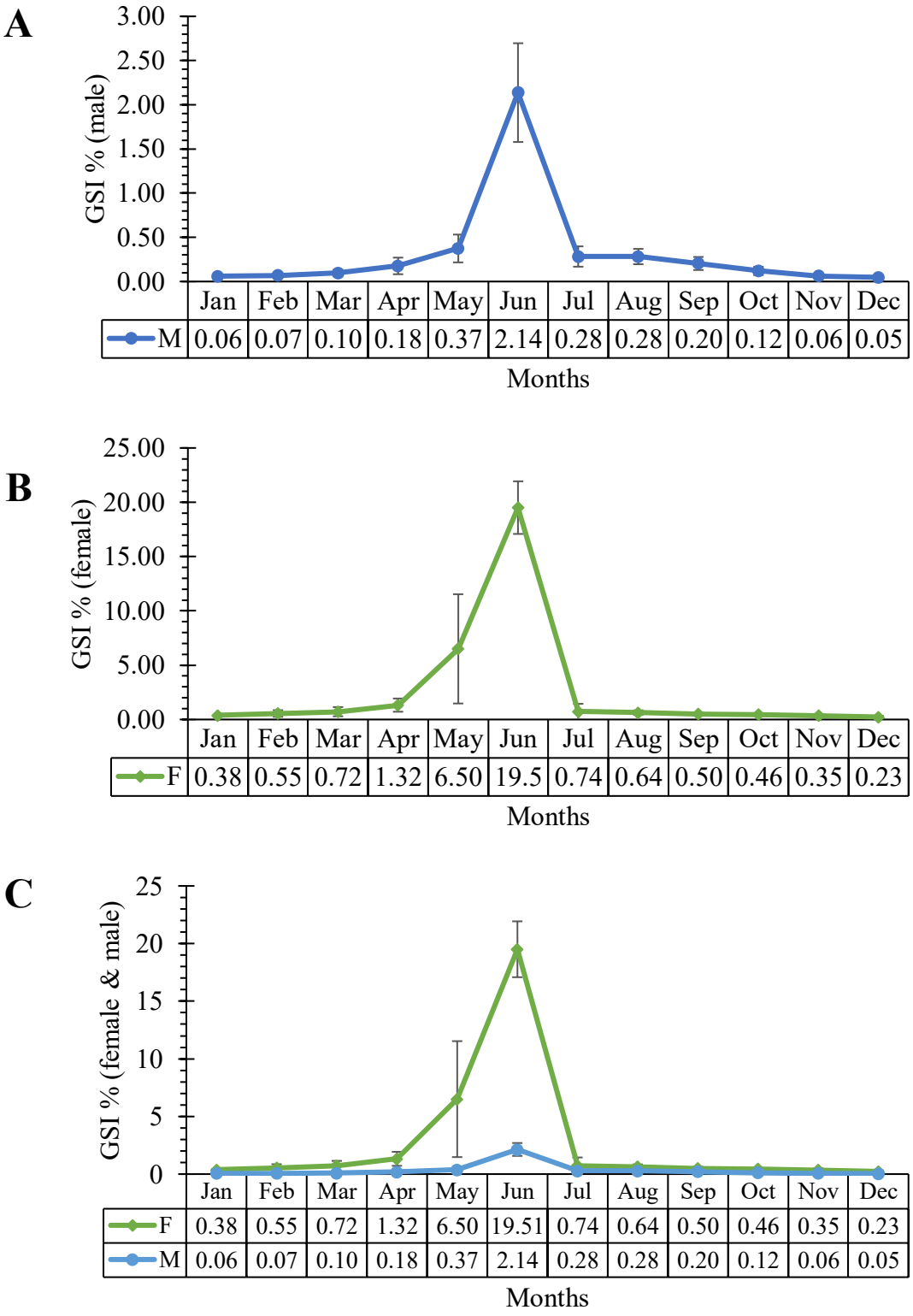



Figure 4: Gonado-somatic index (GSI) of *L. calbasu* collected monthly (November 2020 to October 2021) from the Kaptai lake, Bangladesh, for male (A), female (B), female-male both (C). Values are presented in "mean ± SD" format to represent data (n = 10−15 per month of both sexes). The X-axis represents months, and Y-axis represents GSI% values.

In order to investigate any potential relationship between GSI and HSI in both male and female *L. calbasu*, a correlation coefficient analysis was also carried out. Even so, there was no significant link between GSI and HSI for either male and female fish ($R^2$ = 0.0079 for males and $R^2$ = 0.0099 for females) (Figure 5), despite some opposite patterns in the peak and decline between GSI and HSI value throughout the year (Figure 6).

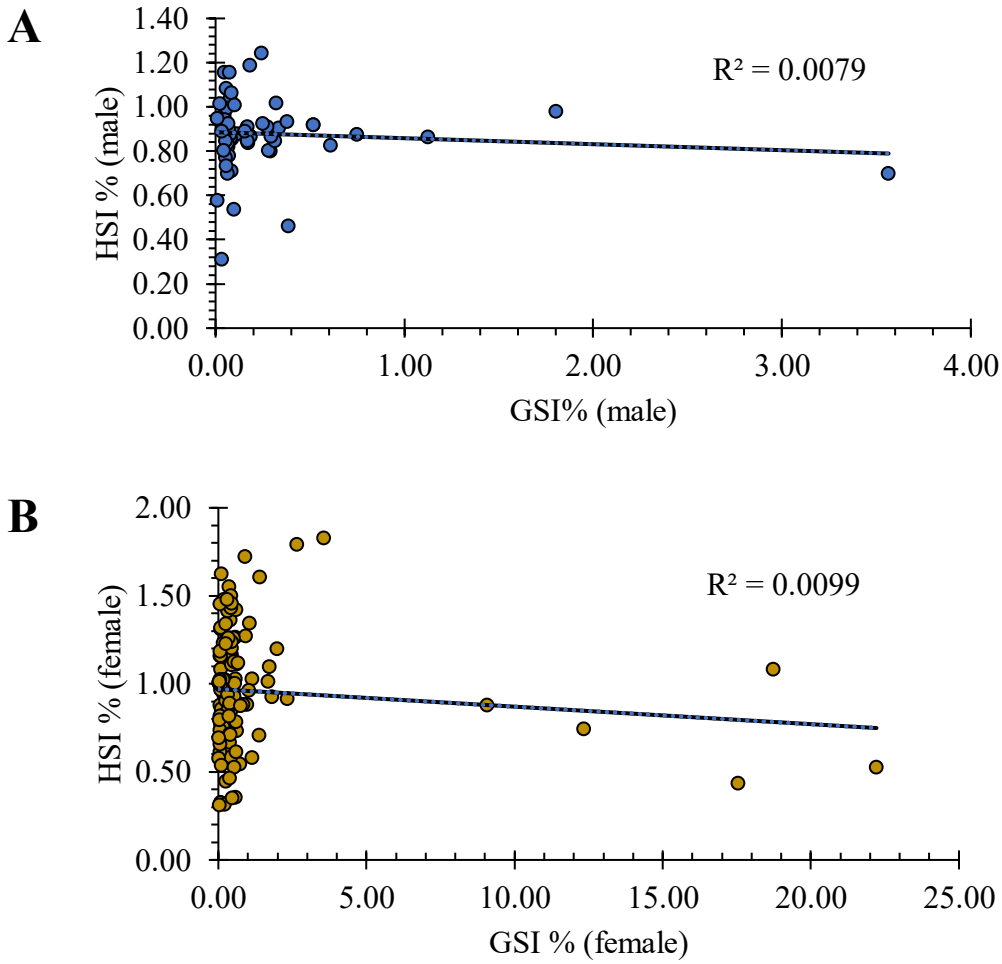



Figure 5: Linear relationship between GSI vs HSI data for males (A) and females (B) shows no significant link between GSI and HSI of *L. calbasu* collected monthly (November 2020 to October 2021) from the Kaptai lake, Bangladesh. The X-axis indicates the GSI% values, whereas Y-axis indicates the HSI% values; the $R^2$ value indicates the coefficient of determination.

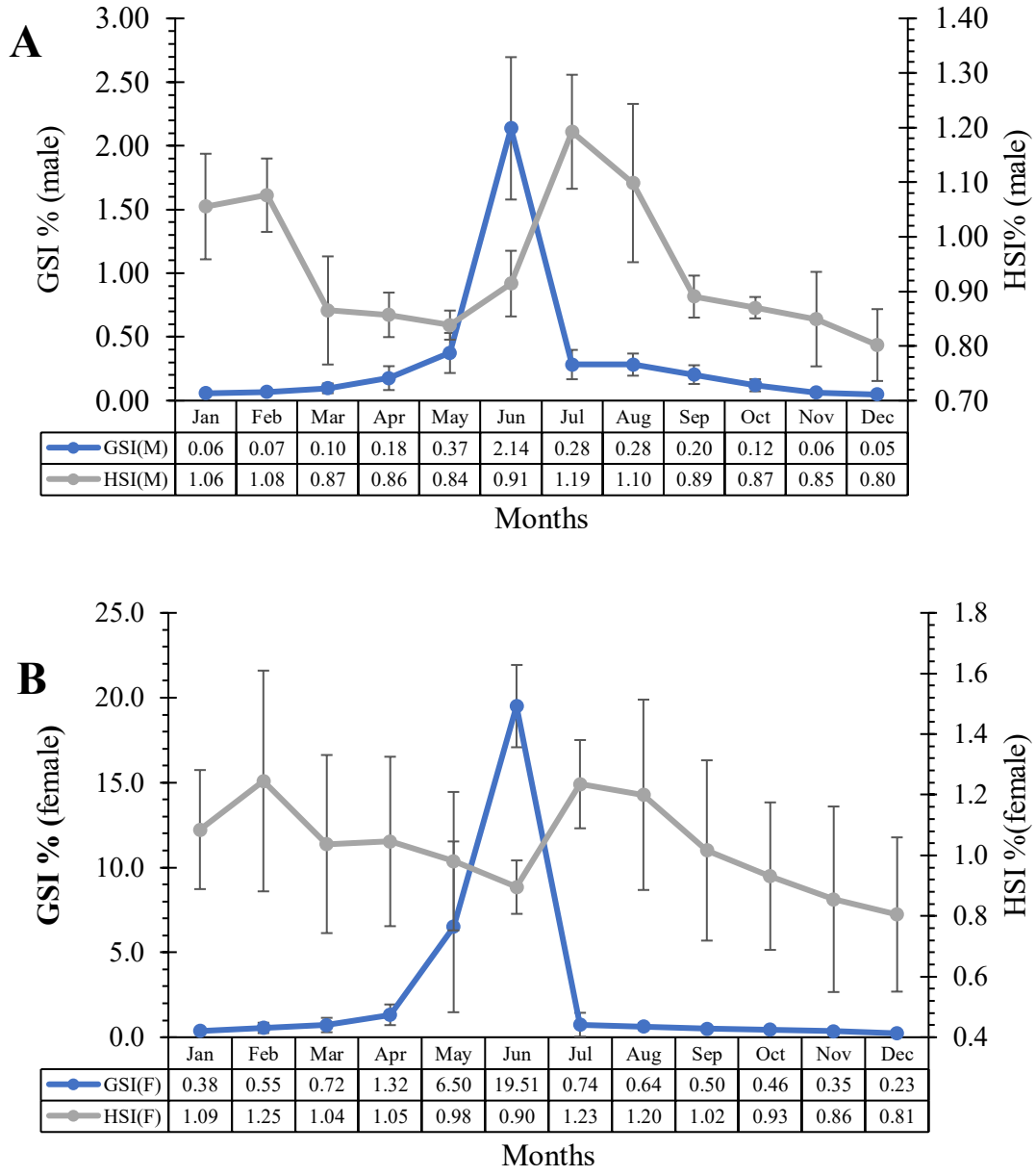



Figure 6: Comparison between gonado-somatic index (GSI) and hepato-somatic index (HSI) of *L. calbasu* for male (A) and female (B) collected monthly (November 2020 to October 2021) from the Kaptai lake, Bangladesh. Values are presented in 'mean ± SD' format. In this combined chart, X-axis = Months, Y-axis = HSI% (left), and second Y axis = GSI% (right). The blue color represents the GSI value, and the grey color represents the HSI value.

### 4.5. Oocyte diameter

This study also examined the oocyte diameter from the monthly collected female *L. calbasu*. Oocyte diameter reached its largest size of 1152.9 ± 115.2 µm in June, which

is the spawning season of *L. calbasu,* and there were no significant changes noticed in size from July to February (Figure 7A). Furthermore, oocyte diameter data show a similar increment pattern with the changes in GSI, suggesting that gonad development is timed to coincide with an increase in egg size (Figure 7B).

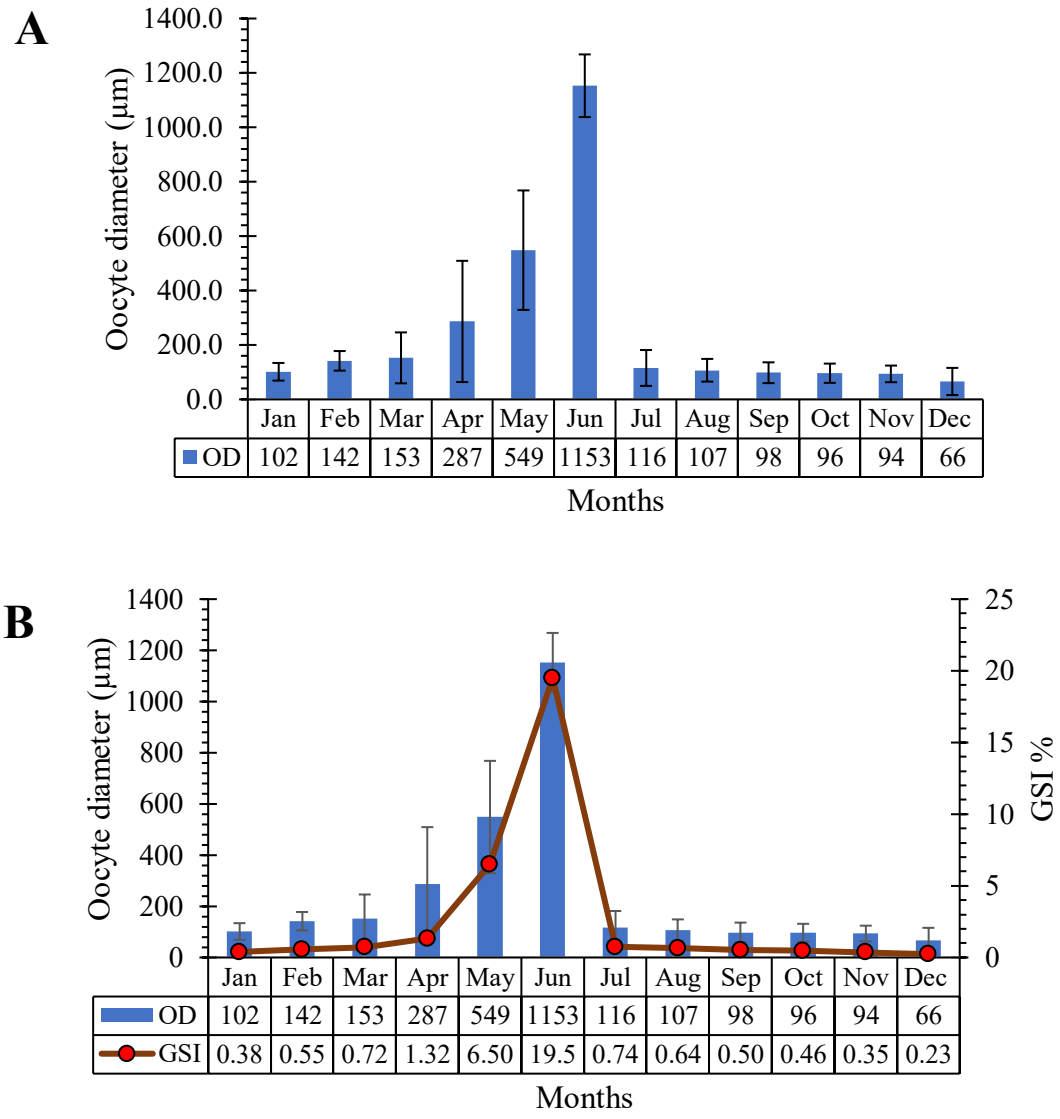
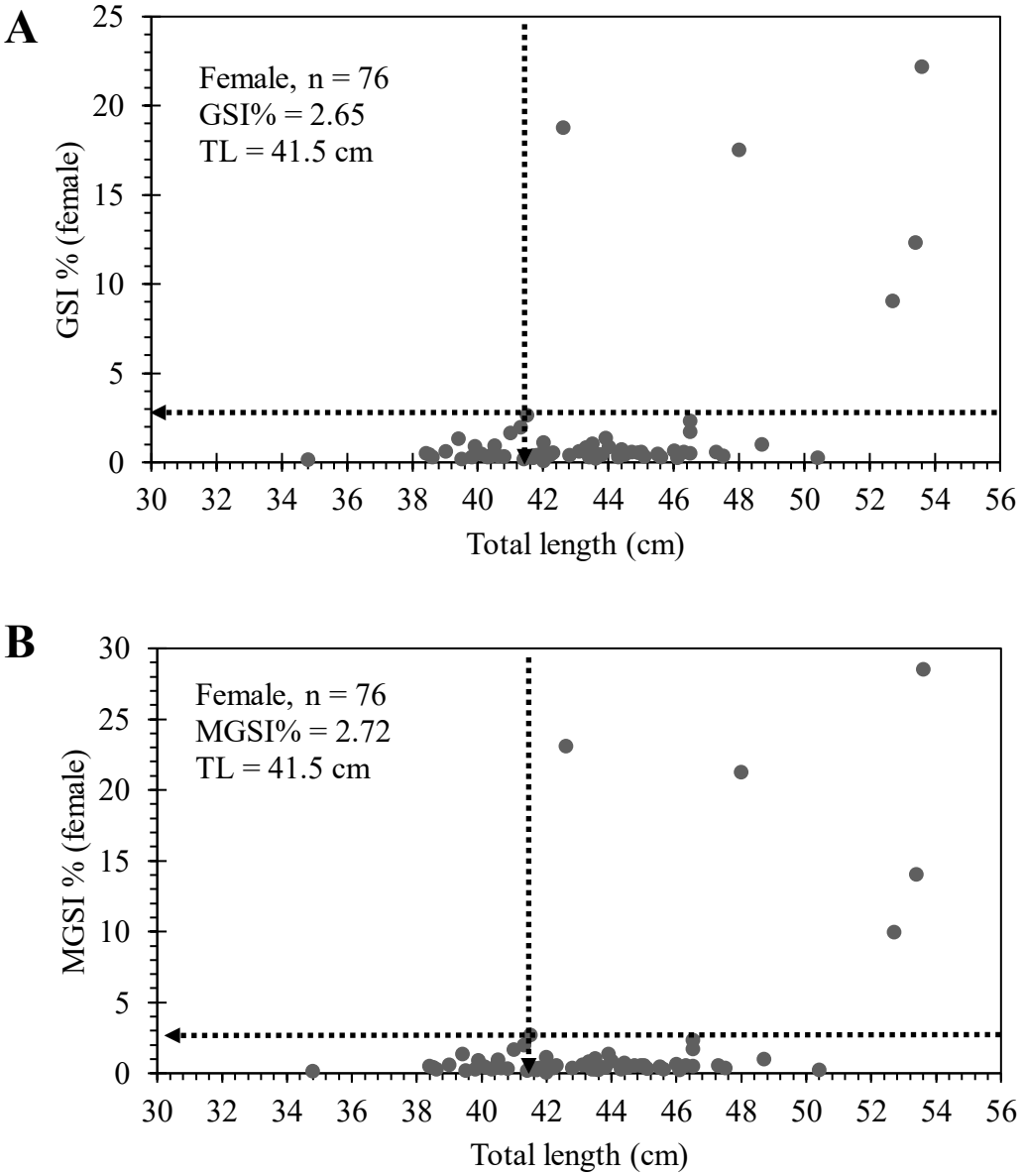



Figure 7: Oocyte diameter (µm) of female *L. calbasu* collected monthly (November 2020 to October 2021) from the Kaptai lake, Bangladesh (A), and comparison between oocyte diameter and GSI (B). X-axis = Months, Y-axis = Oocyte diameter (left), and second Y axis = GSI% (right). Values are presented in 'mean ± SD' format.

### 4.6. Length at first sexual maturity ($L_m$)

A measure of length at first sexual maturity was determined by identifying the point at which GSI suddenly increased. The relationship between total length (TL) to GSI,

MGSI and DI for female *L. calbasu* was analyzed and found that for females smaller than 41.5 cm in TL, the GSI was less than 2.65%, and MGSI was less than 2.72%, and the DI was less than 3.22 at the same length. In females, the GSI, MGSI, and DI all rose sharply just after 41.5 cm TL which was considered the $L_m$ for the females collected from the Kaptai lake (Figure 8).



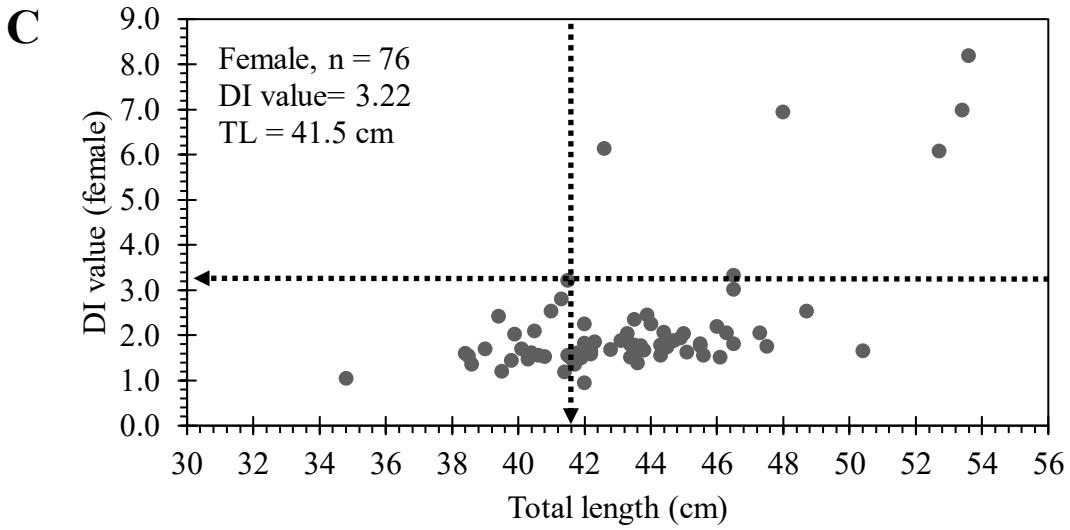
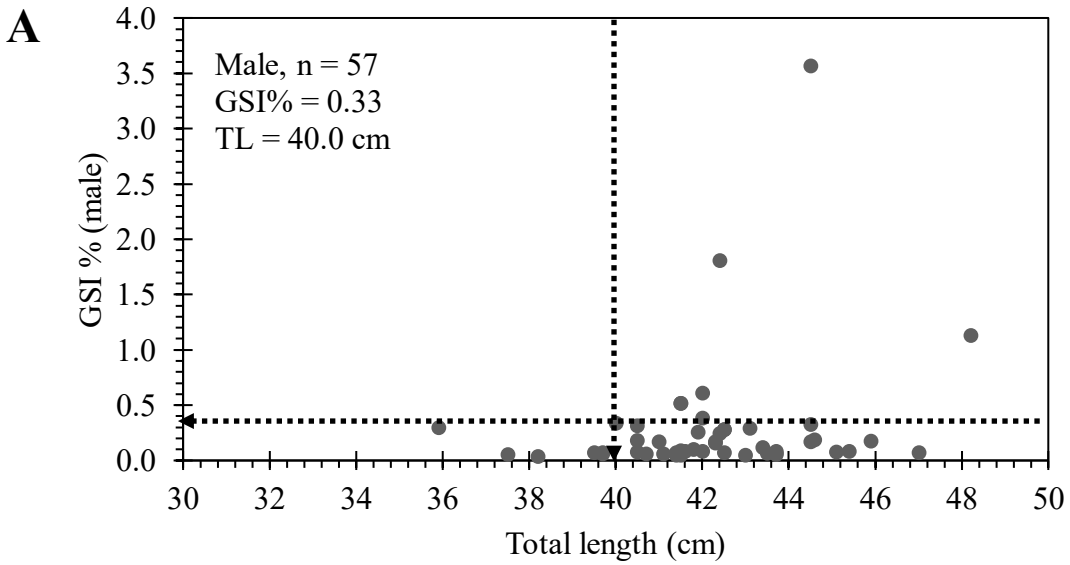



Figure 8: Length at first sexual maturity ($L_m$) from the relationship between gonado-somatic index (GSI%) and total-length (A), modified gonado-somatic index (MGSI%) with total-length (B), Dobriyal Index (DI%) with the total length (C) of female *L. calbasu* collected from the Kaptai lake, Bangladesh. The X-axis shows total length (cm), whereas the Y-axis shows GSI%, MGSI%, and DI values for figures A, B, and C, respectively.

In males, the relationship between TL to GSI, MGSI, and DI revealed that in males smaller than 40.0 cm, the GSI and MGSI and DI were as low as < 0.33%, < 0.34%, and < 1.49, respectively (Figure 9). Besides, in males, the GSI, MGSI, and DI all rose sharply after 40.0 cm TL and were considered the $L_m$ for male *L. calbasu*.



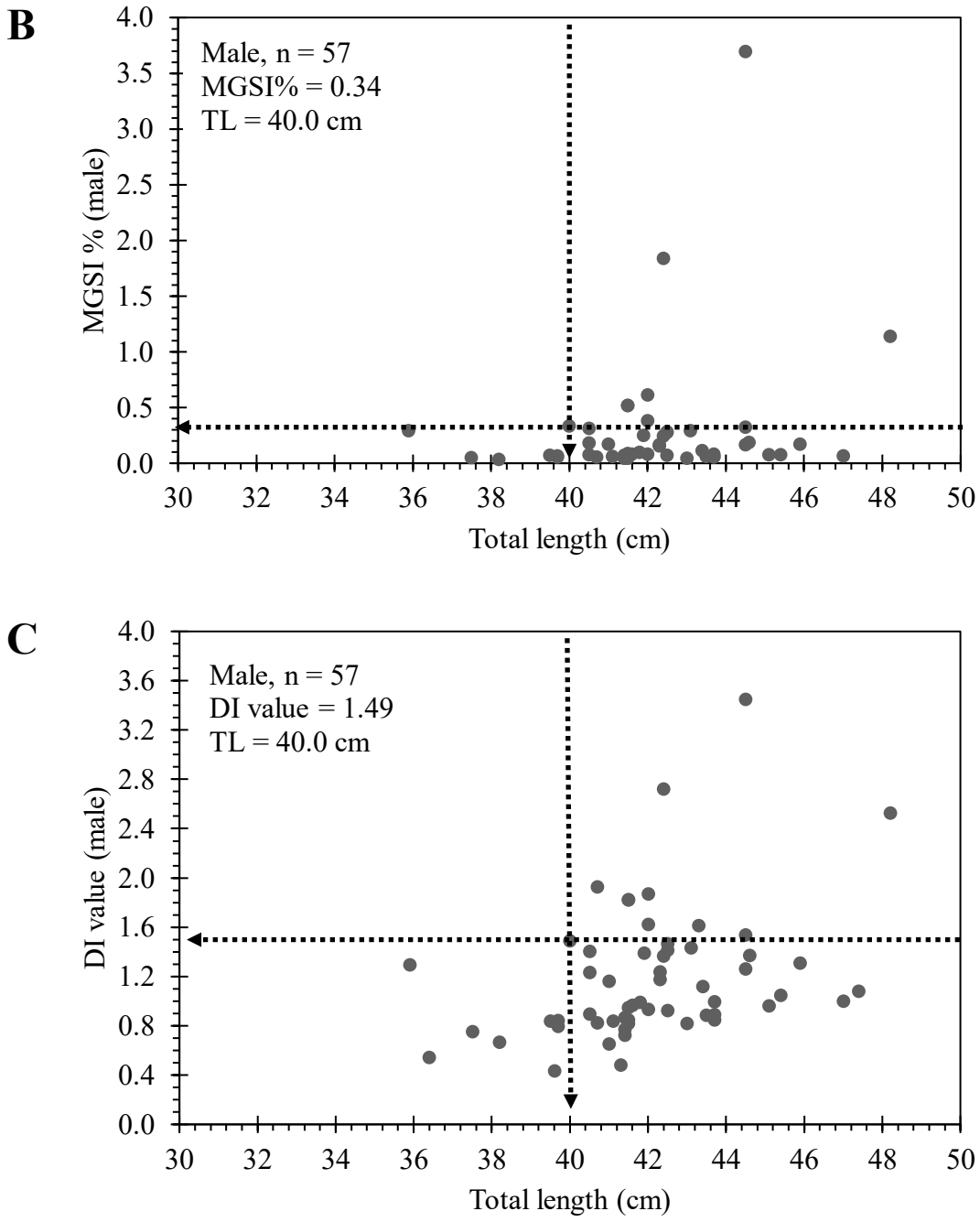



Figure 9: Length at first sexual maturity ($L_m$) from the relationship between gonado-somatic index (GSI%) and total-length (A), modified gonado-somatic index (MGSI%) with total-length (B), and Dobriyal Index (DI%) with the total length (C) of male *L. calbasu* collected from the Kaptai lake, Bangladesh. The X-axis shows total length (cm), whereas the Y-axis shows GSI%, MGSI%, and DI values for figures A, B, and C, respectively.

### 4.7. Fecundity

Randomly selected female *L. calbasu* at the gravid stage was used to calculate fecundity when the ovary of the fish occupies more than 40% of the body cavity, and eggs were observed with naked eyes. *L. calbasu* having a length greater than 41 cm in TL, 1700 gm in BW, and 40 gm in ovary weight (gm), was chosen to calculate the fecundity. As revealed by gravimetric methods, fecundity was observed to vary between 446,264−1,063,644 eggs/individual. Besides, the maximum fecundity of 1,063,644 eggs/individual was calculated from a fish of 2475.9 gm in BW, 550 gm in ovary weight, and 53.6 cm in TL, whereas the average fecundity was 828,198 eggs/per individual *L. calbasu* from the Kaptai lake.

### 4.8. Gonadal development periodicity in females

The gross stages and monthly ovarian development of *L. calbasu* were analyzed throughout the year-long investigation. For each histological section, ovarian maturation was analyzed in detail, and several vitellogenic phases were characterized.

Ovarian development in *L. calbasu* was found to have the following maturity stages as classified based on previous studies (Barbieri et al., 2006; Akhter et al., 2020; Islam et al., 2021).

#### 4.8.1. Pre-vitellogenic stage

Pre-vitellogenic stage of ovarian development, also known as the initial growth phase or the immature stage, was seen to occur between December (A) and January (B). The general appearance of the ovary was small and cylindrical-shaped. It was reddish to blackish transparent in recovering spent females and blackish in immature virgins. In ovarian histology, this stage is characterized by many oogonia (Oo), chromatin nucleolus (Cn), early perinucleolar oocyte (Epo), and some primary growth oocytes. The average oocyte diameter was between 65.9 to 101.6 µm, and the average GSI was 0.306% at this stage. A large, brilliant nucleus with peripheral nucleoli encircled by basophilic cytoplasm indicated that the nucleus had been heavily stained with hematoxylin. In addition, the oocyte's cytoplasm was faintly stained with eosin and considerably small in diameter (Image 8).

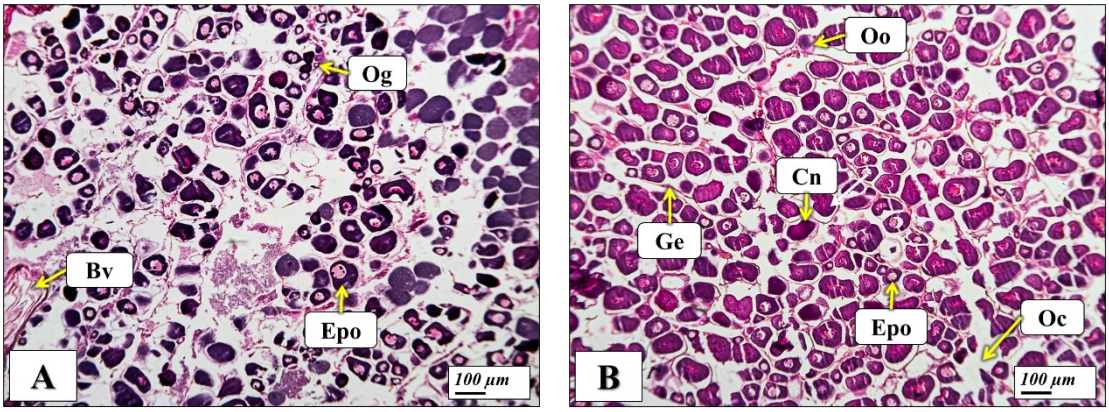



Image 8: *L. calbasu* ovarian development at the pre-vitellogenic stage (A and B). Cn- Chromatin nucleolar, Epo- Early perinucleolar oocyte, Oo- Oogonia, Oc- Ovarian cavity, Bv- Blood vessel, Ge- Germinal epithelium. The scale bar represents 100 µm in length.

### 4.8.2. Early primary vitellogenic stage

The early primary vitellogenic stage is also known as the oocyte's early maturing or secondary growth stage. The size of the ovary increases compared to the previous stage, turning from light yellow to light brown and occupying roughly one-fourth of the peritoneal cavity. At the beginning of the primary vitellogenic stage, a great development in oocyte diameter was seen, and the oocytes also became translucent and pinkish. At this stage, the average GSI and oocyte diameter was 0.552% and 141.8 µm, respectively. In ovarian histology, during this stage, the ovary is packed with early perinucleolar oocytes (Epo) and oocytes in the late perinucleolar stage (Lpo). There were still no blood capillaries visible at this stage. The early primary vitellogenic stage was primarily observed in February (C) (Image 9).

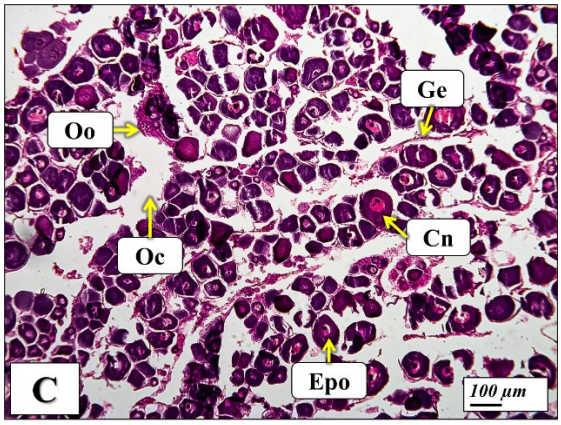



Image 9: *L. calbasu* ovarian development at the early primary vitellogenic stage (C). Cn- Chromatin nucleolar, Epo- Early perinucleolar oocyte, Oo- Oogonia, Oc- Ovarian cavity, Ge- Germinal epithelium. The scale bar represents 100 µm in length.

### 4.8.3. Advanced primary vitellogenic stage

The ovarian histology revealed in this stage that the quantity and size of yolk vesicles significantly increased over previous stages. In addition, the zona radiata emerges on the periphery at the end of this phase. Finally, some mature oocytes get to the primary globular stage. The oocyte appeared to be rounder and to have a smaller vacuole. The ova were visible to the human eye, and the yolk partially covered the nucleus (Image 10). The ovary was clearly in the growth phase in March (D) when the average oocyte diameter was 152.7 µm and the average GSI was 0.721%.

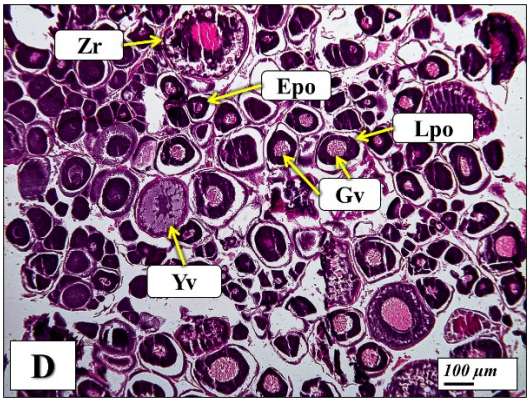



Image 10: *L. calbasu* ovarian development at the advanced primary vitellogenic stage (D). Epo- Early perinucleolar oocyte, Lpo- Late perinucleolar oocyte, Gv- Germinal vesicle, Yv- Yolk vesicle, Zr- Zona radiata. The scale bar represents 100 µm in length.

### 4.8.4. Secondary vitellogenic stage

In the histological examination of the ovary, it is found that Eosinophilic (bright pink) yolk globules build up in the inner cortex as the secondary vitellogenic stage advances. The secondary vitellogenic stage was predominantly observed in April (E) when the average oocyte diameter reached 286.5 µm, and the GSI value was 1.324%. The cytoplasm was coated with huge oil droplets and many yolk globules. The zona radiata now has a greater thickness (Image 11). The ova were round and yellowish or orange in appearance at this stage. In the general appearance of the ovary, some blood arteries have been visible on the ovary's surface. It was possible to count the number of ova using the necked eye.

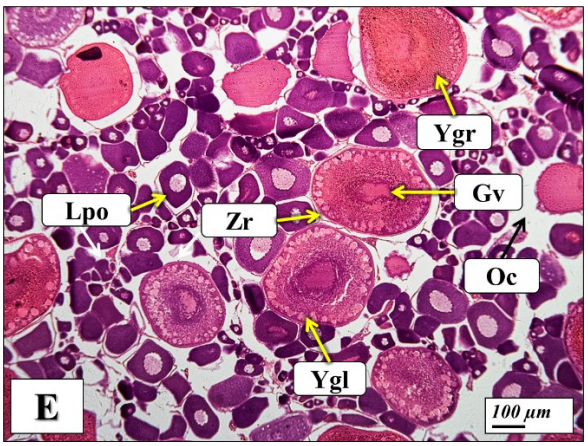



Image 11: *L. calbasu* ovarian development at the secondary vitellogenic stage (E). Lpo- Late perinucleolar oocyte, Gv- Germinal vesicle, Ygr- Yolk granule, Ygl- Yolk globule, Zr- Zona radiata, Oc- Ovarian cavity. The scale bar represents 100 µm in length.

### 4.8.5. Ripe stage

In this stage, most of the abdominal space was occupied by the greatly expanded ovary, and the ovary was greyish to bluish or turquoise in color. The eggs filled the entire space of the translucent ovary. A network of blood vessels encircles the ovary. The oocyte rapidly grew in size as it hydrated itself by taking in much water. In the histological examination of the ovary, many yolk granules were present in the oocyte follicle. Oil droplets combined into a single drop, and the nucleus relocated toward the animal's pole (Image 12). In the general appearance of the ovary, veins and arteries were visible clearly in the outer wall. It was common for large ova to emerge from the vent with only a little pressure applied to the abdomen. This developmental stage was

found to start in May (F) and found fully ripe in June (G) with the maximum oocyte diameter (1152.9 µm) and highest GSI (19.5%) values

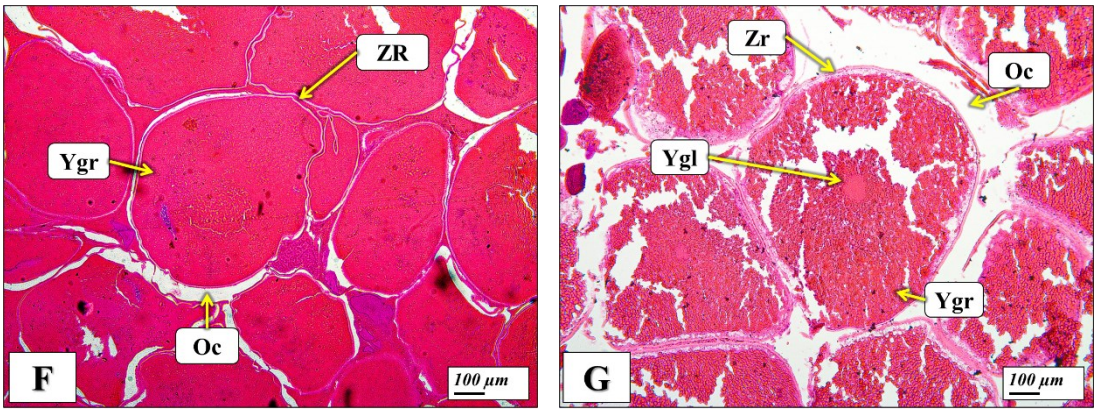



Image 12: *L. calbasu* ovarian development at the ripe stage (F and G). Ygr- Yolk granule, Ygl- Yolk globule, ZR- Zona radiata, Oc- Ovarian cavity. The scale bar represents 100 µm in length.

### 4.8.6. Spent or regressing stage

Due to the release of eggs, the ovary's mass greatly decreased, and the ovaries had completely regressed. The ovaries were reddish and limp, with a constricted, empty, sac-like shape; a few immature eggs and denatured ova were occasionally found. In a fully spent female, the ovarian histology revealed that the ovary contains several degenerating post-ovulatory follicles, some atretic oocytes, and some oogonia and perinucleolar stage oocytes; in a partially spent female, the ovary also contains oocytes in various vitellogenic stages (Image 13). This examination concluded that the spent or regressing stage occurred in July, August, September, October, and November (H, I, J, K and L).

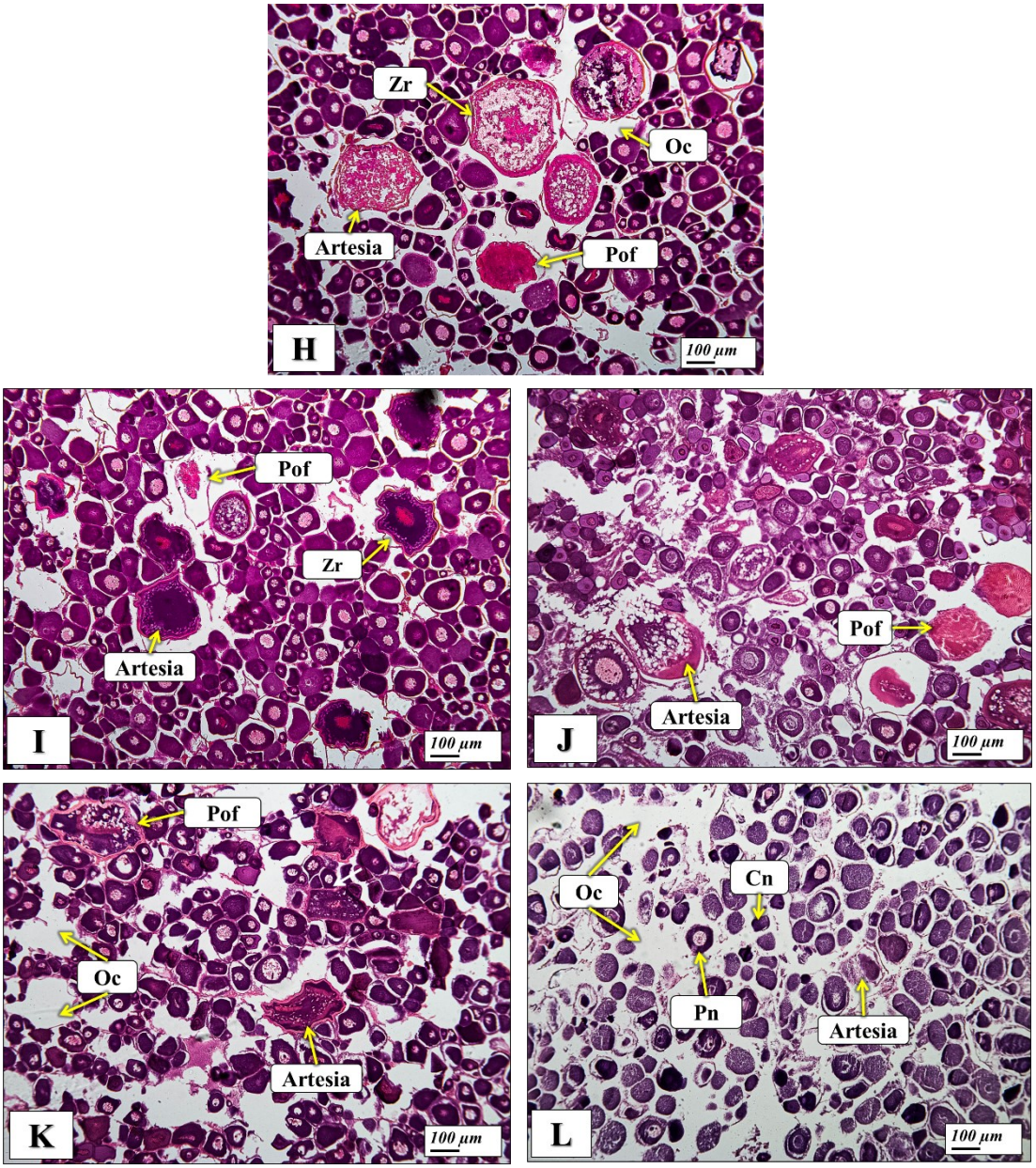



Image 13: *L. calbasu* ovarian development at the spent or regressing stage (H, I, J, K, and L). Pof- Post ovulatory follicle, Pn- Perinucleolar oocyte, Zr- Zona radiata, Oc- Ovarian cavity. The scale bar represents 100 µm in length.

### 4.9. Gonadal development periodicity in males

Based on histological results and the level of spermatogenesis, the annual testicular activity of *L. calbasu* was classified into the following stages based on the classification of previous studies (Brown-Peterson, 2006; Akhter et al., 2020; Islam et al., 2021).

### 4.9.1. Immature stage

The general appearance of testes was small, transparent, thread-like, and pale white at this stage. The mesorchium that held them to the dorsal wall beneath the air bladder constrained them to a tiny portion of the body cavity. The histological examination of the testes revealed in this stage that the continuous germinal epithelium throughout the testes and the seminal lobule of the testes had a large number of spermatogonia but only a small number of spermatocytes. Spermatogonia have a spherical shape and are stained with hematoxylin. In December (A) and January (B), the immature stage was spotted (Image 14).

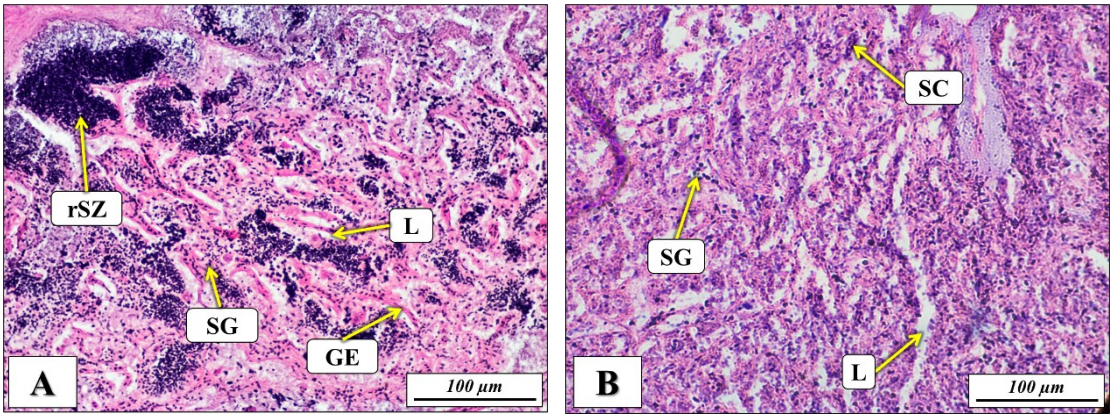



Image 14: Testicular maturation cycles for *L. calbasu* at the immature stage (A and B), GE- Germinal epithelium, L- Lumen of testes, SG -Spermatogonia, SC- Spermatocyte, rSZ- residual spermatozoa. The scale bar represents 100 µm in length.

### 4.9.2. Developing stage

The general appearance of testes was smaller in size, cylindrical in shape, and translucent white at this stage. The histological examination revealed the continuous germinal epithelium in the periphery of the testes, whereas the discontinuous germinal epithelium was in lobules near ducts. Spermatocyte development started. Additionally, secondary spermatogonia and primary and secondary spermatocytes were seen. Some spermatogonium degenerate, while others have big, weakly staining basophilic nucleoli. Fewer spermatozoa are present in the lumen. In February (C), this stage was primarily observed (Image 15).

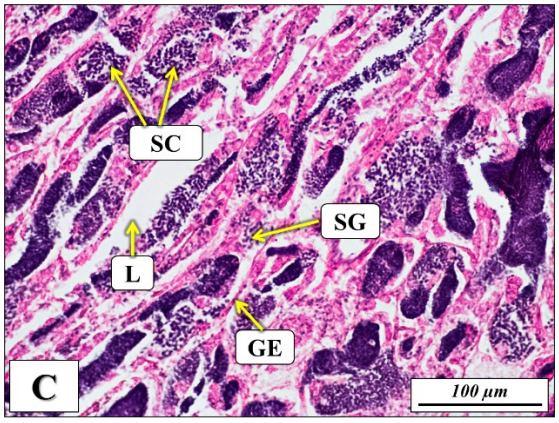



Image 15: Testicular maturation cycles for *L. calbasu* at the developing stage (C), GE- Germinal epithelium, L- Lumen of testes, SC- Spermatocyte, SG -Spermatogonia. The scale bar represents 100 µm in length.

### 4.9.3. Pre-spawning stage

In the histological examination of testes, all phases of spermatogenesis, including spermatogonia, spermatocytes, and spermatids, were typically observed. However, more spermatozoa were found in the lumens of the sperm ducts of testes in late-stage development. In addition, numerous spermatocyte-containing cysts were observed throughout the seminal lobule. The testicles then appeared creamy white in general appearance. This stage was identified in March (D) (Image 16).

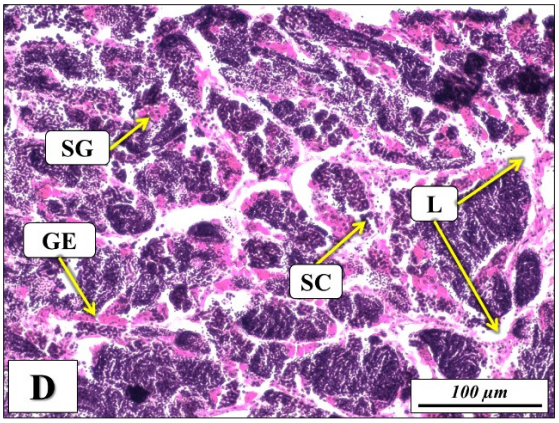



Image 16: Testicular maturation cycles for *L. calbasu* at the pre-spawning stage (D), GE- Germinal epithelium, L- Lumen of testes, SC- Spermatocyte, SG -Spermatogonia. The scale bar represents 100 µm in length.

### 4.9.4. Ripe stage

In April (E) and May (F), the testes were found to be mature and ready for spawning. Because no sperm were discharged from the vent in response to gentle pressure on the

abdomen, this stage was distinguished from the spawning stage. The growth of spermatids and spermatozoa in the seminal lobule lumen and sperm ducts was evident. In addition, the discontinuous germinal epithelium was throughout the testes (Image 17).

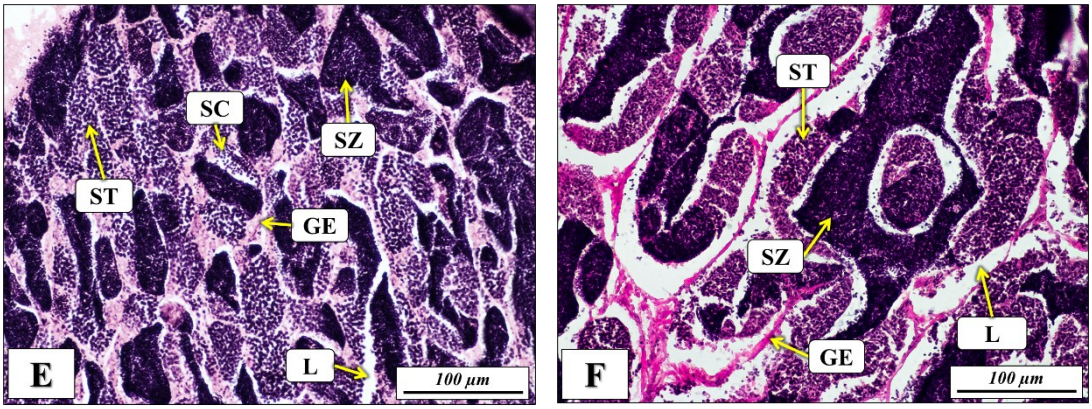



Image 17: Testicular maturation cycles for *L. calbasu* at the ripe stage (E), GE-Germinal epithelium, L- Lumen of testes, SC- Spermatocyte, SG -Spermatogonia, ST-Spermatid, SZ- Spermatozoa. The scale bar represents 100 µm in length.

### 4.9.5. Spawning stage

The study identified spawning males in June (G) and July (H). During this phase, testicles were more opaque, whitish, and large than in previous stages. The sperm was expelled by applying mild pressure to the abdomen in June, while no sperm came out in July. Mature spermatozoa were prevalent in the periphery testes, middle of the sperm ducts, and lumens (Image 18).

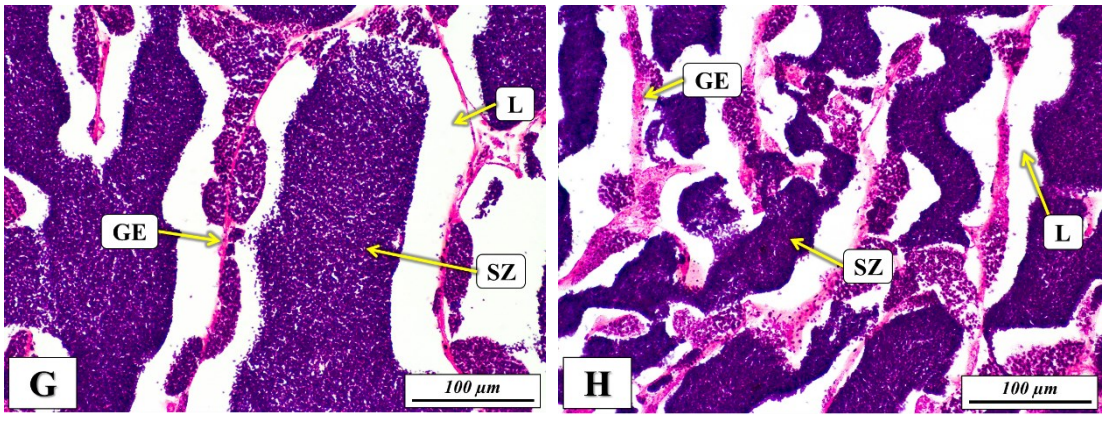



Image 18: Testicular maturation cycles for *L. calbasu* at spawning stage (F and G), GE-Germinal epithelium, L- Lumen of testes, ST- Spermatid, SZ- Spermatozoa. The scale bar represents 100 µm in length.

### 4.9.6. Post-spawning stage

Post spawning stage is divided into two phases, the spent stage and the regressed stage. The GSI value was drastically reduced and found to be minimal in this reproductive cycle. Some spermatozoa are present in partially shrunken testis, but empty spaces characterize fully shrunken testis. Partially spent males were found in July, and fully spent male was found in August (I), whereas regressed males were found in September (J), October (K), and November (L). Histological examination of the testes identified the spent testes with discontinuous GE throughout testes, spermatocytes widely scattered, containing only secondary SC, ST, or SZ. Primary SG appears in the periphery (Image 19). On the other hand, the histology of regressed testes was characterized by continuous GE of only primary SG and the presence of some residual SZ in the lumen.

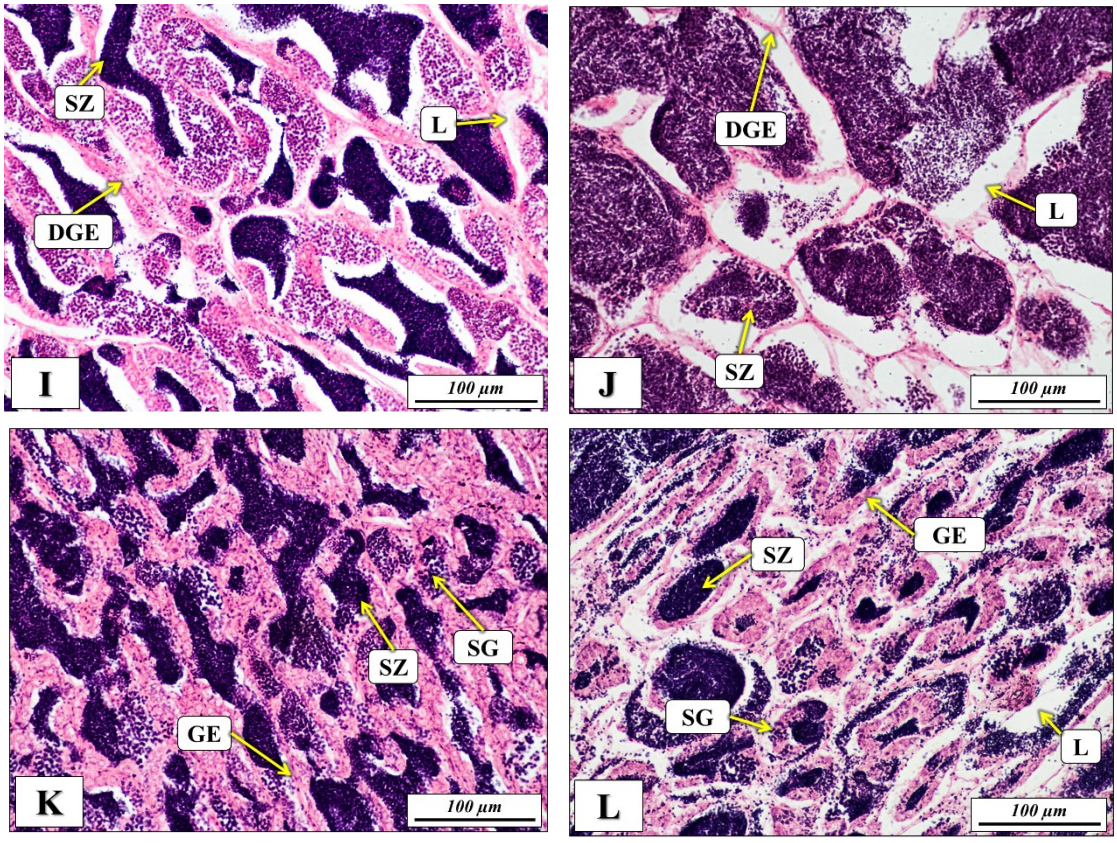



Image 19: Testicular maturation cycles for *L. calbasu* at the post-spawning stage (H, I, J, and K), GE- Germinal epithelium, DGE- degenerated germinal epithelium, L- Lumen of testes, SG -Spermatogonia, ST- Spermatid, SZ- Spermatozoa, TC- Testicular cavity. The scale bar represents 100 µm in length.

# CHAPTER 05

# DISCUSSION

In the present investigation, observed biological indices and the histological examination of male and female gonads demonstrate a clear spawning peak during May−June, considered the peak breeding period of *L. calbasu* in the Kaptai lake. The present study's findings will help to plan a conservation strategy and broodstock improvement plan for this high-valued fish species of Bangladesh.

## 5.1. Length-weight relationship and growth pattern

In this study, the length and weight of the collected *L. calbasu* ranged from 34.8 − 53.6 711.8 − 2762.8 g, respectively. The calculated 'b' value of the *L. calbasu* from the combined male and female data was 2.9035, while it was 3.0077 for females and 2.4481 for males.

The "b" value for pooled data was a little less than 3.0, indicating that *L. calbasu* was collected from the Kaptai lake at 34.8 − 53.6 cm in length and 711.8 − 2762.8 gm in weight, showing negative allometric growth pattern. The growth pattern for males was negatively allometric (b = 2.4481), while, for females, the growth pattern was isometric (b = 3.0077). In the earlier studies, Vahneichong et al. (2018) reported the isometric growth pattern (b = 3.031 and 3.181) for two different size classes from India. Basak and Hadiuzzaman (2019) reported the isometric growth pattern of *L. calbasu* from the Tabalchari area of the Kaptai lake (b = 2.97). Choudhary et al. (1991) reported an allometric (negative) growth pattern for the length classes of 41–50 cm and 51–60 cm, where they found the 'b' values 2.7098 and 2.4164, respectively. On the other hand, Ahmed and Saha (1996) found a positive allometric growth pattern (b = 3.132) for this species from India. The differences in the "b" value might be due to the variation in the sample size, age or size group of the sample, geographical differences, and seasonal variation.

## 5.2. Condition factor (K) and relative condition factor (Kn)

In this study, the average value of the 'K' and 'Kn' was 1.453 and 1.014, indicating that the fish were in good health, especially in January, February, April, May, June, and August, as shown by relative condition values that were equal and above the mean value during these months. The observed maximum value of Kn in these months could be

attributed to the abundance of foods, intense feeding, and favorable environmental factors. On the other hand, the physiological, environmental, dietary, and biological cycles may play a role in the lowest Kn values in December due to low metabolic activity during winter. Male and female condition factors (K) have a similar trend throughout the year, peaking in May (1.631 ± 0.142), indicating that the fish were in good condition before spawning, which is further explained by monsoon floods and temperate weather in May – July in the study area. The relative condition factor (Kn) in male and female *L. calbasu* followed the same pattern as the condition factor (K). In the earlier studies, Basak and Hadiuzzaman (2019) documented the mean condition factor and the mean relative condition factor of 1.396 and 1.02, respectively, of the *L. calbasu* at the Kaptai lake, Bangladesh. In contrast, Choudhary et al. (1991) reported the K and Kn values 1.15 −1.25 and 0.995 – 1.029, respectively, from India, which is similar to the present study's findings.

### 5.3. Hepato-somatic index (HSI)

The HSI value offers insightful data regarding energy reserves, liver and body health, and the effects of water pollution on fish. Fish with low water quality have smaller livers and less energy stored in the liver. In addition, there is a connection between vitellogenesis and liver mass, as egg yolk and lipid droplets synthesize in the liver and transfer to the oocyte during gonadal maturation. In this study, the maximum mean HSI value was found in February for both females (1.25 ± 0.29) and males (1.08 ± 0.20), indicating the time when *L. calbasu* stores the most food before the onset of reproduction. The highest HSI value in February may be due to the favorable environmental conditions and food availability, and fish were willing to eat more after winter for their approaching reproductive activity.

The minimum HSI value was found in June in both females (0.90 ± 0.09) and males (0.91 ± 0.06), indicating that stored food has been used for the reproductive activity, which is an obvious sign of poor environment and lack of food supply in contrast to bodily demand due to heavy rain and turbidity. The HSI value decreases and reaches its lowest in December. It is because fish do not consume energetically while in the resting phase and have the lowest metabolic activity in December and January, owing to the winter season; thus, they use the food stored in their livers. The findings of the HSI in *L. calbasu* will further help to compare the energy reserves for other stocks.

### 5.4. Length at first sexual maturity ($L_m$)

In this study, multiple functions were used to estimate the first sexual maturity length ($L_m$) and found that when the male *L. calbasu* were at 40.0 cm in TL and the females were at 41.5 cm in TL reached the first sexual maturity in the Kaptai lake. Natarajan (1971) and Rao and Rao (1972) analyzed the $L_m$ as 40 cm for males and 45 cm for females from Bhabanisagar reservoir and Godavari River, respectively. Pathak and Jhingran (1977) later analyzed the $L_m$ of *L. calbasu* from the Loni reservoir as 40 cm for males and 33.6 cm for females. Vinci and Sugunan (1981) have reported the $L_m$ of *L. calbasu* 30.6 cm for males and 37.1 cm for females at the Nagarjunasagar reservoir in Telangana, India. The differences in the $L_m$ might be due to variations in the population, geographical region, and the condition of fish.

### 5.5. Oocyte diameter

The current study found that oocyte diameter peaked in June (1152.9 ± 115.2 μm) during their peak spawning phase, and the lowest was in July–December when new eggs start to form for the upcoming spawning season. Comparing oocyte diameter and GSI reveals that the oocyte develops as GSI rises, starting in February, ripens gradually in April, and reaches its peak in June. In the only study on oocyte diameter, Kabir and Quddus (2013) reported that the highest oocyte diameter was 1300 ± 40μm, of a gravid *L. calbasu* in July at a hatchery at Faridpur, Bangladesh, which is consistent with the results of our current analysis.

### 5.6. Fecundity

When managing fish stocks, a species' fecundity is an essential factor to consider. Fecundity is also one of the essential biological characteristics of fish when determining a stock's reproductive capacity (Islam et al., 2008; Begum et al., 2010). In this study, fecundity was found to vary from 446,264 − 1,063,644 eggs/individual with a mean fecundity of 8,28,198 eggs/individual in *L. calbasu* from the Kaptai lake. Previous studies reported the fecundity of the *L. calbasu* was 739,440 eggs/individual, according to Khan (1934). In addition, Sukumaran (1969), Natarajan (1971), Rao and Rao (1972), Pathak and Jhingran (1977), Vinci and Sugunan (1981), Mishra and Saksena (2012) and Kabir and Quddus (2013) and others have identified fecundity ranges of 288,000 – 438,000; 109,700 – 980,700; 40,200 – 517,500; 93,972 – 466,400; 67,500 – 572,460; 312,100 – 657,600; 37,454 – 427,030 oocytes per fish, respectively. Kabir and Quddus

(2013) reported that the fecundity of *L. calbasu* varied from 37,454 to 427,030 eggs/individual, with 230,242 eggs/individual serving as the mean value from Bangladesh. The variation in the fecundity of *L. calbasu* might be due to differences in the size, age of fish, nutritional status, habitat status, and condition of fish (Duponchelle et al., 2000).

### 5.7. Gonado-somatic index, gonadal histology, and breeding periodicity

Determining the spawning period is critical to understanding the exact spawning time (Wilding et al., 2000). The gonado-somatic index (GSI) measures a fish's gonadal maturity and, as a result, its spawning season. The mean GSI value of female *L. calbasu* increased significantly from April onwards and peaked in June (19.507 ± 2.424) (Figure 4). The GSI value dropped dramatically in July and remained low in the subsequent months. In males, the mean GSI value ranged from 0.047 ± 0.010 to 2.137 ± 0.559 and became highest in June (2.137 ± 0.559), then the GSI value drastically decreased in July in males and thereafter months (Figure 4). This pattern indicates that June is the highest spawning month for *L. calbasu* in the Kaptai lake.

Histological examination is the most reliable method for analyzing gonadal maturity (West, 1990). Histological observation of female gonads revealed that from July to October, several post-ovulatory follicles and some degenerated follicles called atresia were found. In November, microscopic observations of histological slides showed that the ovary had a lot of empty space. In November, a small number of oogonia also began to grow. However, Artesia and post-ovulatory follicles in the ovaries during July, August, September, and October mean the peak ripe stage has been gone through during May and June. In June, a greater amount of yolk granules was seen by histological examination of female gonads.

In males, many mature spermatids were found in April, and some spermatozoa began to be seen. However, its peak GSI was in June, and many spermatozoa were seen in May, June, and July, which confirmed the maturity in males and indicated that males mature first than females and remain mature for a prolonged period. Empty testes with residual spermatozoa were observed in August, September, October, and November that indicating the spent stage. In December and January, male *L. calbasu* spermatogonia cells or spermatocytes and primary spermatocytes were seen, indicating immature testes. The study confirmed the relationship in gonadal maturity in males and

females in a synchronized manner in this species, indicating a single spawning period from April to June. Histological examination is the most reliable method for analyzing gonadal maturity (West, 1990). Based on the GSI values and the histological examination of the fish gonads, it may conclude that *L. calbasu* from the Kaptai lake spawns in June.

Kabir and Quddus (2013) determined the GSI value for males ranging from 0.12 ± 0.06 to 1.68 ± 0.11 and the GSI value for females ranging from 0.37 ± 0.06 to 18.58 ± 0.06 with the peak spawning month as July. Gupta and Banerjee (2015) reviewed some previous studies and documented the breeding periodicity of the *L. calbasu* and concluded that the breeding months of *L. calbasu* used to vary with the variation of the monsoon floods at different locations of the world. The breeding season used to vary depending on the location, and it used to coincide with the monsoon floods.

# CHAPTER 06

# CONCLUSIONS

Fish reproductive biological research is crucial for sustainable fishing and aquaculture, the assessment of fisheries stocks, food and nutrition security, livelihoods, economic growth, and environmental preservation. Furthermore, sustainable fisheries and aquaculture management can help protect natural fisheries resources for future generations. Managing open water fisheries requires a thorough understanding of fish population reproductive development, as fish species' reproductive characteristics define their inherent capacity and sustainability of exploitation. Establishing fishing regulations requires a comprehensive understanding of the life cycles and population dynamics of economically significant fish in Kaptai lake. The length-weight relationship, condition factor, relative condition factor, gonado somatic index, oocyte diameter, fecundity, hepato somatic index, length at first maturity, and histological examination of gonads indicate that the breeding season of *L. calbasu* in Kaptai lake begins in April and ends in June, which partially overlaps the current ban period (1$^{st}$ May to 31$^{st}$ July) in that area. Therefore, the ban period for this fish species should begin in April because that is when they begin to be ready for spawning. Therefore, it is crucial to provide *L. calbasu* in the Kaptai lake with enough time to reproduce effectively and to use the correct strategy for the growth of this population.

# CHAPTER 07

# RECOMMENDATIONS AND FUTURE PERSPECTIVES

This study aimed to describe the spawning season and life history characteristics of the *L. calbasu* in the Kaptai lake. This research will aid the fisheries management authority in reorganizing the fishing ban time for *L. calbasu* during its mating season to preserve a viable stock. In addition, this research will also aid in estimating the overall state of the *L. calbasu* in the reservoir. Although a qualitative approach was used to investigate the research's goal, the study has several limitations that the following recommendations can mitigate.

- Because completely randomized samples lead to a definite conclusion for biological research, samples should be gathered from random sources from all over the study area.
- Fresh, well-preserved materials yield a more accurate histological diagram. As a result, samples should be taken directly from the fisherman as soon as possible following the catch.
- During the fishing ban period, sampling activity may be hampered; in that case, there should be issued a certificate from the appropriate authority for fishing during the ban period for research purposes only.
- Mature fishes should not be caught; otherwise, the natural population of this fish species will be lost forever.

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# APPENDICES

Appendix 1: Mean GSI% value of male and female of *L. calbasu* of the Kaptai lake collected monthly (November 2020 to October 2021). Values are presented in the "mean ± SD"

| Months | Mean GSI% value of male | Mean GSI% value of female |
|---|---|---|
| January | 0.06 ± 0.02 | 0.38 ± 0.06 |
| February | 0.07 ± 0.01 | 0.55 ± 0.30 |
| March | 0.10 ± 0.04 | 0.72 ± 0.42 |
| April | 0.18 ± 0.09 | 1.32 ± 0.61 |
| May | 0.37 ± 0.16 | 6.50 ± 5.03 |
| June | 2.14 ± 0.56 | 19.51 ± 2.42 |
| July | 0.28 ± 0.12 | 0.74 ± 0.70 |
| August | 0.28 ± 0.09 | 0.64 ± 0.20 |
| September | 0.20 ± 0.07 | 0.50 ± 0.14 |
| October | 0.12 ± 0.05 | 0.46 ± 0.07 |
| November | 0.06 ± 0.01 | 0.35 ± 0.15 |
| December | 0.05 ± 0.01 | 0.23 ± 0.10 |

Appendix 2: Hepatosomatic index data for male and female *L. calbasu* collected monthly (November 2020 to October 2021) from the Kaptai lake, Bangladesh. Values are presented in 'mean ± SD'

| **Months** | | **HSI% (Female)** | **HSI% (Male)** |
|---|---|---|---|
| January | | 1.09 ± 0.20 | 1.06 ± 0.10 |
| February | | 1.25 ± 0.36 | 1.08 ± 0.07 |
| March | | 1.04 ± 0.29 | 0.87 ± 0.10 |
| April | | 1.05 ± 0.28 | 0.86 ± 0.04 |
| May | | 0.98 ± 0.23 | 0.84 ± 0.03 |
| June | | 0.90 ± 0.09 | 0.91 ± 0.06 |
| July | | 1.23 ± 0.15 | 1.19 ± 0.10 |
| August | | 1.20 ± 0.31 | 1.10 ± 0.14 |
| September | | 1.02 ± 0.30 | 0.89 ± 0.04 |
| October | | 0.93 ± 0.24 | 0.87 ± 0.02 |
| November | | 0.86 ± 0.15 | 0.85 ± 0.09 |
| December | | 0.81 ± 0.10 | 0.80 ± 0.07 |
| **Average** | | 1.01 ± 0.14 | 0.92 ± 0.11 |
| **Range** | Lowest | 0.81 ± 0.10 (Dec) | 0.80 ± 0.07 (Dec) |
| | Highest | 1.25 ± 0.36 (Feb) | 1.19 ± 0.10 (Jul) |

## A brief biography of the author

Muhammad completed B.Sc. in Fisheries (Hon's) from the Faculty of Fisheries of Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh, with a CGPA of 3.76 out of 4.00. His scientific interests include histology, fish breeding season identification, breeding technology development, fish molecular biology, and genetic engineering. He is passionate about fisheries research. He also knows how to use SPSS and MS Excel and has a great understanding of statistical analysis. Now, he is a candidate for the degree of MS in Fish Biology and Biotechnology under the Department of Fish Biology and Biotechnology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU).