Reproductive Biology of Ayre (*Mystus aor*) from the Kaptai Lake of Bangladesh: A Study to Brood Stock Improvement

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Roll No.: 0120/01

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Fish Biology and Biotechnology

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JUNE 2022

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Shifat Ara Noor

June, 2022

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

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LIST OF ABBREVIATIONS

DoF	Department of Fisheries
FAO	Food and Agriculture Organization
g	Gram
cm	Centimeter
µm	Micrometer
MT	Metric Ton
%	Percent
sq	Square
km	Kilometer
M	Meter
°C	Degree Celsius
HSI	Hepato-somatic index
GSI	Gonado-somatic index
S. D.	Standard deviation

ABSTRACT

Life-history information is necessary for effective conservation, management, aquaculture development, and brood stock management of fishing populations, especially for fish with significant monetary exploitation. To characterize the life-history traits of long-whiskered catfish, *Mystus aor* from the Kaptai lake, Bangladesh, this research was conducted. Analysis of morphometric parameters revealed the exponent 'b' value of the length-weight relationship of *M. aor* to be 2.65 (negatively allometric) for the pooled data whereas 3.29 (positively allometric) for male and 2.4491 (negatively allometric) for female respectively. The monthly K values obtained ranged from 1.15 – 1.41 and the Kn value ranged from 0.14 – 0.25 and K was maximum during May (1.41 ± 0.57) and minimum during November (1.15 ± 0.05) whereas Kn value was maximum in July (1.07 ± 0.11) and minimum in the month of November (0.86 ± 0.16). Hepato-somatic index (HSI) values were highest at May (1.03 ± 0.26) and minimum in November (0.6 ± 0.12). Estimated relative fecundity ranged from 18474 − 55504 eggs/per fish. Oocyte diameter was found to be highest in May (0.83µm). Based on the gonado-somatic index, modified gonado-somatic index and Dobriyal Index, the length at first maturity (L_m) for *M. aor* female was 54.1cm and male was 49.5cm. Gonado-somatic index (GSI) values were highest at May for both male and female. Histological analysis also revealed the presence of yolk granules and a number of mature spermatids in April and May, respectively. Based on the findings of the study, it was concluded that April−May is the peak breeding season of *M. aor*. The findings of the current study will serve a baseline for the conservation management and brood stock development in the Kaptai lake of Bangladesh.

Key Words: Condition factor, Relative condition factor, Fecundity, HSI, GSI, Histology of gonad, *M. aor*

CHAPTER: 1

INTRODUCTION

Fisheries management and conservation attempts to ensure long-term stock maintenance of a fish population in an environmentally sustainable manner while also delivering the maximum benefits to fishermen and communities (King, 2003). Scientists and ecologists who study fish conservation may work closely with a species of fish to develop regulations that safeguard the species' future, or they may try to preserve and improve the habitat where fish live. Currently, fish populations are under threat due to overfishing of commercially valuable species, the use of aggressive fishing techniques, and increasing stipulation from eatery and local communities. Fish populations are declining and at risk of extinction due to regional and global environmental problems when combined with overfishing (Jones and Miranda, 1997). To overcome such problems, fisheries stock conservation and management policies need a thorough understanding of a species' basic life cycle patterns, habitat, and exploitation status. Therefore, knowing the fish and fisheries resources including the life-history characteristics of commercially important species is crucial for undertaking any management actions.

The enormous inland water resources of Bangladesh include rivers, canals, lakes, freshwater marshes, estuaries, brackish water impoundments, and floodplains. These waterways have some of the world's richest prospective fish supplies. In the fiscal year 2019–20, the total fish production of Bangladesh was around 4.5 million metric tons which contribute 3.52% to the national GDP (DoF, 2020). The Kaptai lake is one of the most important artificial freshwater lakes in Bangladesh, and the largest in Southeast Asia (Fernando, 1980) which is an important source of natural fisheries resources. With a surface area of 68,800 hectare at full supply level (FSL) (Ali, 1985) it offers a wide range of potentialities and possibilities for the development of fisheries and the enhancement of annual fish production. In the Chattogram Hill Tracts, the river Karnafuli was dammed close to Kaptai town, resulting in the construction of the Kaptai reservoir. The fish fauna that exists in this reservoir closely resembles that of Bangladesh's rivers, haors, and heels system since the fish population of Kaptai reservoir was predominantly dependent on the previous riverine stocks that date back to 36 years following impoundment. The annual fish production of Kaptai lake was 12,696 Metric tons in the fiscal year 2019–20 (DoF, 2020), though the total production was much lower (7,247 Metric tons) 20 years ago (DoF, 2001). But the abundance of large fishes like Indian major carps (*Labeo rohita, Catla catla, Cirrhinus cirrhosus, Labeo calbasu*, and *Tor tor*), catfishes etc. are being dropped dramatically from an initial 81% (1965–66) to roughly 5% now (Alamgir and Ahmed, 2008). Small forager fish (*Corica soborna, Gudusia chapra* and *Ompok pabda*) has increased in popularity from 3% (1965–66) to over 92% currently (Alamgir and Ahmed, 2008). According to recent data, the abundance of carps is below 1% with other forager fishes dominating more than 95% of the total production (DoF, 2020). The major reasons for unbalancing the natural stock of the Kaptai lake is overexploitation, invasive species (explosive growth of clupeids), and water diversion for damming (Ahmed et al., 2006).

Plate 1: Map of the Kaptai lake

The government has enforced 90 days fishing ban (May 1st – 31th July) in Kaptai lake with the aim to increase fish reserves substantially in the lake by allowing the broods to breed successfully and to collect more fishes from the lake after withdrawal of the ban. All forms of fishing, including the capturing, carrying, transporting, selling, buying, marketing, and carrying of fishing nets, are absolutely forbidden during the moratorium. However, further study has to be done on many areas of reproductive biology in order to assure the richness and sustainability of the broodstock as well as the appropriate management of this lake, which is significant as one of the primary sources of freshwater fish production in the nation. A deeper comprehension of fish reproduction is crucial to enhancing the current state of the stock in Kaptai Lake.

Fisheries research, stock assessment, artificial breeding, conservation, and management all benefit from an understanding of fish reproductive biology, which includes the timing and duration of spawning, sex ratio, maturation stages, length and age at maturity, oocyte diameter, and fertility (Brown-Peterson et al., 2011). Fish reproductive biology is essential for managing fisheries (Jakobsen et al., 2009), particularly in developing nations like Bangladesh, where managers rely on size at first maturity and the commencement and duration of the spawning season to manage fisheries stock (Bolger and Connolly, 1989). Most fish have one or two spawning seasons per year, and the spawning season is timed (Medeiros and Maltchik, 2001). The compatibility of spawning and reproductive activities determines the success of reproductive activities. The spawning season must coincide with the best conditions for larval survival for reproductive activities to be successful (Lowerre-Barbieri et al, 2011). Additionally, the broods and larvae must be safeguarded from exploitation throughout the particular spawning period. Moreover, fish stock management highly depends on understanding a species' fecundity (Armstrong and Witthames, 2011). It is both a term for a fish that is first spawning and an assessment of a stock's capability for reproduction. Therefore, obtaining fundamental biological information about spawning season and fecundity is essential for maintaining an effective conservation and management system. Thus, the fish fauna that exists in this reservoir closely resembles that of Bangladesh's rivers, haors, and heels system since the fish population of Kaptai reservoir was predominantly dependent on the previous riverine stocks that date back to 36 years following impoundment.

A small population of any sexually mature animal kept as a source of population replacement or for the creation of new populations is known as broodstock. Natural recruitment and reproduction may restore fish stocks, which are a renewable natural resource (Olusegun, 2011). Any species' ability to reproduce successfully in challenging environmental conditions depends on its ability to recruit. The availability of a sufficient number of brood fish in nature is also critical to the success of reproductive activities. For that, effective management of the broodstock is necessary to assure the availability of ripe broods throughout the breeding season (Olusegun, 2011). For maximal survival, improved gonadal development, and increased fertility, broodstock modification entails modifying the environment around the broodstock (Izquierdo et al., 2001). Therefore, studies into the factors that shape and control the population biology of fish species that are commercially important should be conducted, with a particular emphasis on the breeding season.

Catfish represent one of the largest groups of freshwater fishes, with more than 2,000 species all over the world. They are considered an important group because they serve many different roles, including as food fish in aquaculture, as research animals, as ornamentals and for sport fishing (Jiang et al., 2011). The total production of catfish in Bangladesh was 69,389 Metric tons in the year 2020–21, whereas the production of catfish in the Kaptai lake was 217 Metric tons which is around 1.71% of the total production of the lake (DoF, 2020). Long-whiskered catfish, *Mystus aor,* is one of the naturally occurring and commercially important freshwater catfishes of Bangladesh. The fish, locally known as ayre or guzi ayre, belongs to the order Siluriformes and family Bagaridae (Rahman, 1989). Due to its great nutritional value with strong protein content, low number of intramuscular bones (Chondar, 1999; Talwar & Jhingran, 1991), high market price, and widespread popularity (Chondar, 1999; Khawja, 1966)., it has been regarded as one of the most loved culinary fishes. Though it prefers riverine habitats, it can also be found in ponds, lakes, tanks, channels, and reservoirs. Adults and juveniles reside on the river's bottom and marginal areas; fry lives in shallow marginal pits connected to the river by channels; larvae live in nests constructed amid rocks or soft muddy beds of streams, rivers, and big tanks (Chondar, 1999). Kaptai lake is one of the most important natural habitats of *M. aor*, although the stock is declining gradually due to its geographic position and bottom surface.

Plate 2: Ayre Fish (*Mystus aor*)

A number of studies have been conducted on various aspects of its morphology (Chondar,1999; Ferraris and Runge, 1999; Talwar and Jhingran, 1991), feeding (Ghosh and Chakrabarti, 2013; Azadi et al, 1992; Ramakrishniah, 1992; Sinha and Chakrabarti, 1986), and breeding biology (Azadi et al., 1992; Saigal, 1982; Ranganathan and Radha, 1966) in the past, but no comprehensive report on histological study to understand the reproductive biology of this species is currently available from different waterbodies.

Study on the biology of this catfish especially from the Kaptai lake has been conducted in last century on the basis of oocyte diameter data (Azadi et al., 1992) which is not precise enough for the conservation and management plan as well as broodstock development. In order to improve natural broodstock by identifying their breeding season, more intense observation of the catfish's reproductive biology is required. In the present investigation, the following dimensions will be made on *M. aor* in Kaptai reservoir such as length-weight relationship, condition factor, relative condition factor, size at first maturity, GSI, HSI, fecundity, oocyte diameter and reproductive stages by histological analysis so that the specific breeding season and strategy can be understood.

1.1 Objectives of the Study:

- To understand the basic biological indices (length-weight relationship and condition factor, relative condition factor, hepatosomatic index (HSI), oocyte diameter, fecundity and length at first maturity (L_m), gonadosomatic index (GSI)) of *Mystus aor* of the Kaptai lake; and
- To increase broodstock by reframing the spawning season through investigating the gonadal maturation cycle.

CHAPTER: 2

REVIEW OF LITERATURE

This chapter contains the following data which are gathered to plan the current study and validate the new findings. Length-weight relationship studies are an essential tool for studying the basic biology of fish. A close check at the condition at different body lengths can offer definitive hints about the growth and well-being, whilst a close look at the condition of gonads at different months can give useful information about the maturation and spawning throughout the life cycle of the fish. The previous studies on the length-weight relationship, hepato-somatic index (HSI), gonado-somatic index (GSI), length at first maturity (L_m), oocyte diameter, cyclic variations in gonadal development, and fecundity of *Mystus aor* species are all discussed in this chapter.

2.1 Length-weight relationship

Length-weight relationships (LWR) are used to estimate the weight corresponding to a given length. The determination of a fish population's productivity and biomass requires the establishment of a link between length and weight (Tesch and Ricker, 1968; Safran, 1992; Petrakis and Stergiou, 1995; Dulcic and Kraljevic, 1996; Moutopoulos and Stergiou, 2002) which also indicates the growth patterns of a particular species (Tesch, 1968).

Studies on the LWR have been conducted in a number of catfish species including *M. aor*. The length-weight regression coefficient of *M. aor* in the Kaptai reservoir was found to be 2.89 in males and females (pooled) where males had a coefficient of 2.93, and females had a coefficient of 2.94 (Azadi et al., 1992). The LWR equation for the pooled data was $W = 0.00704693\ TL^{2.89}$ ($r = 0.99$); for male the relationship was $W = 0.00587489\ TL^{2.939}$ ($r = 0.99$) and for female it was $W = 0.00592925\ TL^{2.941}$ ($r = 0.99$) in the same study (Azadi et al., 1992).

Sani, (2010) reported the LWR of another catfish species, *Sperata aor*, from the Betwa and the Gomti river of India. The regression coefficient value was 2.98 and the equation was $W = 0.0059\ TL^{2.98}$ in the Betwa river and for the Gomti river it was 3.02 and $W = 0.0043\ TL^{3.02}$ respectively. The LWR equation for *S. aor* was $W = 0.0042TL^{2.30}$; and $W = 0.0042TL^{2.30}$ in the river Ganga and Gomti river respectively (Sarkar et al., 2013).

Studies on LWR of some other *Mystus* species have also been conducted which are significantly related to our focused species. In *Mystus tengara*, the LWR equation were Log TW = Log 0.0064 + 2.62 Log TL ($r^2 = 0.94$) in the Gomti River, Lucknow (U.P.), India (Kumar et al., 2014); Log TW = Log 0.0158 + 2.80 Log TL ($r^2 = 0.95$) in the Ganges River, Bangladesh (Hossain et al., 2016); and Log TW = Log 0.0239 + 2.41 Log TL ($r^2 = 0.98$) in the Indus River, Pakistan (Muhammad et al., 2017) respectively. In *Mystus cavasius*, the LWR equation were Log TW = Log 0.012 + 2.91 Log TL ($r^2 = 0.96$) in the Betwa River, India (Sani et al., 2010); Log TW = Log 0.0069 + 3.10 Log TL ($r^2 = 0.96$) in the Ganges River, Bangladesh (Hossain et al., 2016) and Log TW = Log 0.01 + 2.92 Log TL ($r^2 = 0.90$) in the Indus River, Pakistan (Muhammad et al., 2017) respectively. In *Mystus bleekeri*, the LWR equation were Log TW = Log 0.0258 + 2.54 Log TL ($r^2 = 0.98$) in the Ganges River, Bangladesh (Hossain et al., 2016) and Log TW = Log 0.0288 + 2.49 Log TL ($r^2 = 0.96$) in the Indus River, Pakistan (Muhammad et al., 2017). In *Mystus gulio*, the LWR equation were Log TW = Log 0.03 + 2.54 Log TL ($r^2 = 0.94$) in the Indus River, Pakistan (Muhammad et al., 2017) and Log TW = Log 0.0091 + 3.10 Log TL ($r^2 = 0.97$) in the Ganges River, Bangladesh (Hossain et al., 2016). Length-weight relationship studies have been taken place on some other commercially important catfishes such as *Sperata seenghala* (Sani et al., 2010; Hossain et al., 2016, Muhammad et al., 2017); *Wallago attu* (Sani et al., 2010; Hossain et al., 2016); *Heteropneustes fossilis* (Hossain et al., 2016) etc. in order to estimate the stock for management and conservation purposes.

The only study on the LWR of *M. aor* in the Kaptai lake was conducted a long time ago by Azadi (1992). But the present scenario of different fish stocks in the Kaptai lake is quite different due to the changes in the climatic condition, stock depletion, availability of the food and so on (Bashar et al., 2015). So, it is the urge of time to validate the length-weight relationship of the fish to assess the stock status and take management actions accordingly in the Kaptai lake.

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

The condition factor (K) is a measure of a fish's physiological status and overall well-being, which includes maturity, spawning, environmental circumstances, and food availability (Tesch, 1968; Brown, 1957). The condition factor (K) demonstrates individual's health condition and can be linked to the reproductive process by reflecting the allocation of energy (mostly proteins) during the spawning phase, when the individual's physiological state changes (Carvalho et al., 2009).

The relative condition factor (Kn) study may be used to assess the plumpness of fish, allowing to compare the weight of fish to a standard calculated weight to see if the fish are in better or worse condition than the average. The relative condition factor can also be used to compare general well-being, body fatness, and gonad development (Thomas, 1969). Sarkar et al., (2013) found the average condition factor of another catfish species, *Sperata aor,* to be 0.97 ± 0.29 in Gomti river, India and *Sperata seenghala* to be 2.16 ± 0.48 in river Ganga, India.

To date, studies on K of *M. aor* is not available for any part of the Bangladesh including Kaptai lake. The only study on the relative condition factor of *Mystus aor* was conducted in Kaptai lake where males had a relative condition factor (Kn) of 0.95–1.04, females had a Kn of 0.93–1.04, and for the pooled data the Kn was 0.97–1.04 (Azadi et al., 1992).

The seasonal variation in the K was studied in *Mystus tengara* by Gupta and Banerjee, (2013) where they found that females and males reach to peak once a year in June. The lowest value of the K was recorded in December; then it began to rise from January onwards, peaking in June; then it fell in July, returning to the lowest value in December (Gupta and Banerjee, 2013). The mean condition factor of *Mystus bleekeri* from the Surma river, Bangladesh varied from 1.45 ± 0.23 to 0.89 ± 0.18 in different size groups (Sultana et al., 2021). Begum et al., (2010) found two peaks of condition factor in *Mystus gulio* in 18–20cm length and 22–24cm length where the first peak corresponded to the time of sexual maturation, which exhibited a rapid growth rate, whereas the second peak corresponded to the size at breeding maturity.

Though the relative condition factor of *M. aor* in Kaptai lake was studied by Azadi et al., in the year (1992), there is still a gap in condition factor data. So, it is essential to elucidate both condition factor and relative condition factor data at the present time to understand the reproductive stages of fish.

2.3 Fecundity

Fecundity refers to the number of eggs laid by a species in a single season. The quantity of eggs produced by fish is extremely important in fish culture because it determines the number of rearing facilities and the extent to which various types of equipment are required. Fecundity and spawning behaviors are two key components of fish biology that must be understood in order to explain population fluctuations and to make attempts to enhance fish harvest (Das et al., 1989), and the development of sexual maturity and fecundity determination are also fundamental aspects of fisheries study (Brown, 1957).

Numerous research has been conducted on the fecundity of *Mystus aor* in multiple locations and at different times. In a study by Saigal (1964) recorded a fecundity range 45,000–1,22,500, while Ranganathan and Radha (1966) reported fecundity range of 21,490–38,400 from Bhabanisagar reservoir, India. *M. aor* from the Kaptai reservoir in Bangladesh had a fecundity range of 12,560–48,635 (Azadi et al., 1992). They also found a favorable relationship between fecundity and body length and also between body weight and ovary weight. In a recent study, the fecundity of *M. aor* found to varied from 59,255–70,586 with an average value of 64,920 from the North-East Bangladesh (Jabed et al., 2021).

Fecundity of some other *Mystus* species has also been studied. The fecundity of *Mystus seenghala* and *Mystus cavasius* varied between 20,064–46,443 and 3,314–63,135 respectively. In *Wallago attu* and *Heteropneustes fossilis* it was varied between 66,070–4,53,148 and 1,375–46,737 respectively (Bhatt et al., 1977). The mean fecundity of *M. bleekeri* from Padma River, Bangladesh was recorded as 23,611±15,427 with a range from 4,652 – 57,932 eggs (Musa and Bhuiyan, 2007). The fecundity of *Mystus tengara* ranged from 6,770–21,708 with an average of 13,365±7,260 (Gupta and Banerjee, 2013). Lal et al., (2016) documented the fecundity of *M. gulio* from 5,950–1,41,210.

The physiology and biology are quite different and the physicochemical parameters of the Kaptai lake are changing gradually (Bashar et al., 2015) which has a direct impact on the fecundity of fish. For that reason, determining the fecundity of *M. aor* in the Kaptai lake is important.

2.4 Hepatosomatic index (HSI)

Hepatosomatic index (HSI) is the main indicator of metabolic activity in animals. The HSI provides valuable information about the condition of liver and body and also about the impact of water pollution on fish body. HSI also provides an indication on status of energy reserved in fish. Variations in the HSI are connected to the liver's glycogen storage capacity, which reflects physiological circumstances and the availability of energy (mostly lipids) for reproduction (Carvalho et al., 2009).

HSI of some *Mystus* species have been studied so far. The mean HSI of *Mystus gulio* was 1.5 ± 0.01 and there was a positive linear correlation was observed between the body weight and HSI (Sabbir et al., 2017). In *M. vittatus*, the mean HSI value was 1.72 ± 0.24 (Verma and Prakash., 2017).

But there is no record found about the HSI value of *Mystus aor*. That's why it is important to study the HSI of *M. aor* to understand its' condition, glycogen profile and status of pollution of its' surrounding environment.

2.5 Length at first sexual maturity (L_m)

Length at first sexual maturity (L_m) is the length at which 50% of the fish have reached maturity. For effective fish population management and conservation, it is essential to understand the fish's length at maturity and breeding season since it reveals when and at what length the fish should be protected (Hunter et al., 1992). Harvesting only adult individuals is one of the fundamental rules that must be followed to ensure sustainability.

Previous studies in the Kaptai lake found that L_m for female *M. aor* was 48cm (Azadi et al., 1992). Saigal, (1964) found the minimum size at maturity for *S. aor* was 84 cm. Ramakrishniah, (1992) recorded a length of 57.3 cm for *S. aor* at initial maturity, and he also said that *S. aor* matured at the age of 4 years.

The research findings from a study held in 1992 by Azadi on the L_m of *M. aor* in the Kaptai lake should be reconsidered for today's situation. That's why, this research is focusing on the latest information about the size at first maturity of *M. aor* in the Kaptai lake.

2.6 Gonadosomatic index (GSI) and oocyte diameter (OD)

The gonado-somatic index (GSI) indicates the level of maturity and the start of the spawning season and is used to track a species' reproductive cycle at monthly or smaller intervals throughout the year. When compared to the overall mass of the fish, this index suggests that an ovary grows in size as the fish develops. The condition of the female, its' ovaries, and the size of the eggs (oocyte diameter) are all important factors in determining the breeding season. The GSI and oocyte diameter rises with the development of fish, peaking during the period of peak maturity and then dropping sharply when the fish are exhausted after releasing the eggs and sperm (Lal et al., 2016).

The GSI and oocyte diameter of the *M. aor* from Kaptai lake was studied by Azadi et al., (1992) and concluded that gonad weight changes roughly correspond to the three gametogenic stages: (1) a gradual increase in GSI during the pre-spawning period, November-February; (2) a marked increase reaching a peak during the spawning period, April-July; and (3) a gradual decrease in the postspawning period, August-October. The oocyte diameter varied from 0.3504 to 0.9510 mm (Azadi et al., 1992).

The GSI values of *Sperata aor* were fluctuated between 0.47 ± 0.04 to 3.0 ± 0.02 and 1.08 ± 0.01 to 0.12 ± 0.01 for female and male respectively during the period of August to July. Higher GSI peak values were found from June to August, ranging from 2.6 ± 0.03 to 3.0 ± 0.02 and 0.86 ± 0.01 to 1.08 ± 0.01 for female and male respectively (Jabed et al., 2021). GSI was shown to peak once a year in the month of July in both *M. tengara* male and female. The GSI was recorded at its lowest point in December, began to rise from January on, peaked in July, then began to decline in August before returning to its lowest point in December (Gupta and Banerjee, 2014). Oocyte diameter of *M. tengara* was found ranging from 33µm to 1629 µm in the Ganges River of Rajshahi, Northwestern Bangladesh (Mitu, 2017). GSI values of both male and female *M. gulio* increase from March onwards reaching a peak in July followed by a gradual decrease up to December (Sarker et al., 2002; Lal et al., 2016). The highest mean GSI for *M. cavasius* male was 3.41 in early July and the lowest was 0.05 in late September and for female it was 24.54 in late July and the lowest was 0.19 in October from the 'Haor' basin in Northeastern Bangladesh (Maya et al., 2018). The GSI of female *M. bleekeri* revealed a sharp increase from minimum (August) to a maximum in November. In December, it dropped to a lower level, indicating that spawning had taken place. However, from December, the GSI again started to increase up to April and then gradually decreased (Sultana et al., 2021).

The only information about the GSI and oocyte diameter of *M. aor* from kaptai lake is recorded in 1990. Due to the Physico-chemical and environmental changes in the lake within this long period of time, the characters and onset of gonadal development may be altered which is required to be studied.

2.7 Reproductive biology and spawning season of *Mystus aor*

Studies on the reproductive biology are essential to assess the culture potential of a fish species. When a species' spawning season is known, stopping fishing at that time is an appropriate management action that has been used for a long time. Knowledge of a species' gonadal development and spawning season allows for future studies on the population's spawning frequency, which is crucial for management (Chakraborty and Singh, 1963). It is critical to examine the annual breeding cycle of culturable fishes in order to be successful in fish farming (Stoumboudi et al., 1993). Fish spawn during a certain stage of their reproductive cycle; some breed once a year, while others do so at regular intervals throughout the year. The study of sex ratios, length at initial sexual maturity, maturation cycle, and spawning frequency, among other things, are important element of reproductive biology (Reddy, 1979; Vazzoler, 1996).

According to Azadi et al., (1992), the breeding season for *Mystus aor* in Bangladesh's Kaptai reservoir extends from April to July on the basis of oocyte diameter study. The mating season for *S. aor,* according to Saigal, (1964), lasts from March to August, with the spawning peaks occurring in April and June in Ganga Rivers. Ranganathan and Radha, (1966) reported February to May as the breeding season for this fish species in the Bhavanisagar reservoir. In Nagarjunasagar reservoir, April to October was the breeding season (Ramakrishniah, 1992). Histological study on the gonadal development of *S. aor* from the northeast Bangladesh (Sylhet region) revealed that the breeding season of *S. aor* lasts for long duration from April to August with peak spawning in July to August (Jabed et al., 2021).

Studies on some other *Mystus* species have also been conducted which provides relevant information about the reproductive biology and spawning season of these species. The breeding season of *M. gulio* was found at March–November with July as the peak spawning month (Sarker et al., 2002). Spawning capable females of *M. tengara* were reported between May −July, peaking in June (Mitu, 2017). Study on the gonadal development of *M. cavasius* observed that the occurrence of mature stage together with highest GSI value in late July which indicates peak breeding season of this fish in late July. The ovaries contained developing oocytes in post-monsoon September indicating spent phase of the ovary (Maya et al., 2018). The maturation cycle and the stages of maturity of *M. bleekeri* indicated that this species spawns twice annually between April–May and October–November with five maturity stages (Sultana et al., 2021).

Saigal (1964) found seven gonadal maturity stages in *S. aor* based on macroscopic and microscopic scales - immature, intermediate, early maturing, late maturing, advanced maturing, ripe, and spent. The maturation stages of ovary of *M. tengara* were divided into six stages including reproductively inactive ovaries, early developing ovaries, late developing ovaries, spawning capable ovaries, spawning active ovaries, and regressing phase ovaries (Mitu, 2017).

Such of the above-mentioned studies have had a great impact on the recommendation of reinforcing the national fish moratorium for the conservation of different commercially important fish species of different region. We hope that, this study will be helpful in recommending the ban period of fishing *M. aor* in the Kaptai lake for the conservation of this delicious and highly demanded fish species.

CHAPTER: 3

MATERIALS AND METHODS

3.1 Sampling site and collection of samples

Fish samples (both male and female *Mystus aor*) were obtained from the local Banarupa Bazar fish market and fish landing center of the Bangladesh fisheries development corporation (BFDC), Rangamati, Bangladesh. During the study period, a total of 136 fish (average weight 1040.2 ± 233.7 gm, and total length 55.09 ± 4.43 cm) were collected monthly during November/20 – October/21. The collected fish were kept in an insulated iced box. Then the samples were brought to the Faculty of Fisheries of the Chattogram Veterinary and Animal Sciences University for further analysis.

3.2 Collection of length-weight data and determination of length-weight relationship

Total length (TL, from tip of the snout to the end of the caudal fin) and standard length (SL, from tip of the snout to the caudal peduncle) of each fish was measured by using a measuring scale and recorded as centimeter (cm). Weight of the fish was measured using an electric balance (Redwag, WPT1211NV, Poland) and recorded as gram (g). Length-weight relationship was determined by fitting the data to a potential relationship based on the exponential equation according to Le-Cren (1951).

$$TW = aTL^b.$$

Where,

TW is the total weight (expressed in g);

TL is the total length (expressed in cm);

"a" is a coefficient related to body form and

"b" is an exponent.

Then the measured values of constant 'a' and 'b' was estimated from the log transformed length and weight for equation (Le Cren, 1951).

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

Plate 3: Length and weight measurement

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

Condition factor and relative condition factor were calculated from the monthly samples, which was used to detect seasonal variations in the condition of fish. Condition factor was calculated by using the following equation (Lima-Junior et al., 2002).

$$K = W/L^b$$

Where,

K = condition factor;

W = weight calculated from length-weight relationship;

L = length

Relative condition factor "Kn" (Le-Cren, 1951) was estimated by using the following formula:

$$Kn = W_0 / Wc$$

Where,

W_0 = Actual weight of fish (g);

Wc= Expected weight,

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

W* = Average of W

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

After recording length-weight data the fish was cut ventrally to collect the gonad and liver and weighted in "g" by an electric balance (Maks HR-250A, India). The samples were kept in the Bouin's fixative for further histological study.

Monthly GSI was calculated by using the equation of Kumar et al., 2014.

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

Hepato-somatic index (HSI) was calculated on monthly basis by using the equation (Schereck and Moyle, 1990).

$$HSI\ (\%) = (\text{Liver weight (g)}) / (\text{Total body weight of the fish(g)}) \times 100$$

3.5 Determination of length at first sexual maturity (L_m)

The L_m was estimated by using multiple functions such as the relationship of (i) gonado-somatic index (GSI) vs total length (TL), (ii) modified gonado-somatic index (MGSI) vs TL, and (iii) Dobriyal index (DI) vs. TL. The GSI, MGSI, and DI which were calculated as-

$$MGSI\ (\%) = (GW/BW-GW) \times 100$$ (Nikolsky, 1963) and

$$DI = (GW)^{1/3}$$ (Dobriyal et al., 1999)

Where,

BW= body weight,

GW= gonad weight

3.6 Determination of fecundity and oocyte diameter

Gravimetric method was used to estimate the fecundity. At first 3–5 sub-samples from different part of the ovary were cut and then weighed the subsamples. After weighing, the numbers of eggs were counted for each sample and fecundity was determined by the following formula:

$$\text{Fecundity} = (\text{Total ovary weight}) / (\text{Weight of sub sample}) * \text{no. of eggs in subsample}$$

The diameter of the oocyte was measured by ocular microscope (Carl ZEISS Microscope Gmblt, Optica B-190 Series) with installed software (Optika Vision Lite 2.1, Italy)

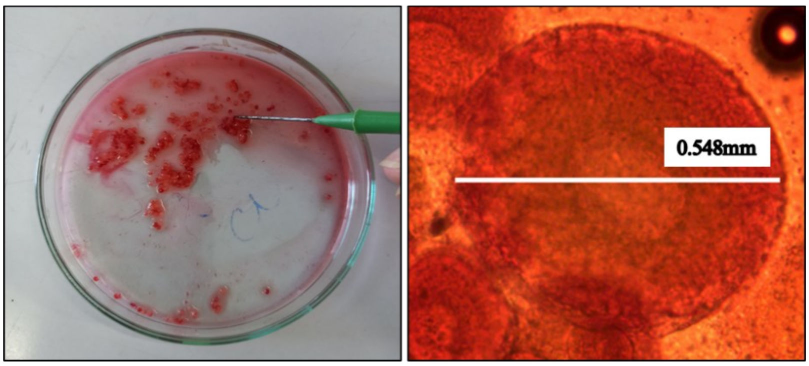


Plate 4: Egg counting and determination of oocyte diameter

3.7 Histological analysis of gonad

For histological study, the gonads were preserved into the Bouin's fixative for 24hr to maintain the integrity of the sample during sample collection in each month. Gonad samples were then transferred to 70% ethanol and kept in room temperature until histological study. When starting the samples for histological assay, the samples were dehydrated by different grades of alcohol (70, 80, 90, 95, 100 and 100%). After dehydration, samples were embedded by paraffin wax. Then, embedded wax was sectioned by using microtome machine (KD 2258, China). The sections were stained by standard staining procedure using hematoxylene and eosin and then were subjected to a histological examination under microscope (Carl ZEISS Microscope Gmblt, Optica B-190 Series, USA). The description of the detailed histological analysis is as follows-

3.6.1 Collection and preservation of gonad

At first, the fish was placed on a tray and then it was cut from the anus towards the lower jaw by using a scissor. The gonad and liver were carefully gathered using a forceps after the undesirable fat, blood arteries, and intestine were removed. Then the sexes of the collected gonads of the fishes were determined by using the acetocarmine gonad squashing method (Guerrero and Shelton, 1974). The weight of the gonad of the individual fish were measured carefully by using an electrical balance (EK600 Dual, U.A.E) and the data were recorded for further study. Individual gonad sample was kept in a vial and preserved in Bouin's fixative solution and kept at room temperature for histological analysis.

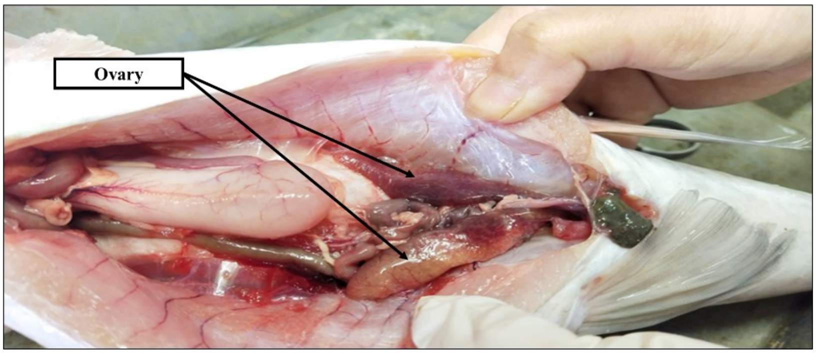


Plate 5: Dissection of fish

Plate 6: Fixation of gonads

3.6.2 Washing of fixed tissue

After fixation, excess fixatives were washed out from the tissue to prevent interference with subsequent processes during the histological procedure. Washing was done in running water about 2–3 hours before dehydration.

3.6.3 Dehydration

After washing, the gonads were taken out and cut into small pieces (about 1cm) and put into pre labelled cassettes separately. Then the tissue blocks were passed through successive ascending concentration of graded alcohol for dehydration (Table-1).

Table-1: Dehydration schedule

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

3.6.4 Cleaning

After dehydration the blocks were passed through successive changes of xylene until the alcohol from the tissue is replaced by xylene (Table-2).

Table -2: Cleaning schedule

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

3.6.5 Infiltration

After cleaning, the tissue blocks were placed in melted paraffin in the oven at 60ºC. Heat causes evaporation of xylene and the space in the tissue become infiltrated with paraffin.

Table -3: 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

3.6.6 Embedding

After infiltration, each tissue sample was taken from the melted paraffin and put in a block and filled with molten paraffin. Then the embedded blocks were allowed to cool in room temperature for overnight.

3.6.7 Trimming

In the process of trimming, the unwanted wax layers of the embedded blocks are cut away to produce the right blocks. Trimming helps in easy sectioning of the samples. In this step, both side trimming and surface trimming were conducted.

3.6.8 Sectioning

The small embedded paraffin blocks with tissue are sectioned by the hand rotatory microtome (KD-2258 Rotary Microtome, China) at 5µm size. After sectioning, the ribbons like sections were floated in water bath at (42°C) temperature for 3–4 min.

3.6.9 Attachment of section on glass slide and drying

The well spread ribbons of section from water bath are transferred to glass slides treated with adhesive substanced (gelatin, egg albumin). The glass slides with section (s) were then dried in a slide warmer (XH-2003, USA) at temperature 37°C for overnight.

3.6.11 Staining

After drying, the slide with tissue section was ready for staining. Following steps (Table-4) were followed in staining the tissue for hematoxylin and eosin stain.

Table – 4: Staining schedule

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

3.6.12 Mounting

After staining, tissue section with glass slide was protected by thin cover slip. Required drop of DPX (Qualikems Fine Chemical Pvt. Ltd., India) was put on each slide followed by attachment of cover slip and mounted slides are allowed to harden.

3.6.13 Microscopic examination and identification of maturation stages

The mounted slides were observed under microscope (Optika; B-190 Series, Italy), which was connected to computer with digital camera. By the help of this mechanism numerous photographs were taken at different magnifications. According to the morphometric appearance and gamete stages, changes in the gonadal phases including types, organization, egg sizes, wall structure, presence of atresia, spermatids, yolk vesicle, yolk granules, and post-ovulatory follicles throughout the year were described as per Gupta and Banerjee, (2013); Maya et al., (2018); Jabed et al., (2021).

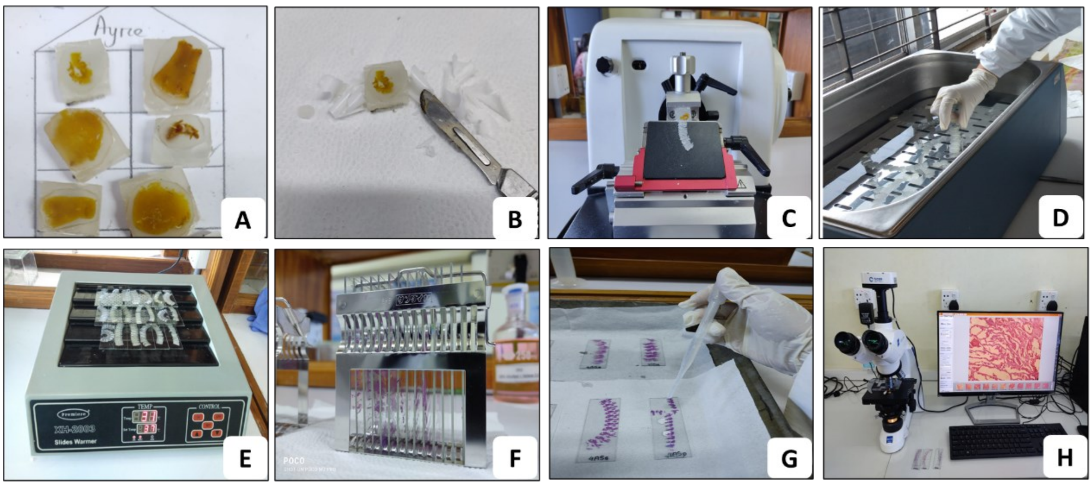


Plate 7: Histological stages: A. Embedding, B. Trimming, C. Sectioning, D. Floating sections in water bath, E. Drying of glass slides in warmer, F. Stained slides, G. Mounting, H. Microscopic observation

3.7 Statistical analysis:

Values are presented as mean ± standard deviation (SD). Homogeneity and distribution of the data were check. Pearson regression (r) analysis were conducted to establish the correlation among the GSI and HSI. Statistical analysis was performed using Microsoft excel and data were visualized using Microsoft power point. The data gathered in various months were compared for significant differences using one-way analysis of variance (ANOVA). Student t-tests were used to determine whether there were any significant differences between male and female *Mystus aor*.

CHAPTER: 4
RESULT

This section contains detailed information of the present research work about length-weight relationship, condition index, fecundity, oocyte diameter, HSI, GSI and the histological changes in the gonads of *M. aor* throughout the year.

4.1 Length-weight relationship

The logarithmic equation using total body weight (TW) and standard length (SL) was found as TW = 3.29SL – 2.36 (r^2 = 0.8212) for male *M. aor* collected from the Kaptai lake, Bangladesh. The slope (b) and intercept (a) for male was 3.29 and 2.36 respectively. For female, the logarithmic equation was TW = 2.45 SL – 0.99 (r^2 = 0.74). The slope (b) and intercept (a) for female was 2.4491 and 0.9889, respectively (Figure 1A & B).

In this experiment, the established logarithmic equation using pooled data (male and female) was TW = 2.65SL – 1.31 (r^2 = 0.8212) while the slope (b) and intercept for the was 2.64 and -1.31, respectively (Figure 1C).

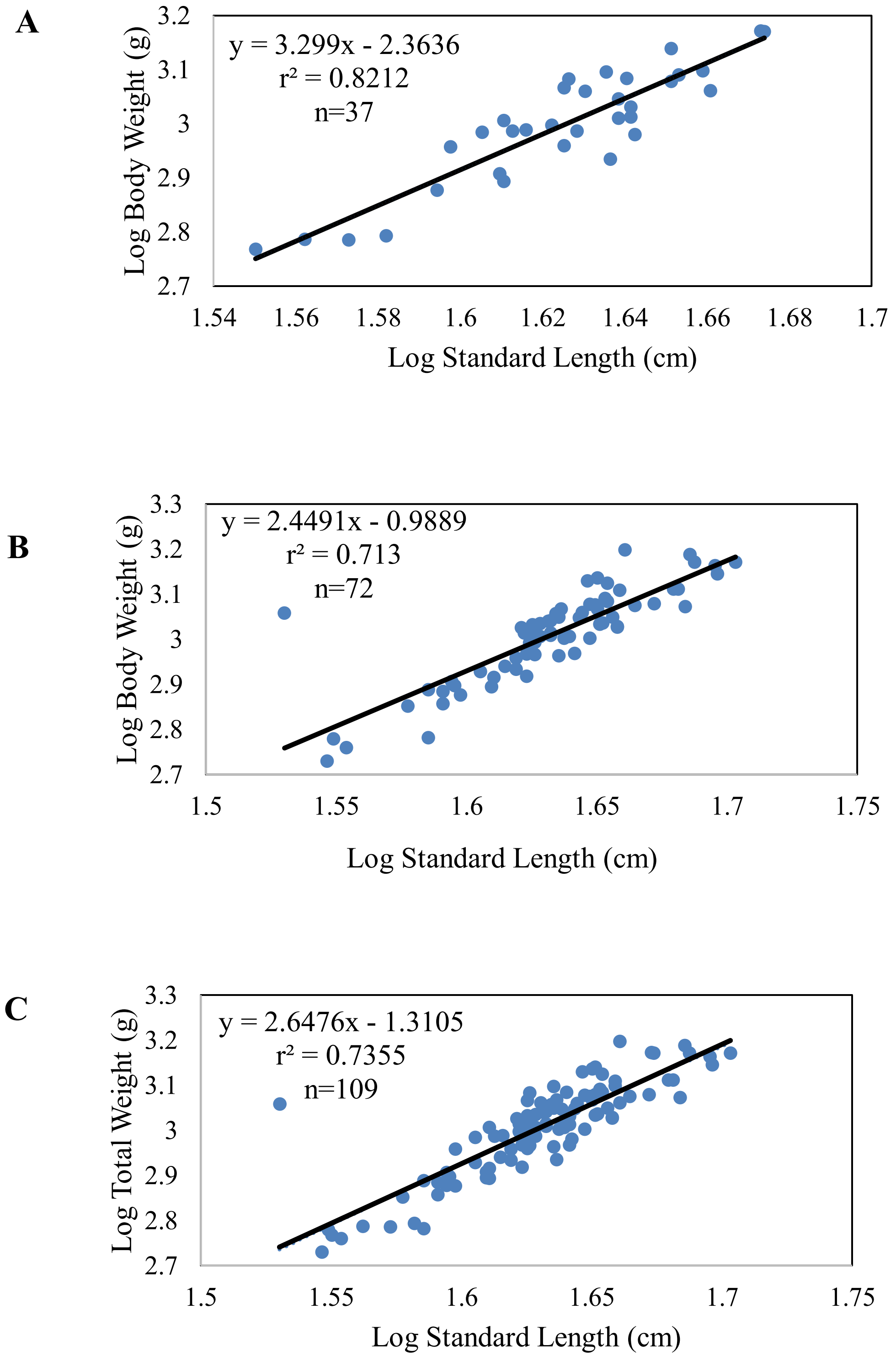


Figure 1: Logarithmic relation between the standard length and weight of A) male; B) female and C) pooled data of *M. aor* collected from the Kaptai lake, Bangladesh. X-axis indicates the log-transformed standard length (SL), Y-axis indicates log-transformed total weight (g) of the fish and r^2 indicates coefficient of determination (n = 37 for male, n = 72 for female and n = 109 for polled data).

4.2 Condition factor and relative condition factor

The monthly collected *M. aor* from the Kaptai lake was used to calculate the mean values of the condition factor (K) and relative condition factor (Kn). The monthly K values varied between 1.15–1.41 and the Kn value varied between 0.14–0.25. K was highest in May (1.41±0.57) and lowest in November (1.15 ± 0.05) whereas Kn value was highest in July (1.07 ± 0.11) and lowest in November (0.86 ± 0.16) (Figure 2).

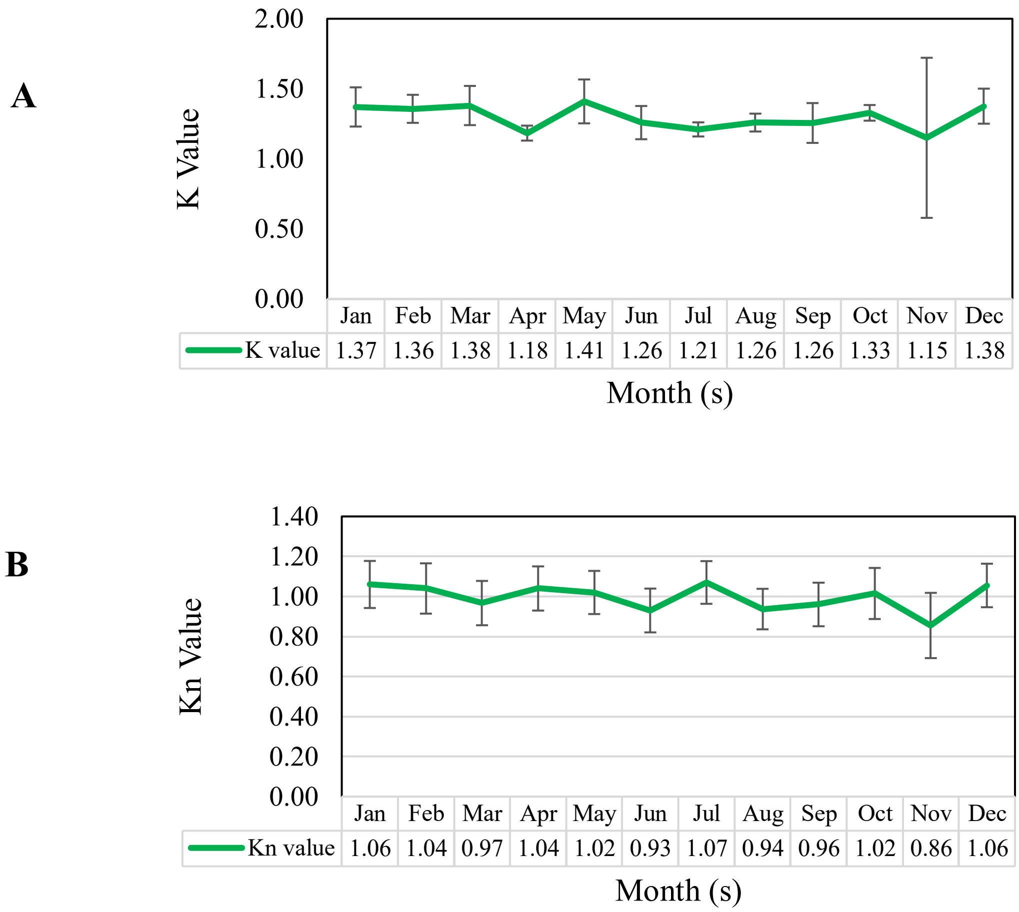


Figure 2: A) Condition factor and B) relative condition factor of *M. aor* collected from the Kaptai lake, Bangladesh based on pooled data. Data are presented as mean ± standard deviation (SD) (n = 109, 8 – 12 for each month).

For males, K value was peaked in March (1.5 ± 0.08) and for females, it was peaked in November (1.52 ± 0.69). The lowest K value was found in November (0.91 ± 0.02) for male whereas, it was lowest in July (1.14 ± 0.07) for females. On the other hand, the Kn value was maximum and minimum in April (1.14±0.06) and March (0.86 ± 0.02) respectively for males. For females, Kn was maximum in July (1.13 ± 0.4) and minimum in November (0.85±0.07) (Figure 3). Student t -test found significant differences between male and female K values in May ($p < 0.05$), whereas in case of Kn, it was significantly varied in April, May and July between male and female (Figure 3).

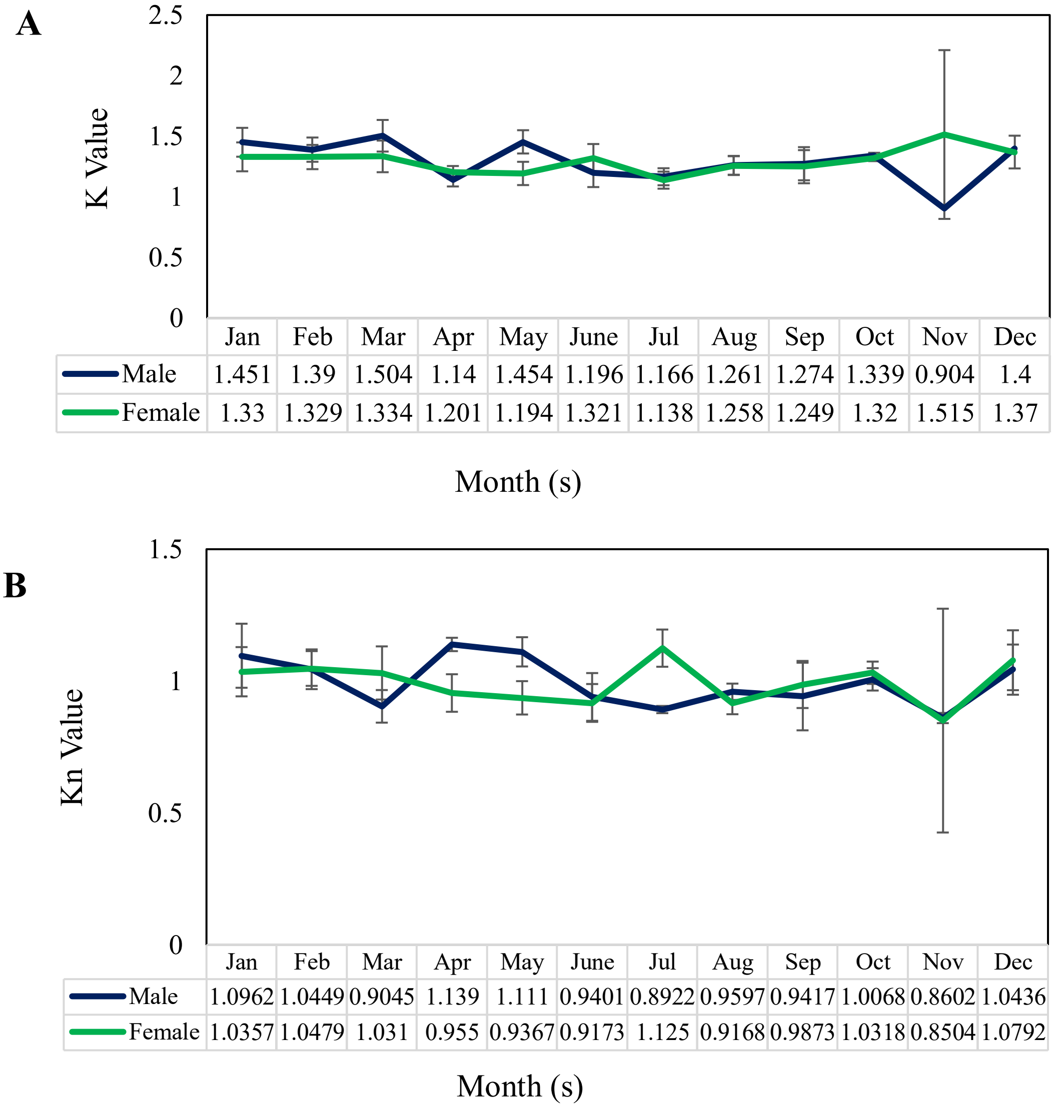
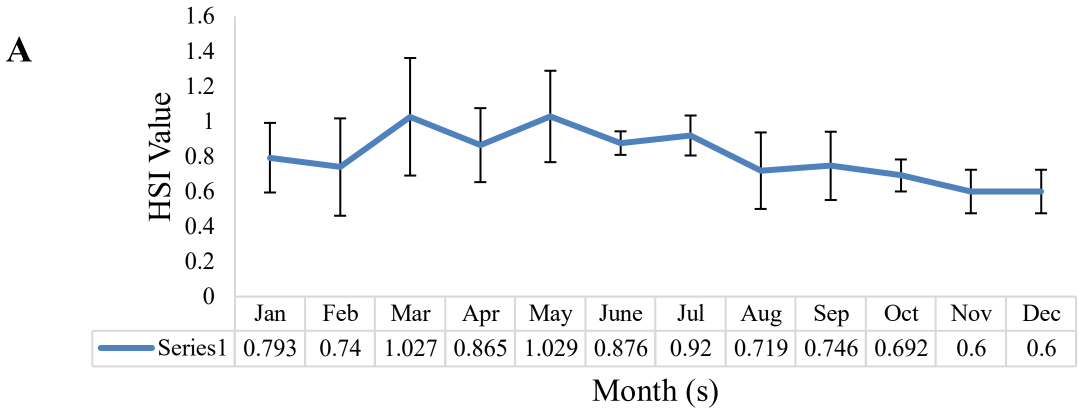


Figure 3: A) Condition factor and B) relative condition factor of *M. aor* collected from the Kaptai lake, Bangladesh. The blue color indicates males and the green color indicated females. Data are presented as mean ± standard deviation (SD) (n = 37 for male, n = 72 for female).

4.3 Hepato-somatic index (HSI)

All through the year, monthly variations in the HSI values for *M. aor* were recorded. For pooled data, the HSI value ranged from 0.60 – 1.029 with the maximum value in May (1.029 ± 0.26) and minimum in November (0.6 ± 0.12). For male, the value of HSI ranged from 0.297 − 1.132 with highest HSI value in March (1.164 ± 0.07) and lowest in February (0.518 ± 0.15). In females, the value of HSI ranged from 0.518 – 1.164 with highest value (1.132 ± 0.25) in May and the lowest (0.67 ± 0.27) in October (Figure 4).



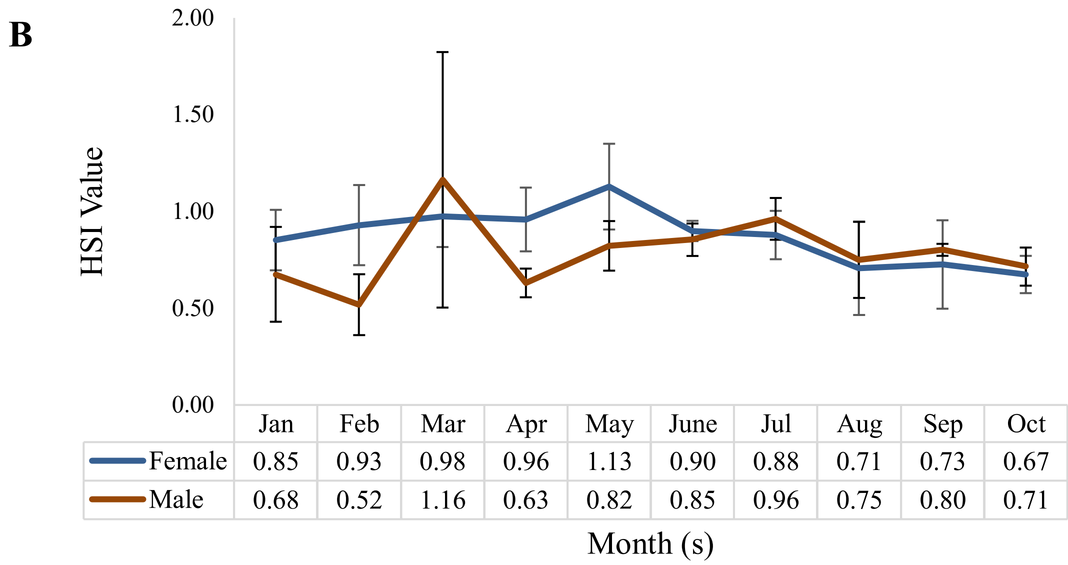


Figure 4: Hepato-somatic index for (A) pooled data and (B) male and female of *M. aor* collected from the Kaptai lake, Bangladesh. Data are presented as mean ± standard deviation (SD) (n = 37 for male, n = 72 for female and n = 109 for polled data).

4.4 Gonado-somatic index (GSI) and oocyte diameter

Monthly changes in the GSI values were observed for *M. aor* throughout the year. The value of GSI ranged from 0.028 – 0.78 in males and 0.233 – 2.319 in females. The lowest value of GSI in male was recorded 0.028 ± 0.002 in October and the highest was 0.908 ± 0.05 in May. In female, the peaked GSI was found in May (2.319 ± 0.367) and lowest in November (0.233 ± 0.21) (Figure 5).

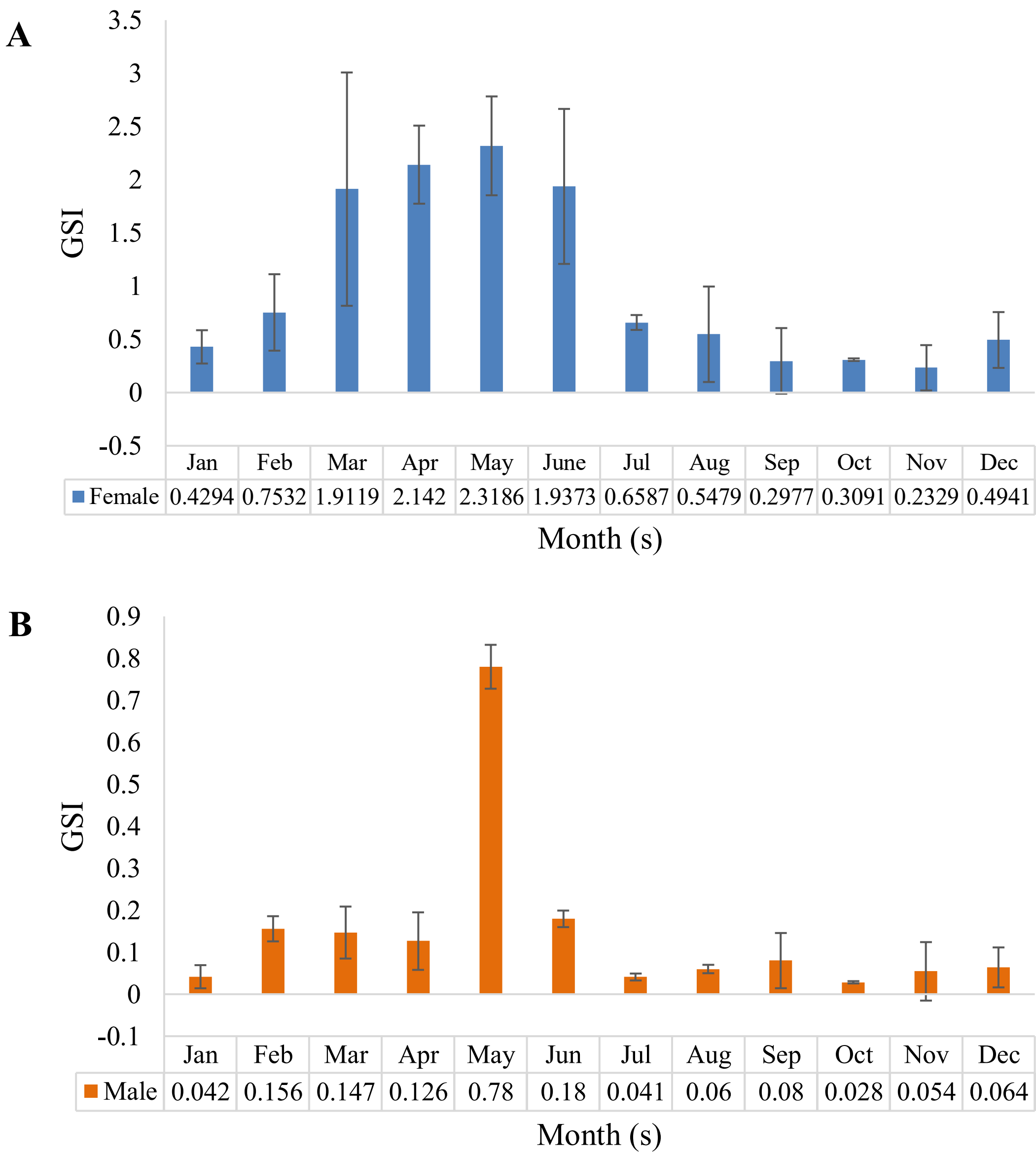


Figure 5: Gonado-somatic index (GSI) for (A) female and (B) male of *M. aor* collected from the Kaptai lake, Bangladesh. Data are presented as mean ± standard deviation (SD) (n = 37 for male and n = 72 for female).

Oocyte diameter was measured from the monthly collected female *M. aor* using light microscope. The highest oocyte diameter was recorded in May (0.830 ± 0.1095 µm). There was a strong positive correlation (r^2 = 0.9023) between the monthly oocyte diameter and the GSI of *M. aor* (Figure 6).

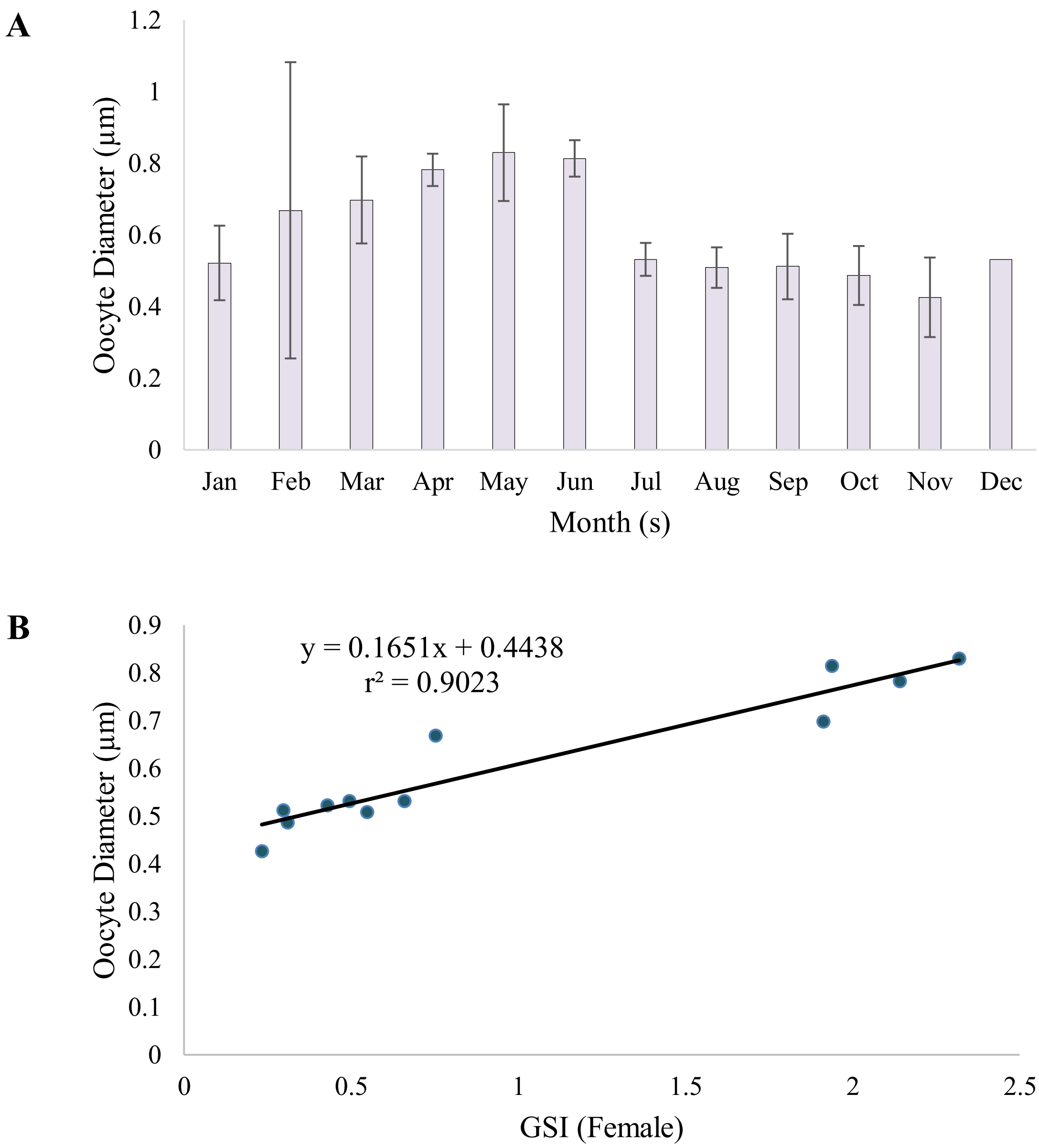


Figure 6: A) Oocyte diameter of *M. aor* collected from the Kaptai lake, Bangladesh. Data are presented as mean ± standard deviation (SD) (n = 72). B) correlation between oocyte diameter and GSI of female *M. aor* where X axis indicates the GSI values and Y-axis indicates the oocyte diameter (µm).

4.5 Fecundity

A random sample of gravid female fish weighing between 536.8 and 1480.4g, total length ranged from 46.7cm to 59.2cm and ovaries weighing between 4.21g and 41.21g was used to calculate the fecundity. The fecundity was found to vary from 18474 to 55504 eggs/female.

4.6 Length at first sexual maturity (L_m)

L_m for *M. aor* was calculated using the multiple relationship between TL vs GSI, MGSI, and DI (Figure 7&8). The GSI and MGSI in females was low (< 2%) less than 54.1 cm in TL had low level. DI was also low (< 2) at the same length. The GSI, MGSI (> 2%) and DI (> 2) rose sharply around at 54.1 cm TL in female hence considered as L_m for females (Figure 7).

In case of male, the GSI and MGSI was very low (< 0.1%) for fishes smaller than 49.5 cm in TL (Figure 8). DI value was also low (< 0.1) at the same length. The GSI, MGSI (> 0.1%) and DI (> 0.1) rose sharply around at 49.5 cm TL in male (Figure 8).

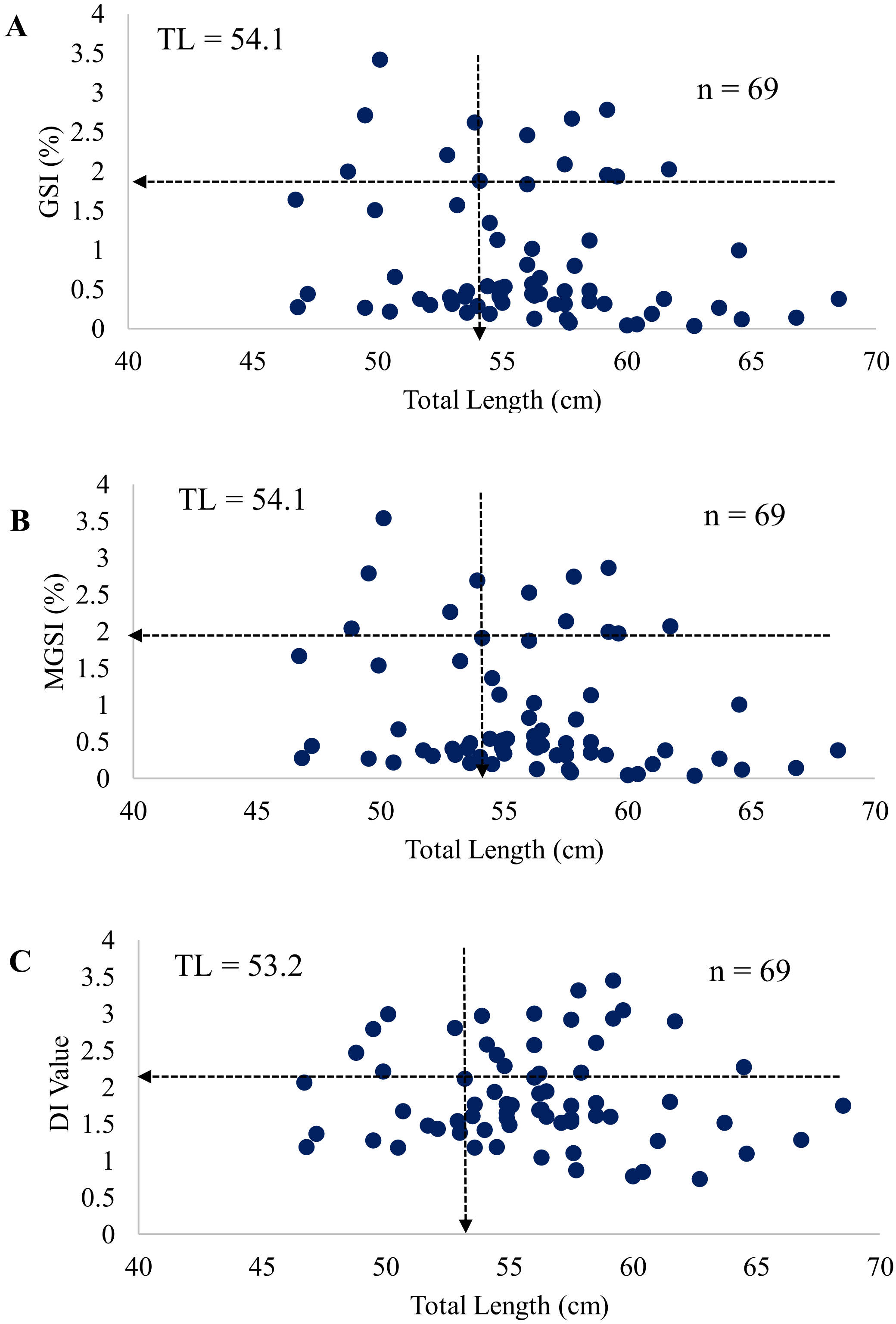


Figure 7: Relationship between A) Gonado-somatic index, GSI (%) and total length B) modified gonado-somatic index, MGSI (%) and total length and C) Dobriyal index and total tength of female *M. aor* collected from the Kaptai lake, Bangladesh (n = 69).

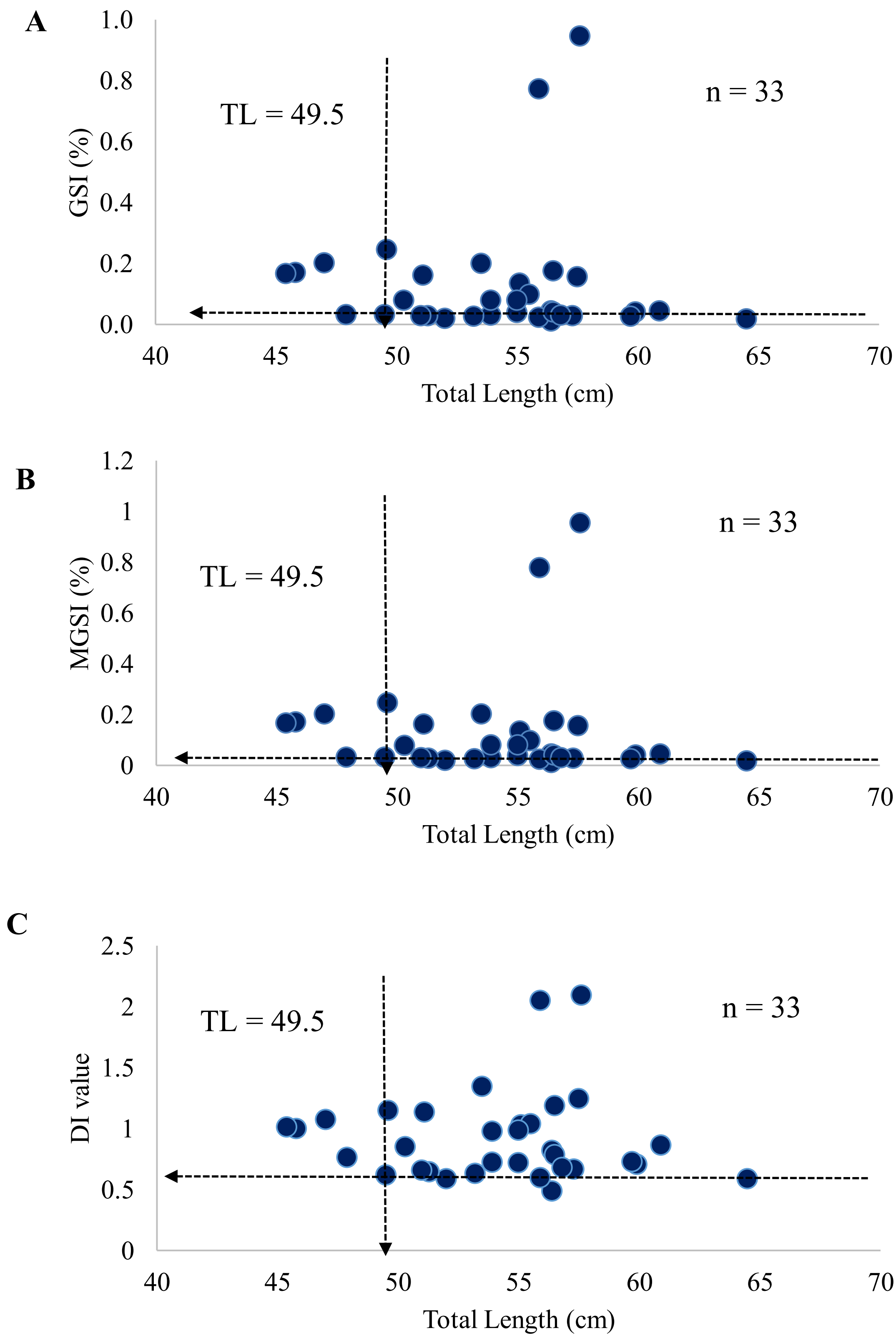


Figure 8: Relationship between A) Gonado-somatic index, GSI (%) and total length B) modified gonado-somatic index, MGSI (%) and total length and C) Dobriyal index and total tength of male *M. aor* collected from the Kaptai lake, Bangladesh (n = 33).

4.7 Gonadal maturation stages in females

4.7.1 Pre-vitellogenic stage: This stage was characterized by a number of oogonia (Oo), chromatin nucleolus (CN), early perinucleolus (EPO) and a number of primary growth oocytes. Oocytes were very small in diameter (0.5105 µm ± 0.05) and the cytoplasm was densely stained with hematoxylin as evidenced by a large and bright nucleus containing peripheral nucleoli. The GSI value at this stage was 0.415±0.03 and predominantly observed in the month of August and September (Plate 8).

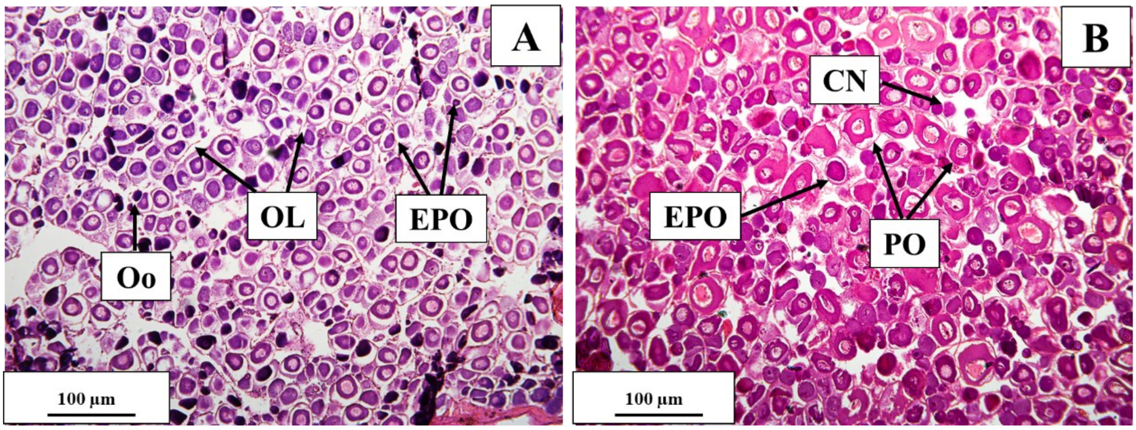


Plate 8: Ovarian maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the pre-vitellogenic stage (A, B). CN = Chromatin nucleous, EPO = early perinuclear oocyte, PO = perinuclear oocyte, Oo = oogonia, OL = ovarian lamellae. Scale bar = 100 µm

4.7.2 Early primary vitellogenic stage: Ovary was larger in size with yellowish coloration. Early perinuclear oocytes (EPO) and late perinuclear stage oocytes (LPO) were abundant in this stage. Vascularization in ovary started to appear. This stage was found in the month October and November. The average oocyte diameter was 0.4565 µm ± 0.086 and the GSI was found to vary from 0.309 to 0.233 (Plate 9).

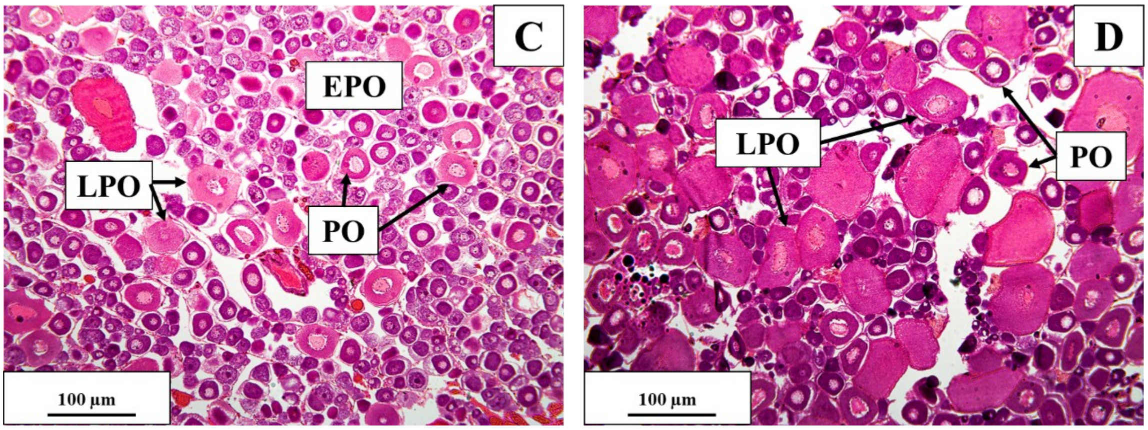


Plate 9: Ovarian maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the early primary vitellogenic stage (C, D). EPO = early perinuclear oocyte, PO = perinuclear oocyte, LPO = late perinuclear oocyte. Scale bar = 100 µm

4.7.3 Advanced primary vitellogenic stage: Zona radiata became visible in this stage. Yolk granules started to deposit. Number and size of yolk vesicle increased. Ova became visible in naked eyes and the vascularization was prominent. This stage was seen in the month of December and January with an average oocyte diameter of 0.526 µm ± 0.08 and the GSI was 0.429 (Plate 10).

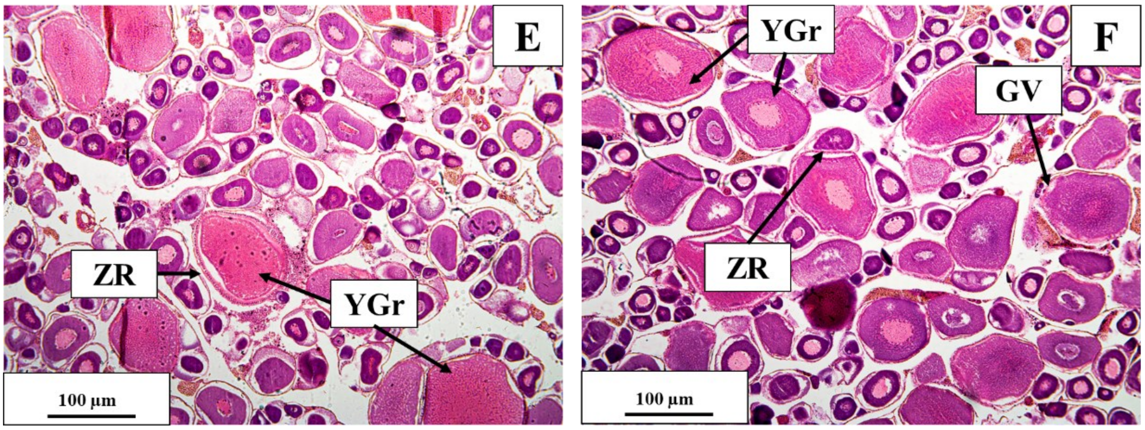


Plate 10: Ovarian maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the advanced primary vitellogenic stage (E, F). YGr = yolk granule, GV = germinal vesicle, ZR = zona radiata. Scale bar = 100 µm

4.7.4 Secondary vitellogenic stage: The secondary vitellogenic stage was accompanied with the accumulation of eosinophilic yolk globule in the inner cortex. The cytoplasm was mostly covered with yolk globules. The nucleus contained some peripheral nucleoli. The zona radiata had increased its thickness. Ova in this stage were spherical and yellowish or orange in color. The average oocyte size was 0.6835 µm ± 0.3 and the GSI in this stage was 1.3321. Blood vessels were appeared in the surface of the ovary. The number of ova could be counted by necked eye. This stage was found in the month of February and March (Plate 11).

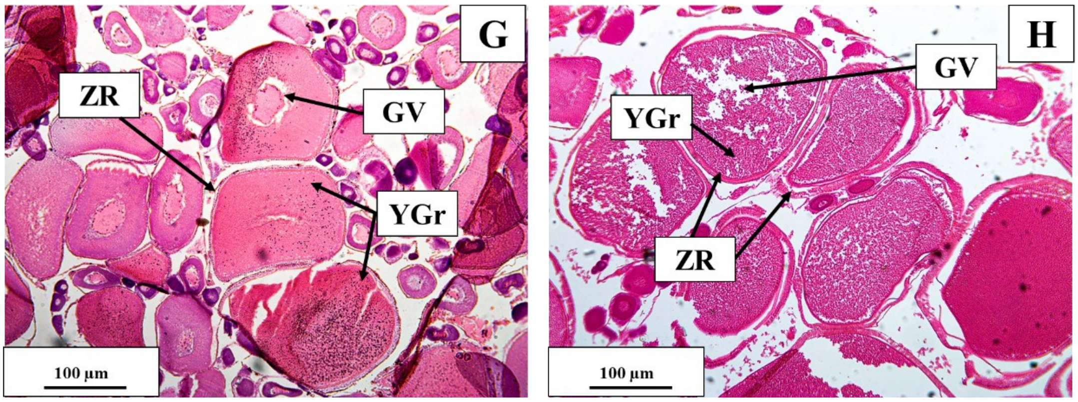


Plate 11: Ovarian maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the secondary vitellogenic stage (G, H). YGr = yolk granule, GV = germinal vesicle, ZR = zona radiata. Scale bar = 100 µm

4.7.5 Ripe stage: Ovary was largest, dense and light yellow in color having GSI at its highest (2.31± 0.36). Vascularization was highly conspicuous. Ovary occupied most of the body cavity. Oocyte diameter increased to highest in this stage (0.830 µm ± 0.045). Post ovulatory follicles were also present. This stage was seen in the month April and May (Plate 12).

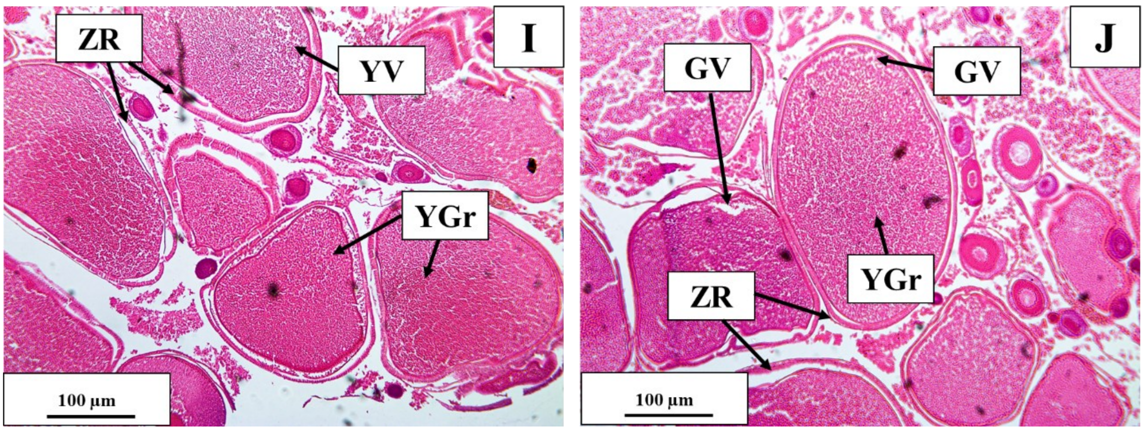


Plate 12: Ovarian maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the ripe stage (I, J). YGr = yolk granule, GV = germinal vesicle, ZR = zona radiata, YV = yolk vessicle. Scale bar = 100 µm

4.7.6 Spent/ regressing stage: A sudden decrease in the ovary was seen. The ovary became shrunken, hollow, sac-like structure. There were abundant number of partially spent ovaries present. Oocytes in different vitellogenic stages were also found. Oocyte diameter in this stage was 0.673 µm ± 0.085. GSI value experienced sudden fall (0.658) in this stage which was found in the month June and July (Plate 13).

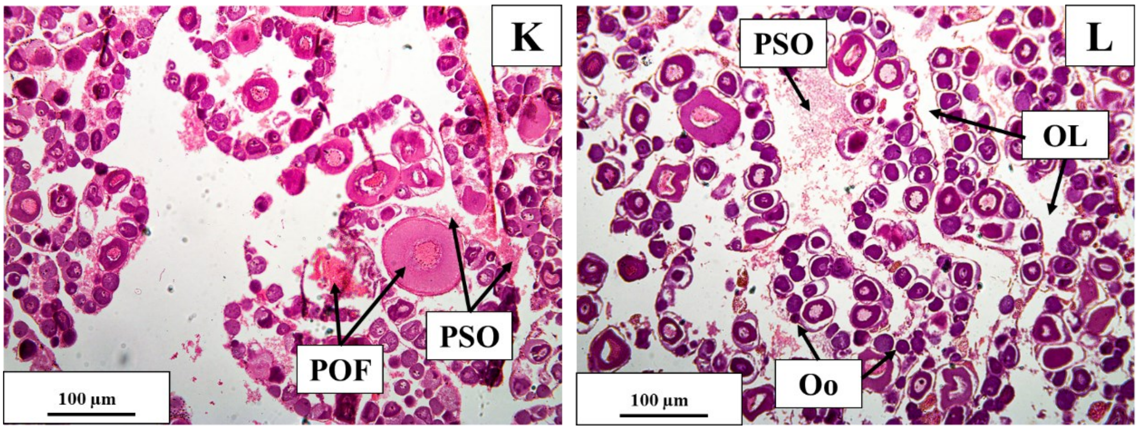


Plate 13: Ovarian maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the spent and regressing stage (K, L). POF = post ovulatory follicle, PSO = partially spent ovary, Oo = oogonia, OL = ovarian lamellae. Scale bar = 100 µm

4.8 Gonadal maturity stages in male:

4.8.1 Immature: Testes in this stage were small and creamy whitish in color. The seminal lobule of testes contained a large number of spermatogonia with few numbers of spermatocyte. Spermatogonia were spherical in shape and stained with hematoxylin. Prevalence of primary spermatogonia was seen. Lumen of the tubules was imperceptible. This stage was found in the month of September and October with an average GSI value of 0.0535 (Plate 14).

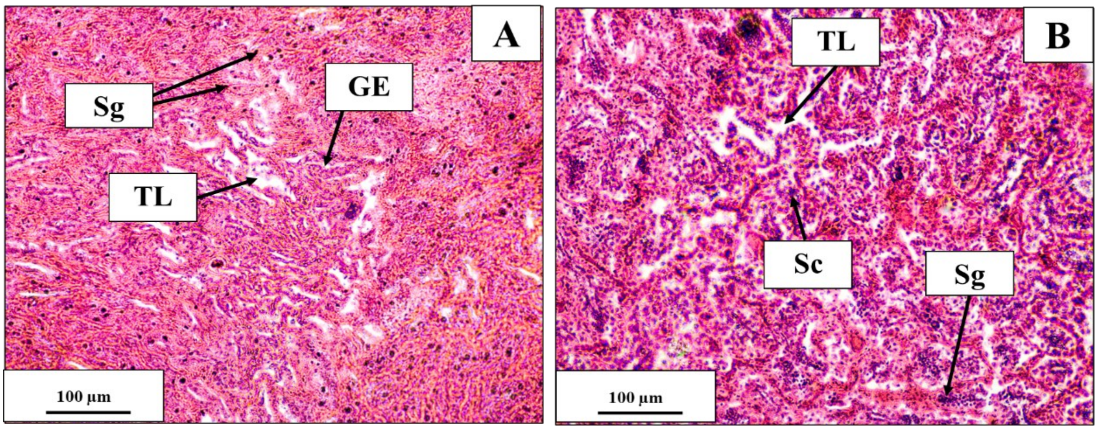


Plate 14: Testicular maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the immature stage (A, B). TL= testiclar lumen, GE= Germinal epithelium, Sc= Spermatocyte, Sg= secondary spermatogonia. Scale bar = 100 µm

4.8.2 Developing: Testes were slightly larger, flat and translucent in color. Germinal epithelium was seen throughout the testes in this stage. Spermatocytes formation started. Primary spermatocyte, secondary spermatogonia, secondary spermatocyte was also seen. This stage was found in the month of November and December with the GSI value of 0.059 (Plate 15).

4.8.3 Pre-spawning: Testes were in late developing stages with spermatozoa in the lumens of the sperm ducts. All stages of spermatogenesis like spermatogonia, spermatocyte and spermatids were usually seen in this stage, among them spermatocytes were predominant in the sperm tissue. Many cysts containing spermatocytes were seen throughout the seminal lobule. Testes were then looking creamy white and soft. In this stage, the GSI was 0.0989 and predominantly seen in the month January and February (Plate 15).

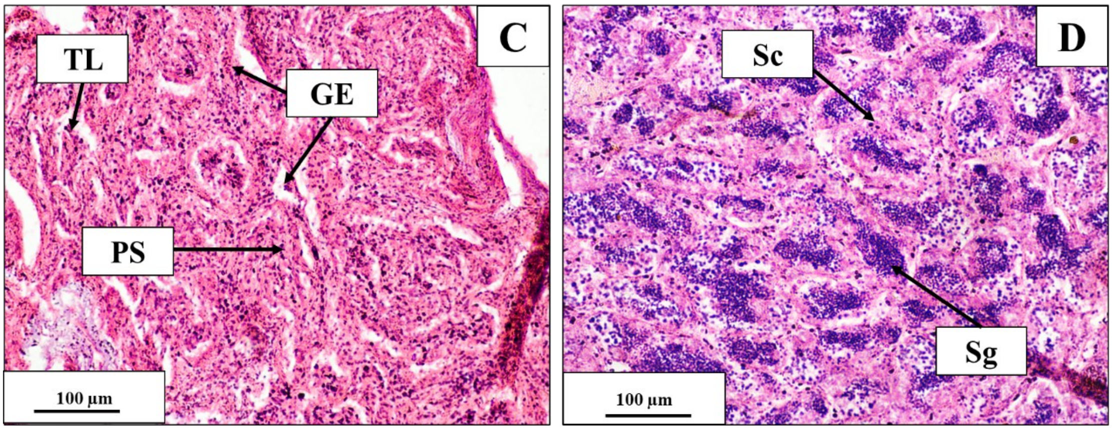


Plate 15: Testicular maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the developing (C) and pre-spawning stage (D). PS= primary spermatogonia, TL= testicular lumen, GE= germinal epithelium, Sg= secondary spermatogonia. Scale bar = 100 µm

4.8.4 Ripe: Number of spermatids in the seminal lobules and spermatozoa in the lumen were increased. The number of spermatogonia and spermatocyte decreased with increase of the spermatids. The spermatozoa were spherical shaped. This stage was found in the month March and April with an average GSI of 0.137 (Plate 16).

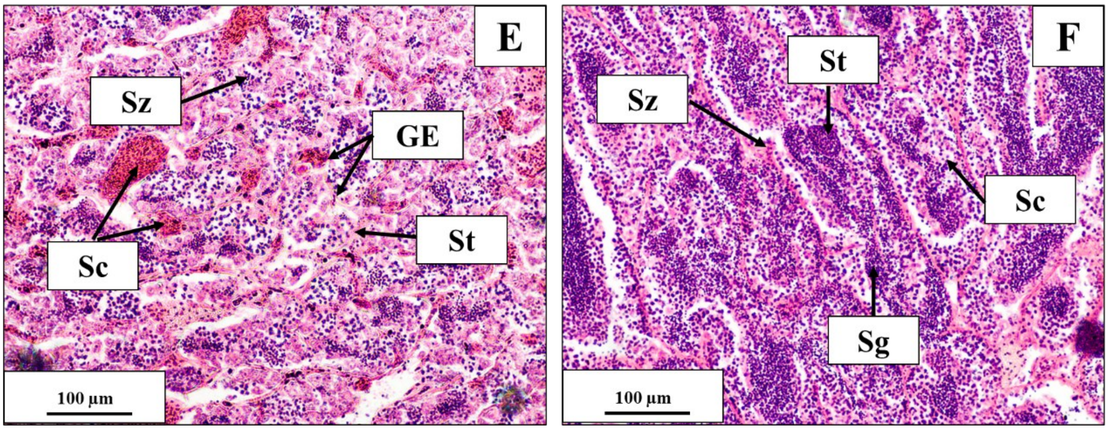


Plate 16: Testicular maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the ripe stage (E and F). TL= testicular lumen, GE= germinal epithelium, Sg= secondary spermatogonia, St = spermatocyte. Scale bar = 100 µm

4.8.5 Spawning: Testes in this stage was large, maximum in GSI (0.4795), creamy white and soft. Histological slides were abundant with mature spermatozoa in the peripheral and central ducts of testes and throughout its lumen. This stage was found in the month May and June (Plate 17).

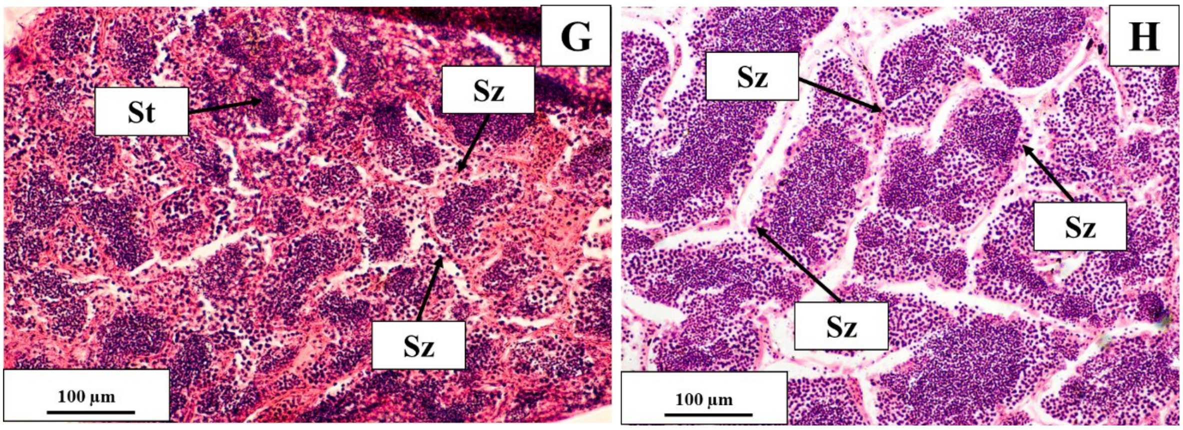


Plate 17: Testicular maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the Spawning stage (G and H). Sz = Spermatozoa, St = Spermatocytes. Scale bar = 100 µm

4.8.6 Post-spawning: The GSI value decreased suddenly. Testes was found to be very thin and transparent. Some spermatozoa and residual spermatids were present, but empty spaces show the regressing stage of testes. This stage was found in the month July and August (Plate 18).

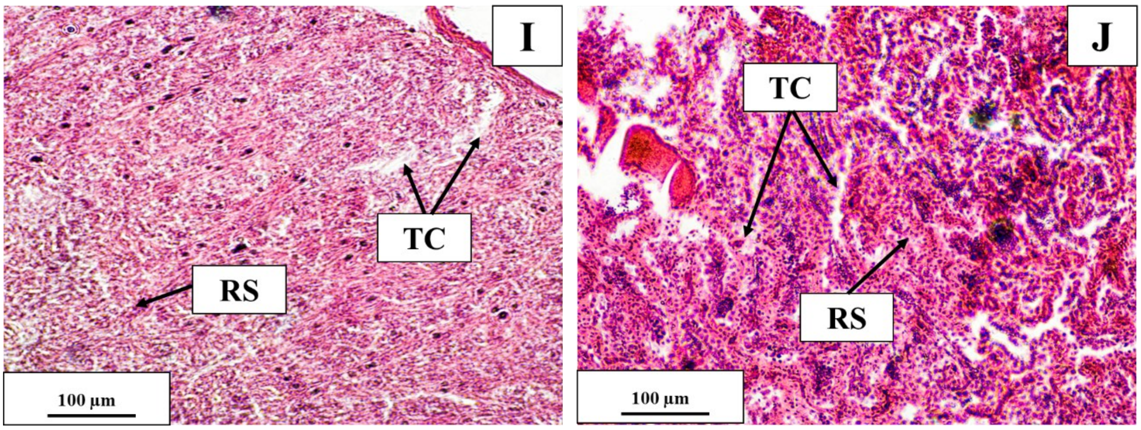


Plate 18: Testicular maturation stages of *M. aor* collected from the Kaptai lake, Bangladesh showing the post-spawning stage (I and J). RS = Residual spermatid, TC = Testicular cavity. Scale bar = 100 µm

CHAPTER: 5

DISCUSSION

Studies on reproductive biology are essential for sustainable conservation and management; also, a fundamental tool to assess culture potential of a fish species, as it is an assessment of general circumstances of fish regarding reproductive biology (Froese, 2006; Tsoumani et al., 2006; Britton and Davies, 2007). This study observed the reproductive biology and gonadal maturation stages along with some morphometric parameters such as length-weight relationship, condition factor and relative condition factor, hepato and gonado-somatic index, length at first maturity (L_m), oocyte diameter and fecundity of *Mystus aor* from the Kaptai lake of Bangladesh. The results show the breeding season to be in May and June with good health condition and reproductive potentiality.

5.1 Length-weight relationship

Length-weight relationships are employed to determine the weight associated with a given length. In fisheries management and research, biometric relationships are frequently used to transform field data into usable metrics. As direct weight measurements take time in the field, biomass is frequently calculated using length-weight regression coefficient (b) values. Positive allometric growth occurs when the value of "b" is larger than 3.0, while isometric growth occurs when it is equal to 3.0. Conversely, negative allometric growth occurs when the value of "b" is less than 3.0, and results in a population where fish become less rotund as length grows (negative allometric growth).

The computed "b" value for the male and female participants in the current study was 2.4491 and 3.2999, respectively, whereas it was 2.6467 for the pooled data. For the pooled data and for females, the "b" value suggests a negative allometric growth pattern, but for males, the growth is positive allometric. A lower exponential value in the female suggests a slower rate of growth in *M. aor*, whereas a higher exponential value in the male indicates a faster rate of weight gain relative to its length. This change in the b value was brought on by differences in the physiology of the male and female, the environment, the availability of food, and the ecological state of the habitats (Le Cren, 1951). However, the combined data for *M. aor* revealed a negative allometric growth trend, indicating that weight gain in either species is not proportionate to growth in body length.

Growth in *M. aor* was discovered to be negatively allometric in the study by Azadi et al., (1992) in both males (b = 2.939), females (b = 2.941), and pooled data (b = 2.890). Additionally, Kumar et al., (2014) reported the negative allometric growth pattern in S. aor. However, Sani, (2010) observed an isometric growth pattern of *S. aor* in their research in the Betwa (2.98) and Gomti (3.02) revers of Indian. The variations in sample size, age or size group of the sample, geographic differences, and seasonal change may all contribute to the differences in "b" values.

5.2 Condition factor

The condition factor is a measurement of the physiological status and general well-being of fish and it takes into account things like maturity, spawning, environmental conditions, and availability of foods (Brown, 1957). Information on seasonal variation in a fish's condition in respect to both the internal and exterior environment becomes crucial in fisheries biological research because the condition of fish can change substantially due to physiological, environmental, nutritional, and biological cycles.

Since most fish do not comply to the cube law in their natural habitat, correlation and interpretation of these results can often be challenging. The function of fatness and gonad state are indicated by the condition factor, which can be better addressed by using the relative condition factor "Kn" (Le Cren, 1951). The Kn value depends upon the stage and maturity of the gonads and length of the fish (Chakrabarty and Singh, 1963). The Kn value more than 1 indicates good health of the fishes and Kn less than 1 indicates poor condition of fish Le Cren, (1951),

In the present study, the monthly K values of *M. aor* from the Kaptai lake ranged from 1.15 ± 0.05 to 1.41 ± 0.57 and the Kn values ranged from 1.06 ± 0.11 to 0.93 ± 0.10. K was maximum in July (1.41 ± 0.57) and minimum in November (1.15 ± 0.05), whereas Kn value was maximum in July (1.06 ± 0.11) and minimum in the month of December (0.93 ± 0.10) which indicate highest growth in the monsoon season and lower growth during winter. This is due to the suitable temperature, habitat condition and nutrition profile available during the monsoon season in comparison to the condition in winter in the Kaptai lake. The values of K and Kn in the present study indicate the good health condition of the fish in the Kaptai lake as they are above 1. This finding coincides with the values of Kn of *M. aor* from the Kaptai lake (Azadi et al., 1992) and the values of Kn for *S. aor* from the Gomti river (Sarkar et al., 2013).

5.3 Fecundity

The fecundity of a species is an essential aspect to consider when managing fish stocks. Fecundity is also crucial biological traits for assessing the reproductive potential of fish (Begum et al., 2010). By using randomly selected gravid female, fecundity was calculated from *M. aor* and the fecundity of the fish found to vary from 18474 – 55504 eggs/female weighting from 536.8 – 1480.4g.

Azadi et al., (1992) reported the fecundity of *M. aor* from the Kaptai reservoir in Bangladesh as 12,560 – 48,635 eggs/female. Ranganathan and Radha, (1966) reported the fecundity range varied from 21,490 – 38,400 eggs/female from the Bhabanisagar reservoir, India. But Saigal, (1964) found the fecundity to be 45,000 – 1,22,500. In a recent study from the North-East Bangladesh, the fecundity of *M. aor* varied from 59,255 – 70,586 with an average value of 64,920 eggs/female (Jabed et al., 2021). Variations in the fecundity might be due to the differences in stocks, size of fish and nutritional status of fish and the habitats characteristics (Duponchelle et al., 2000).

5.4 Hepato-somatic index (HSI)

The condition of the liver affects the metabolic activity and overall health status of fish. The ration between the liver weight to body weight is a sign of energy reserves in fish under varied environmental conditions. So that the hepato-somatic index (HSI) is crucial for figuring out the physiological status of fish.

In the present investigation, HSI of *M. aor* was recorded monthly and found highest in March (1.164 ± 0.07) and lowest in February (0.518 ± 0.15) in male whereas the value was found highest in May (1.132 ± 0.25) and lowest in October (0.67 ± 0.27) in females. The increased HSI value of males and females during the breeding months indicate a good reserve of lipid for reproductive expenditure. The HSI value rises when the food is readily available in large quantities and the environment is in suitable conditions. *M. aor* may have higher HSI values in May and March as a result of the good water quality, the surrounding environment, and the availability of food. This is the first record of HSI from the Kaptai lake of Bangladesh which could further help in sustainable management.

5.5 Length at first sexual maturity (L_m)

It is necessary to adjust the catch size group by regulating the mesh size and allowing the fish to reproduce for better stock maintenance (Lucifora et al., 1999). Understanding the size of the fish at maturity and the season in which they reproduce is crucial for efficient fish population management and conservation (Hunter et al., 1992). In this study, length at first sexual maturity (L_m) was estimated by using multiple functions such as the relationship of (i) GSI vs.TL; (ii) MGSI vs. TL; and (iii) DI vs. TL and found that the length at first maturity was 49.5 cm and 54.1cm in male and female respectively. According to Azadi et al., (1992), the L_m of a female *M. aor* was 48 cm The L_m for *S. aor* was 84 cm according to Saigal (1964), and 57.3 cm according to Ramakrishniah (1992) which is close to our findings.

5.5 Gonado-somatic index (GSI) and oocyte diameter

The gonadosomatic index (GSI) is a good indicator of reproductive activity and is also related to the stages of gonadal maturation (Le Cren 1951, Hismayasari et al., 2015). It is used to improve the accuracy in determining the maturity stages and breeding time of fish species. In this study, a gradual increase in GSI was observed from the month of December to February, reaching a peak in March to May and then decreasing from June to November. GSI value was highest in May for both male and female *M. aor* indicating the peak spawning season. Similar cyclic changes were found in *M. aor* from Kaptai lake by Azadi et al., (1992) with a prolonged peak time from April to July.

A gradual increase in oocyte diameter was also observed in *M. aor* in association with the GSI as they approached towards gonadal maturity. The oocyte diameter and GSI values in *M. aor* delineate a homogenous growth pattern while reaching the highest oocyte diameter value in May (0.830 ± 0.109 µm). Findings from earlier studies on *M. aor* from Kaptai lake was comparable to our findings on oocyte diameter. However, the value was relatively higher in the study conducted by Saigal (1964) where they found oocyte diameter ranged between $1.60 - 2.15$ µm during spawning months.

5.7 Gonadal histology and spawning periodicity

The spawning season is essential for deciding when to reproduce for fish. Fishery biologists and management planners typically estimate the phases of fish maturity by macroscopic and microscopic inspection to pinpoint when spawning takes place and then analyze and set objectives for management action. The most accurate way to assess gonadal maturation is thought to be by histological examination (West, 1990). The gonads of *M. aor* were examined histologically in the present study, and it was discovered that females had much more yolk granules from April to June. In April and May, mature eggs began to come out from the belly upon light pressure, which is a sign that the ovaries will begin to mature in the coming months. Multiple post-ovulatory follicles were noticed throughout the ovaries in the months of June and July. The ovary included many atresia and empty areas, according to histological studies obtained in July. In September, a small number of oogonia also started to grow, indicating the start of a new gonadal maturation cycle. The months of October and November are particularly rich with chromatin nuclear and perinuclear oocytes, which continued to grow until the beginning of February, when yolk granules first appeared.

Male spermatogonia were prominent in September and October, and from November through February, they progressively began to mature into secondary spermatogonia and spermatocytes. In the months of April and May, mature spermatozoa were detected in the peripheral and central ducts of testes as well as throughout their lumen, which suggests that males were spawning at this particular period. Numerous empty spaces with some spermatozoa still present in the testis were found in June and July, which is a sign that spawning is regressing. This finding predicts that gonadal maturation will peak between April and June and then begin to decline.

Azadi et al., (1992) reported that April to July as the breeding season by studying the oocyte diameter for *M. aor* in Kaptai reservoir, Bangladesh which coincides with our findings. A histological study on the gonadal development of *S. aor* from the North-east Bangladesh revealed that the breeding season of *S. aor* lasts for a long duration from April to August and peaks in July to August in hatcheries (Jabed et al., 2021). Ramakrishniah, (1992) found a prolonged breeding season, starting from April to October. The knowledge obtained through histological studies of gonads of *M. aor* provides the information that the breeding period of this species is from April to June with peak in May in the Kaptai lake which will further help in sustainable management of this species.

CHAPTER: 6
CONCLUSIONS

In fisheries research, stock assessment, and management, reproductive biology is crucial. Sustainable, productive fisheries and aquaculture enhance income and improve livelihoods, promote economic growth, and safeguard the environment and natural resources, all while improving food and nutrition security. A sustainable approach to fisheries and aquaculture will aid in the conservation of natural resources and the preservation of fish populations for future generations. Because reproductive features of any species influence its intrinsic capability and sustainability of exploitation, open water fisheries management heavily relies on information about distinct stages of reproductive development of any fish population. Fishing laws must be based on a thorough understanding of the reproductive cycles and population dynamics of the area's commercially significant species. Experimental results on length-weight relationship, condition factor, relative condition factor, GSI (Gonadosomatic Index), HSI (Hepatosomatic Index), fecundity, oocyte diameter and histological study of gonad delineate that breeding season of *M. aor* starts from April and ends at May which partially coincide with the existing ban period. So, the ban period should be advanced for one month to ensure proper broodstock management and improvement.

CHAPTER: 7

RECOMMENDATIONS AND FUTURE PERSPECTIVES

This study set out to characterize the *M. aor*'s life cycle traits and spawning season in the Kaptai lake. The results of this study will be useful for the fisheries management authority in setting the *M. aor* fishing ban duration and maintaining a sustainable stock. Additionally, the establishment of artificial breeding will be possible from this work. Although a qualitative approach was followed to explore the objective of the research, there are some limitations of the study which can be minimized by following the recommendations:

- Fresh and properly preserved samples give better histological diagram. So, samples should be collected directly from the fisherman immediately after catch.
- Different sample collection sites should be followed to identify the differences of spawning season based on the geographical location.
- Further research on intensive breeding biology may be conducted with the samples collected from different breeding sites.
- Artificial propagation of *M. aor* may be possible following its breeding biology.

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APPENDICES

Appendix 1: Data of total length, standard length, body weight of collected *M. aor* fishes

Month	Total Length (TL)	Standard Length (SL)	Body Weight (BW)	Log 10 TL	Log 10 SL	Log 10 BW
November, 2020	64.5	45.8	1150	1.80956	1.660865	3.060698
	56.4	43.8	1030	1.751279	1.641474	3.012837
	55.1	40.7	808.8	1.741152	1.609594	2.907841
	64.6	44.8	1080	1.810233	1.651278	3.033424
	56.5	33.9	1141	1.752048	1.5302	3.057286
	66.8	50.5	1480.8	1.824776	1.703291	3.170496
	57.7	40.8	821.4	1.761176	1.61066	2.914555
	56.3	41.2	870.4	1.750508	1.614897	2.939719
	49.5	38.5	772.6	1.694605	1.585461	2.887955
December, 2020	55.5	42.7	1149.2	1.744293	1.630428	3.060396
	53.9	45.6	1254.6	1.731589	1.658965	3.098505
	53.6	43.1	1140.6	1.729165	1.634477	3.057133
	57.5	44	1114.8	1.759668	1.643453	3.047197
	56.3	43.3	1167.8	1.750508	1.636488	3.067368
	53.5	42.5	1014.8	1.728354	1.628389	3.00638
	63.7	47.8	1291.8	1.804139	1.679428	3.111195
	61.5	48.5	1540	1.788875	1.685742	3.187521
	58.5	45.8	1575.4	1.767156	1.660865	3.197391
	51.7	40.3	846.2	1.713491	1.605305	2.927473
January, 2021	53.9	42.2	1164.8	1.731589	1.625312	3.066251
	56.4	43.2	1248	1.751279	1.635484	3.096215
	52	40.8	1013.6	1.716003	1.61066	3.005867
	55.9	42.2	910.8	1.747412	1.625312	2.959423
	58.5	44.7	1165.2	1.767156	1.650308	3.0664
	54.4	44.3	1348.2	1.735599	1.646404	3.129754
	56.2	44.9	1086.6	1.749736	1.652246	3.03607

	54.9	42	927.4	1.739572	1.623249	2.967267
	54.9	42.2	1075.8	1.739572	1.625312	3.031732
	54.9	42.9	1097.8	1.739572	1.632457	3.040523
	55.1	42.9	1020.4	1.741152	1.632457	3.00877
	60.4	43.6	1012.4	1.781037	1.639486	3.005352
February, 2021	47.9	44.8	1378.4	1.680336	1.651278	3.139375
	51.3	40.3	965	1.710117	1.605305	2.984527
	55.9	43.5	1113.2	1.747412	1.638489	3.046573
	56.5	44.8	1198.8	1.752048	1.651278	3.078747
	55	42.5	970	1.740363	1.628389	2.986772
	53.2	41.3	973.4	1.725912	1.61595	2.988291
	56.2	42.9	1033	1.749736	1.632457	3.0141
	57.9	45.1	1331.8	1.762679	1.654177	3.124439
	55	42.3	983.8	1.740363	1.62634	2.992907
	54.5	42.5	1082.4	1.736397	1.628389	3.034388
	56	44.6	1189.6	1.748188	1.649335	3.075401
	56.2	45	1232.8	1.749736	1.653213	3.090893
	68.5	49.7	1397.4	1.835691	1.696356	3.145321
March, 2021	51.1	39.6	907	1.708421	1.597695	2.957607
	55	43.7	1212.8	1.740363	1.640481	3.083789
	53.5	42.3	1210.4	1.728354	1.62634	3.082929
	56.5	42.3	923.4	1.752048	1.62634	2.96539
	50.1	39.4	789	1.699838	1.595496	2.897077
	56	42.8	1095.4	1.748188	1.631444	3.039573
	57.8	44.7	1366.4	1.761928	1.650308	3.135578
	53.9	43.4	1004.8	1.731589	1.63749	3.00208
	54.8	41.8	1059.8	1.738781	1.621176	3.025224
	61.7	47	1198	1.790285	1.672098	3.078457
	55	41.9	1031.2	1.740363	1.622214	3.013343
	50.3	40.8	781.8	1.701568	1.61066	2.893096
	56.5	43.9	954.8	1.752048	1.642465	2.979912
	59.2	48.7	1480.4	1.772322	1.687529	3.170379

April, 2021	57.5	46.2	1188.6	1.759668	1.664642	3.075036
	54.1	43.2	919	1.733197	1.635484	2.963316
	59.2	48	1290.4	1.772322	1.681241	3.110724
	48.8	39.6	752.4	1.68842	1.597695	2.876449
May, 2021	45.9	40.2	975.2	1.661813	1.604226	2.989094
	57.6	41	969.8	1.760422	1.612784	2.986682
	49.5	39.3	805.2	1.694605	1.594393	2.905904
	59.6	49.6	1457.2	1.775246	1.695482	3.163519
	52.8	44.4	1005.4	1.722634	1.647383	3.002339
	56	43.8	928.2	1.748188	1.641474	2.967642
June, 2021	47	37.4	610.2	1.672098	1.572872	2.785472
	45.8	35.5	585.6	1.660865	1.550228	2.767601
	45.4	38.2	620.6	1.657056	1.582063	2.792812
	46.4	36.3	612.8	1.666518	1.559907	2.787319
	44.3	34.5	595.7	1.646404	1.537819	2.775028
	46.7	35.2	536.8	1.669317	1.546543	2.729813
July, 2021	55.1	41.8	875.6	1.741152	1.621176	2.942306
	52.5	40.3	754.8	1.720159	1.605305	2.877832
	54.1	39.1	685.3	1.733197	1.592177	2.835881
	54.5	41.6	857.8	1.736397	1.619093	2.933386
	53.6	40.7	784.8	1.729165	1.609594	2.894759
	53.2	38.5	604	1.725912	1.585461	2.781037
August, 2021	49.6	36.5	612.2	1.695482	1.562293	2.786893
	57.3	43.5	1024.8	1.758155	1.638489	3.010639
	56.8	43.8	1073.8	1.754348	1.641474	3.030923
	49.9	39	719	1.698101	1.591065	2.856729
	50.7	37.8	710.6	1.705008	1.577492	2.851625
	50.5	39	764.8	1.703291	1.591065	2.883548
	46.8	35.4	601.6	1.670246	1.549003	2.779308
	47.2	35.8	575.2	1.673942	1.553883	2.759819
	52.9	41.6	908.4	1.723456	1.619093	2.958277
	53	42	827.2	1.724276	1.623249	2.917611

September, 2021	60.9	47.2	1482.4	1.784617	1.673942	3.170965
	57.5	45	1231.2	1.759668	1.653213	3.090329
	59.9	43.3	860.8	1.777427	1.636488	2.934902
	60	42.2	1044.4	1.778151	1.625312	3.018867
	58.5	44.4	1195.2	1.767156	1.647383	3.077441
	61	45.5	1066.8	1.78533	1.658011	3.028083
	62.7	45.5	1063.2	1.797268	1.658011	3.026615
	57.1	43.2	1118.8	1.756636	1.635484	3.048752
	57.6	45.3	1117.4	1.760422	1.656098	3.048209
	64.5	48.3	1182.2	1.80956	1.683947	3.072691
	57.5	44.1	1147.4	1.759668	1.644439	3.059715
October, 2021	51	41.9	993.8	1.70757	1.622214	2.997299
	49.5	39.3	754.4	1.694605	1.594393	2.877602
	59.7	47.1	1485.8	1.775974	1.673021	3.17196
	57.5	45.1	1211.4	1.759668	1.654177	3.083288
	59.1	45.6	1283.4	1.771587	1.658965	3.108362
	54	42.1	980.8	1.732394	1.624282	2.99158
	52.1	42.1	964.4	1.716838	1.624282	2.984257

Appendix 2: Condition Factor (K) and Relative Condition Factor (Kn) data of *M. aor* (Male and Female)

Month	Mean K ± S.D.		Mean Kn ± S.D.	
	Male	Female	Male	Female
November, 2020	0.904±0.019	1.515±0.696	0.904±0.019	1.125±0.424
December, 2020	1.399±0.108	1.369±0.135	1.0434±0.095	1.079±0.114
January, 2021	1.451±0.161	1.330±0.119	1.096±0.121	1.036±0.093
February, 2021	1.389±0.098	1.329±0.100	1.045±0.075	1.047±0.066
March, 2021	1.504±0.082	1.334±0.131	1.139±0.062	1.031±0.101
April, 2021	1.139±0.016	1.201±0.054	0.860±0.025	0.955±0.072
May, 2021	1.454±0.066	1.193±0.096	1.111±0.055	0.937±0.063
June, 2021	1.196±0.101	1.321±0.115	0.940±0.090	0.917±0.072
July, 2021	1.166±0.029	1.138±0.070	0.892±0.014	0.850±0.071
August, 2021	1.261±0.017	1.258±0.078	0.959±0.031	0.917±0.042
September, 2021	1.274±0.187	1.249±0.137	0.941±0.128	0.987±0.089
October, 2021	1.339±0.090	1.320±0.025	1.001±0.042	1.0318±0.042

Appendix 3: Month wise hepato-somatic index (HSI) and gonado-somatic index (GSI) of *M. aor* (both male and female)

Month	HIS (Mean ± S.D.)		GSI (Mean ± S.D.)	
	Male	Female	Male	Female
November, 2020	0.602±0.069	0.599±0.151	0.054±0.070	0.233±0.213
December, 2020	0.684±0.138	0.846±0.265	0.064±0.048	0.494±0.262
January, 2021	0.675±0.245	0.220±0.199	0.042±0.028	0.429±0.157
February, 2021	0.518±0.157	0.929±0.207	0.156±0.302	0.753±0.359
March, 2021	1.163±0.660	0.975±0.159	0.147±0.062	1.912±1.096
April, 2021	0.631±0.074	0.958±0.165	0.126±0.069	2.142±0.367
May, 2021	0.822±0.128	1.132±0.256	0.909±0.052	2.177±0.392
June, 2021	0.854±0.084	0.899±0.05	0.180±0.020	1.898±1.321
July, 2021	0.962±0.108	0.878±0.125	0.041±0.008	0.659±0.792
August, 2021	0.750±0.197	0.705±0.241	0.645±1.068	0.548±0.449
September, 2021	0.802±0.031	0.726±0.229	0.080±0.066	0.298±0.308
October, 2021	0.715±0.098	0.674±0.096	0.028±0.003	0.309±0.012

Brief Biography of the Author

Shifat Ara Noor, daughter of Md. Nurul Islam Mazumder and Sayeda Naznin Akter, is from Sadar South Upazila under Cumilla district of Bangladesh. She has completed her B.Sc. in Fisheries (Hons.) Degree in 2020 from Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh obtaining 3.96 out of 4.00. Now, she is a candidate for the degree of M.Sc. in Fish Biology and Biotechnology under the Department of Fish Biology and Biotechnology, Faculty of Fisheries, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh. She has research interest in the field of histology, fish reproductive biology and biotechnology.