STUDY ON THE MORPHOMETRIC TRAITS AND BREEDING BIOLOGY OF *Meretrix meretrix* COLLECTED FROM MOHESHKHALI CHANNEL, COX'S BAZAR, BANGLADESH

Roll No. 0121/03

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A thesis submitted in the partial fulfillment of the requirements for the degree of Master of Science in Marine Bioresource Science

Department of Marine Bioresource Science

Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University

Chattogram-4225, Bangladesh

AUGUST 2023

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Supervisor

Co-supervisor

Dr. Sadequr Rahman Khan
Chairman of the Examination Committee

Department of Marine Bioresource Science
Faculty of Fisheries

Chattogram Veterinary and Animal Sciences University
Chattogram-4225, Bangladesh

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The Author

DEDICATED TO MY BELOVED PARENTS AND FAMILY MEMBERS

Table of Contents

List Of Tables

List Of Figures

LIST OF APPENDICES

ABBREVIATIONS

FAO	Food and Agricultural Organization
WHO	World Health Organization
TW	Total Weight
STW	Soft Tissue Weight
CI	Condition Index
GSI	Gonadosomatic Index
ANOVA	Analysis of Variance
MPEDA	Marine Products Export Development Authority

ABSTRACT

The potential local and international markets, suitable habitats and by-product use has made the Asian hard shell clam, *Meretrix meretrix* as one of the potential species that may enhance the blue economy of Bangladesh. The fundamental aspects of reproductive biology, biometric, and morphometric traits for this genus in the coastal waters of Bangladesh remain unexplored. Therefore, this study was conducted to delineate the morphometric traits and breeding biology, including the Gonadosomatic Index (GSI) and Condition Index (CI), of Asian hard clam *M. meretrix*. A total of 2267 samples were collected from Chowfaldandi canal, near the Moheskhali Channel, over a year (from December, 2021 to November, 2022) during full-moon cycle at low tide. The positive correlations were observed between shell length- total weight, shell height- total weight-shell-width ($p< 0.001$). These relationships suggested that specific morphometric traits were linked to clam growth patterns, where allometric growth patterns were observed ($W= 0.046* L^{1.63}$, $b=2.021$). The sex ratio male to female for this species was 1:1.66 ($P > 0.001$) determined through *Welch F test.* The gametogenesis cycles was observed through histology showed that spawning periods for males were from February to April 2022, and for females from March to May 2022.The gametogenesis cycles of male and female *M. meretrix* exhibited stages of development and maturation, with males showing a tendency to initiate gametogenesis earlier than females. GSI value reached its peak in November-December indicating initiation of spawning period and reached its lowest point in June-July, signifying a period of inactivity or rest. The highest condition index value was noted in March 2022 (5.44±2.05), while the lowest was recorded in May,2022(2.16±1.31). Interestingly a high CI value in July despite being associated with the resting phase, emphasizing that CI values are influenced by factors beyond reproductive changes, such as water parameters and environmental conditions. Overall, this comprehensive study sheds light on the morphometric traits, breeding biology, and reproductive cycles of *Meretrix meretrix* providing valuable insights into its life history and potential reproductive periods.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Mollusks account for a significant portion of global fisheries (FAO, 2011). According to FAO 2020, the global mollusk production is 17.511 MT. Of all the molluscan shellfish produced worldwide, marine bivalves make up about 14% of the total (FAO,2016).The majority of marine bivalve production, with an annual economic value of US $20.6 billion whereas only as small portion (11%) comes from wild capture harvest (FAO, 2016). In 2015, the worldwide harvest of marine bivalves, considering both capture fisheries and aquaculture, exceeded 16 million metric tons. Among these, oysters constituted 24%, mussels 18%, scallops 25%, and clams 33% of the total harvest (FAO, 2017). Bivalves are the second largest mollusk class with about 10,000 living species are known throughout the world (Wye, 1989). Bivalve meats are consumed for their nutritional content as well as their flavor (Wijsman, 2019). It is a prominent and highly desirable food item in China, Korea, Thailand, Japan, and the European Union due to increased need for proteins from a growing population and rising living standards (Smaal *et al*.,2019). Bivalves are good source of vitamins such as vitamin A, vitamin D & vital minerals like iodine, selenium, and calcium (Dong, 2001; Krzynowek, 1989). Bivalves muscles are also low in fat and high in omega-3 fatty acids, all of which have been attributed to a variety of health benefit (Orban 2002; Schug 2009; EFSA 2014). Minerals play a crucial role as essential components of hormones, enzymes, and enzyme activators in human nutrition. Insufficient intake of minerals can lead to biochemical, structural, and functional pathological alterations (Aliasgharpour *et al.*, 2013). The utilization of bivalves for their nutritional value, bioactive compounds and flavors has opened a new horizon for blue economy in the coastal areas of the world (Kartika, 2014).

Bangladesh has exceptionally rich marine waters that cover an area of 165,887 km2, which is more than its land area with 166,000 km2 EEZ towards open sea (Hoq *et al*, 2014). Its coastal areas which covers about 23% of the country are one of the most productive in the world due to its geographical location and climatic conditions (Islam, 2003). Bangladesh's current annual marine fisheries capture is 5,46,333 t, which represents approximately 16% of the BoB's overall marine catch (Siddiqui KU,2007). Siddiqui *et al.* (2007) described 142 marine and 6 freshwater bivalve species have found in Bangladesh. Among the bivalve resources found in Bangladesh, economically significant species include the clam (*M.*

meretrix), green mussel (*Perna viridis and* oyster (*Crassostrea sp*.) (Shahabuddin, 2010). These species are high valued and commercially important all around the world. Around 62% of shellfish is harvested seasonally, whereas 38% is collected year-round and used to fulfill local market demand. (Shahabuddin, 2010). Among mollusks Asian hard clam is abundant in the coastal areas of Bangladesh.

M. meretrix serves as a delectable, cost-effective, and protein-abundant dietary option for individuals residing in coastal areas (Babu, 2012). This species holds substantial economic significance and carries considerable cultural potential across various countries (Zhang, 2002). In Bangladesh, the consumption of *M. meretrix* is primarily seen among tribal populations inhabiting the coastal zone. Nevertheless, there exists an internal demand for mollusk meat among approximately two million tribal individuals, alongside an increased requirement for mollusk shells within the poultry industry (Shamsuzzaman *et al*., 2017). Promising local markets have been identified for clams, including the Rakhine, Buddhist, and Chakma communities in Cox's Bazar, as well as among tribal populations in the Hill Tracts regions (Gosling, 2003). There are also potential export markets in Japan, Thailand, Malaysia, Cambodia, and China, where clams are popular (Solaiman, 2005). The availability of suitable molluskan habitats, such as coastal channel, rivers sandy, muddy shores, tides, mangrove areas, and coral reefs may provide an ideal environment for natural growth, recruitment and farming of *M. meretrix*.It is important to actively acknowledge the cultivation of *M. meretrix* as a pivotal element of coastal aquaculture. This species holds significant promise and could potentially tap into export markets if successfully cultivated in suitable areas of the Bay of Bengal. Additionally, this culture could create alternative employment and livelihood avenues for a substantial portion of the coastal population. Till now clam is collected from wild sources. There is no farming technology been developed for the coastal areas of Bangladesh. For this, studying *M. meretrix* morphometric information & reproductive biology are a must for developing sustainable farming technology and providing relevant scientific guidance for the management of this valuable resource.

Apart from understanding the reproductive cycle, it's crucial to have precise assessments of harvestable biomass, maturity length, and other population parameters to establish sustainable fisheries. The identification of morphometric correlations, such as the relationship between shell length and total weight, among individuals within a population, serves as an initial step in furnishing this information (Newell & Hidu 1982). Similar to other bivalves, *Meretrix sp.* adjust their body weight as time passes, influenced by shell size parameters like length, width, and height (Hemachandra and Thippeswamy, 2008; Singh, 2017). Such a connection between two quantifiable attributes, body weight and shell size, is often referred to as allometry. In fisheries biology research, the study of length-weight correlations and dimensional connections is significant because it establishes a mathematical linkage, allowing one variable to be derived from another (Hibbert, 1977). Often growth is estimated by measuring shell dimensions or the volume of the animal because they are simple and non-destructive methods (Bailey,1988). Once the allometric connection has been established, Shell measurement is an adequate alternative for estimating biomass and total flesh output (Hibbert, 1977; Rodhouse, 1984). Hence, investigations into the allometric growth of marine bivalves are necessary to effectively ascertain the point at which harvesting can be enhanced for achieving optimal aquaculture production (Palmer, 1990).

To ensure the sustainable management of harvested populations, it is essential to assess the natural stocks' ability to regenerate (Olver *et al*.,1995). This is initially based on a comprehensive perception of the species reproductive cycle and life history (Baron, 1992). We need to know not just the exact timing of gametogenic development and spawning, but also sex-ratios for a wide variety of size classes. Bivalves have an annual reproductive cycle that includes gametogenesis, followed by a single or many spawning events (Mouneyrac 2008; Ben Salah 2012; Crnčević 2013; Sawant and Mohite, 2013; Morillo and Manalo, 2016). For obtaining information about the reproductive cycle, the most accurate and comprehensive data can be acquired through histological examination of the gonads (Seed and Suchanek, 1992). In clams, the process of gametogenesis primarily occurs within the mantle tissue, although genital tissue can be distributed throughout the entire body (Seed and Suchanek, 1992). Male and female are practically impossible to differentiate during the non-breeding season, even with histological preparation (Seed and Suchanek, 1992). By utilizing histological sectioning, it becomes possible to accurately identify different stages within the reproductive cycle (Buchanan, 2001).

M. meretrix has been found in coastal waters around Bangladesh's south-eastern coastlines, particularly in the Moheshkhali Channel (Shahabuddin, 2010). In Bangladesh, limited studies have been conducted on the Asian hard clam (*M. meretrix*), which encompass investigations into the biochemical composition of *M. meretrix* originating from the Bakkhali river estuary in Cox's Bazaar (Chowdhury, 2010) & Biometric analysis, encompassing length-weight relationships and condition indices, as well as assessments of growth and mortality rates, was conducted on collected *M. meretrix* from the Moheshkhali channel (Amin, 2009). Previous studies on mollusk show that clams are mostly found and utilized around South-east coast of Bangladesh adjacent to Bay of Bengal where there is suitable habitat,potential market for clam. The reproductive biology, allometric relationships of *M. meretrix* are not known for the local aspect in Bangladesh. Therefore, we studied the gonadal histology and morphometrics of clam to have baseline data on reproductive seasonality, sex ratio, gonadal development and allometric relationship of clam (*M. meretrix*) from south-east coast of Bangladesh.

1.2 Research Objective:

The objectives of the research are:

(i) To study the key morphometric variables of *M. meretrix* to comprehend the allometric relationship

(ii) To investigate the reproductive biology of *M. meretrix* in a multidisciplinary way including GSI, Condition Index (CI) and gonad histology.

CHAPTER TWO

REVIEW OF LITERATURE

*M. meretrix*is an economically potential species for the blue growth of Bangladesh with the achievement of 1,18,803 sq km of marine areas from Myanmar and India in 2012 & 2014 respectively. Government has given emphasis on the coastal and marine aquaculture. To develop aquaculture technique and sustainable management of clam in Bangladesh basic knowledge on clam reproductive biology and allometric relationship are necessary. In this section, this reproductive biology and allometric relationship of clam have been studied based on previous literature.

2.1 Taxonomy details of *M. meretrix* (Linnaeus, 1758)

Phylum: Mollusca

Class: Bivalvia

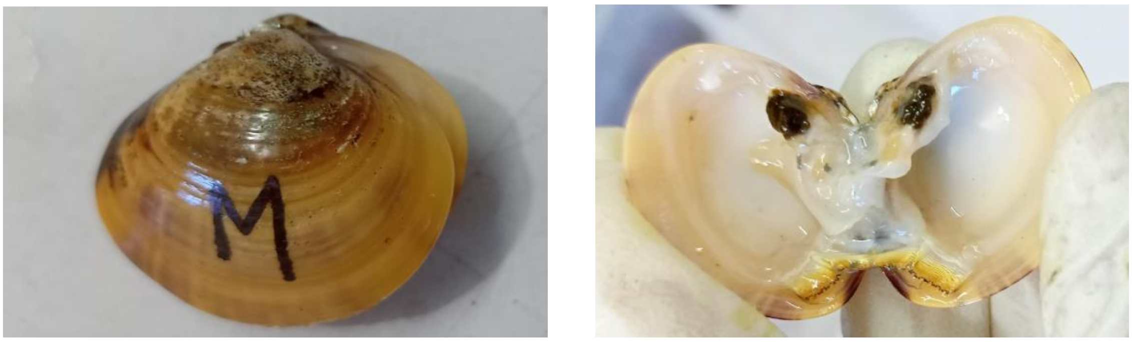
Subclass: Autobranchia

Order: Veneroida

Family: Veneridae

Genus: *Meretrix*

Species: *Meretrix. meretrix*



(a) **(b)**

Figure 1: (a) *M. meretrix* (Linnaeus, 1758) outer shell (b) *M. meretrix* internal organ

2.2 Biological characteristics and Distribution of *M. meretrix*

There has been an estimation of 31,000 different bivalve species worldwide (Ferreira,2014). They have a more uniform shape than gastropods because they are formed of two shell valves that are joined to one another by hinge teeth and a filament structure called a ligament. The bivalves' mantle and shell are growing as they develop. All sections of the body contain internal organs, with the exception of the mantled and feet. The majority of bivalves live in saltwater, however certain species may also be found in brackish and freshwater. Because to their frequent filter-feeding habits, bivalves are rarely observed on land. Bivalves exhibit the capacity to thrive in marine environments due to their ability to consume phytoplankton, which is abundant in such habitats (Kongsa *et al.*, 2019). Similarly, *M. meretrix* shares this characteristic with other bivalve species, utilizing hard shells as a protective covering against potential threats and environmental variations. Typically, the structure of clam bodies is divided into four main sections: the head, foot, visceral mass, and mantle. Among Molluscan species, the mantle stands as the sole organ responsible for producing the shell that encases the visceral mass. The body of the shell, which is protected by a mantle, lacks a head, and has gills that filter food particles out of water intake. For locomotion and burrowing, it has a robust foot muscle. It is buried close below the surface of muddy sand flats, has short siphons, and lives there.

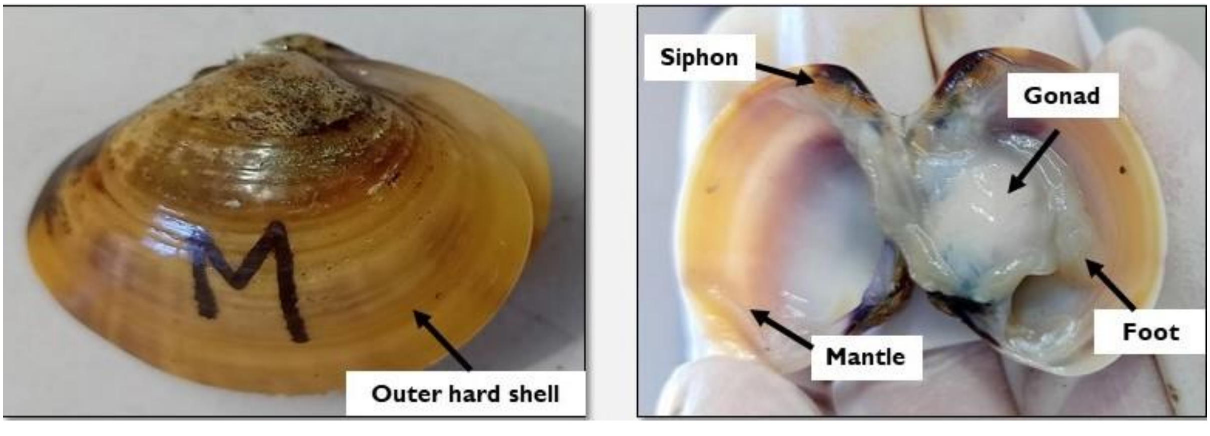


Figure 2: *M. meretrix* body parts

Various surveys were carried out to identify the mollusk species present and their abundance throughout Bangladesh's coast (Ali 1975; Ali and Aziz 1976; Ahmed *et al.*, 2010). It was suggested that in Bangladesh, *M. meretrix* is widely distributed throughout the coastal areas, including St. Martin's Island, Bakkhali river estuary, Sahaparirdip, Teknaf, Cox's Bazar, Moheskhali, and Kutubdia in the Cox's Bazar district. It can also be found in Galachipa and Kalapara in the Patuakhali district, Amtoli, Borguna, and Paterghata in the Borguna district, Mongla port and Saronkhola in the Bagherhat district, as well as Dakop, Koira, and Paikgacha in the Khulna and Satkhira districts (Chowdhury, 2019).

2.3 Mophometric traits of *M. meretrix*:

M. meretrix, a bivalve mollusk found across the Indo-Pacific region, has garnered substantial attention due to its ecological role and commercial importance. Morphometric traits, including shell size, shape, and growth patterns, play a vital role in determining the species' ecological interactions and responses to environmental changes. The shell morphology of *M. meretrix* has been extensively studied to understand its adaptation to different habitats and its responses to various ecological factors (Amin *et al.,* 2009; Narasimham *et al,*.1988) Research has shown that shell size varies across different populations, with variations influenced by factors such as water temperature, salinity, substrate type, and food availability (Abraham, 1953; Adjei-Boateng *et al.*, 2013; Bailey *et al.*, 1988).

In *M. meretrix*, understanding its morphometric traits is essential for understanding its ecological roles, adapting conservation strategies, assessing potential impacts from human activities, and ensuring the sustainable use of this valuable species. The combination of morphometric data with other biological and environmental information enhances our ability to comprehensively study and manage populations and ecosystems

2.4 Reproductive system of Bivalves sex and reproduction

Bivalves have a very basic reproductive system: the gonads are comprised of branching tubules, and gametes bud off the tubules' epithelial lining. Tubules combine to form ducts, which then branch into longer ducts and ultimately terminate in a short gondoic. In the most of bivalves, the gonoduct enters the mantle cavity near the nephridiopore through a pore. During external fertilization, the gametes are discharged through the exhalant orifice of the mantle (Gosling, 2008).

Most bivalves are dioecious, meaning they have separate male and female individuals. Often, there are approximately equal numbers of males and females, although distinguishing between the sexes is challenging based on physical characteristics (Gosling, 2008). The gonad of *M. meretrix* is dispersed within its visceral mass around the internal organs.

2.5 Gametogenesis and reproductive cycle in bivalves

The gametogenic cycle in clams takes place either once a year or multiple times within a year, influenced by the state of water quality, particularly variations in temperature and salinity (Vakily, 1989). This cycle encompasses several stages, starting with the initiation of the gonad, progressing through gametogenesis, leading to the release of gametes, and culminating in the gradual decrease of the gonad (Seed, 1969).Bivalves can be categorized into two primary groups based on their reproductive system: (1) gonochoristic bivalves, where sexes are distinct within individual organisms, such as *Cuspidaria Nado* and *Nacula Lamarck;* and (2) hermaphrodite bivalves, characterized by having both male and female gonads within the same individual, like *Ostrea Linnaeus, Crassostrea Sacco, Anodonta Lamarck, and Pecten Muller* (Uppatham *et al.*,1995). *M. meretrix* is a dioecious or gonochoristic bivalve species, but determining the sex through external traits is not feasible. Reproduction involves external fertilization, with eggs and sperm being released into the water column Gametogenesis is the biological process responsible for producing reproductive cells or gametes (such as oocytes or eggs and spermatozoa). This process involves multiple stages of oocyte and spermatocyte development (Lee *et al.*, 2007). Examining the reproductive cycle of bivalves through histological analysis to discern gonad differentiation (gametogenesis) provides more dependable insights into the timing of spawning seasons compared to relying on overall characteristics or gonad color. Consequently, the approach of histological analysis has gained extensive use in studying the reproductive cycles of diverse bivalve species from different geographical regions.

Bivalves follow a yearly reproductive cycle that encompasses gametogenesis, succeeded by either a single or multiple spawning event (Mouneyrac, 2008; Crnčević,2013; Sawant and Mohite, 2013; Morillo-Manalo, 2016). Seasonal gonadal changes were observed histologically in softshell clam (*Mya arenaria*) in Co. Wexford, Ireland, where it had a single spawning phase in November (Cross, 2012). Victor *et al.,* (1988) addressed that the gametoganic cycle of *Donax cuneatus* on the Madras coast started in November-December, with spawning began in February and last until July. An examination of the gonad development of *Lutraria philippinarum*, collected from North Bais Bay in the Philippines, showed that the processes of gametogenesis, maturation, and spawning season were observed consistently throughout the year. Notably, there were two distinct peaks in spawning, one in January and another in June (Bantoto *et al.*,2012). The reproductive cycle of the baby clam, *Marcia opima* collected from the Indian coast revealed two spawning seasons: May to July and September to December (Suja *et al.*,2007).

Prior research on the gonadal development of *M. meretrix* along the northeast coast of India has unveiled that this species typically exhibits separate sexes, maintaining a sex ratio of 1:1.36 (male:female). The reproductive cycle occurs twice a year, specifically in January to April and June to October, with the highest spawning activity observed during January to February and September (Narasimham,1988). In the east coast, spawning in *M. meretrix* occurs twice a year, around the beginning of September and again in May (Hornell, 1922). Biannual spawning events have also been observed in Thailand. (Thuaicharoen,1986), Western Korea (Chung,2007), Tokyo bay, Japan (Namakura, 2010) and Kerala of India. On the other hand, Single spawning of *M. meretrix* have been recorded along the Mumbai coast of India (Sharma, 2005), Kalbadevi estuary, Ratnagiri, south-east coast of India and South Zhejiang, China (Zhihua,2004). So previous studies on gonad histology have revealed that *M. meretrix* could either have a single spawning activity or two within a year. However, under favorable condition spawning may occur throughout the year (Rai, 1932). For example, under favorable condition, this species spawns for eight months from February to September in Vellar estuary, India (Jayabal and Kalyani, 1986) and in Bombay coast it may spawn all the year round except for the monsoon (Rai,1932).

Table 1: Reproductive season of different clam species

Species	Reproductive season	Methods	Average size of male & female	Country	References
M. meretrix	January-April; June-October	Condition Index	Male:9.91 cm Female:9.6 cm	Korampallam creek, India	(Narasimham, 1988)
M. meretrix	October-November	Gonad histology	Male& Female: 4.5-5 cm	Kalbadevi estuary,India	Mane*et al.*,1988
M. meretrix.	Late May to mid-August; Late June to late July	Gonad histology	Male:7.1 cm Female: 7.02 cm	South Zhejiang	Zhihua *et al.*, *(*2004)

M. meretrix.	October	Length-weight relationship	Male:3.2cm Female:3 cm	Madras, India	Jayabal and Kalyani, (1987)
M. meretrix	June-August; November-January.	GSI, Gonad histology	Male& Female: 2.00-5.77 cm	Thailand	Thuaicharoen *et al.*, (1986)
Meretrix lyrata	May, October & March	Condition Index	Male: Female:	Sarawak. *Malaysia*	Hamli *et al.*, (2017)
Marcia recens	November – February	Gonad histology,GSI	Male: 19.51 mm, Female: 20.09	Andaman sea coast, southern Thailand	Kongasa, W. (2019)
Meretrix lusoria	June to September	Gonad histology	Female: 50 mm	Simpo, Korea	Chung, E. Y. (2007).
M. meretrix		GI, CI	Male:5cm Female: 7cm	Marudu Bay, Malaysia	Duisan (2021).
Meretrix petechialis	December and January	Gonad histology, GI	Female: 50.1mm	Western Korea	Jun *et al.*, (2012)
M. meretrix	February to September	Gonad histology	Male:5cm female: 7cm	Vellar estuary, India	Jayabal and Kalyani, 1987
M. meretrix	January-April and June-October	Gonad histology	Male:5cm female: 7cm	North-east coast of India.	Narasimham,1988
Meretrix lyrata	September& May	Gonad histology	Male:5cm female: 7cm	East coast, India	Hornell, 1922

2.6 Condition index and Gonadosomatic Index:

The Condition Index (CI), which represents the ratio of soft tissue dry weight to shell weight, seems to be an effective indicator of growth rate when employed to signify development (Yap, 2002; Thebault, 2005).CI rises when the gonad enters the maturation phase, the amount of meat is higher, and declines during the spent and rest periods, when the amount of meat is low. The CI could be used to evaluate the stages of gonad development without the use of chemicals. In eco-physiological research employs a range of indicators, including physiological or biochemical traits that reflect the metabolic condition of bivalves. Condition indices (CIs), similar to allometry, serve as fundamental metrics for assessing the physiological well-being of marine bivalves, alongside gauging the allocation of resources between tissue and shell growth, CIs are frequently used to indicate the quality of a marketed product, but in eco-physiology, they are used to characterize the health state. They're also typically studied to assess a species' well-being, i.e. meat portion (fattiness), on the concept that organisms with a lot of weight on a certain length are in great shape (Froese, 2006). Therefore, a comprehensive understanding about these parameters and their linkage to different eco-physiological factors is very essential for the commercial exploitation of this species from the aquaculture farms or nature.

The process of spawning or gametogenesis requires energy, and different species employ various strategies to acquire it (Gosling, 2003). Some organisms utilize recently absorbed energy, while others draw from energy reserves stored in different organs (Gabbott, 1976; Bayne, 1976). There exists a significant connection between the gametogenic cycle, condition index (CI), and the utilization of stored reserves, particularly glycogen and meat quality (Gabbott, 1976). When combined with an estimation of the bivalve's biochemical composition, the CI method is perhaps the most practical and straightforward approach for monitoring gametogenic activity (Okumus and Stirling, 1998). Additionally, CIs have found utility in the management of bivalve exploitation, both from wild populations and within aquaculture settings, driven by economic and eco-physiological considerations (Lucas and Beninger, 1985)

The gonad development of the marsh clam *Polymesoda expansa* has been studied using CI in Sarawak, Malaysia (Azimah, 2013). Other species namely *Meretrix casta* and *Meretrix lusoria* have also been studied using CI in India and Japan, respectively (Rao, 1951 ; Nakamura, 2010).Previous studies on clam using CI showed positive relation between gonad maturity and condition factor(Durve, 1964;Alagarswami, 1966).However, research on *M. meretrix* in Mumbai waters did not support this trend; instead, they revealed that the condition factor varies seasonally(Sharma, 2005) which is also similar in *Perna viridis* (*Thippeswamy et al*.,1988). In Bangladesh, such information using CI on *M. meretrix* is still unidentified.

CHAPTER THREE

MATERIALS & METHODS

3.1 Sample collection and transportation:

Wild samples were collected once in every full moon from subtidal zone of Chowfaldandi. Moheshkhali canal adjacent to Cox's bazar (21°30′20″ N and 91°59′19″ E) from December 2021 to November 2022 (Figure 3). Local fishermen were hired to collect clams from these collection site. A total of 200 live samples were collected per month, which were immediately transported to the oceanography laboratory, CVASU using supported with saline water collected from the sampling site. Sampling was consistently conducted during low tide in all instance.

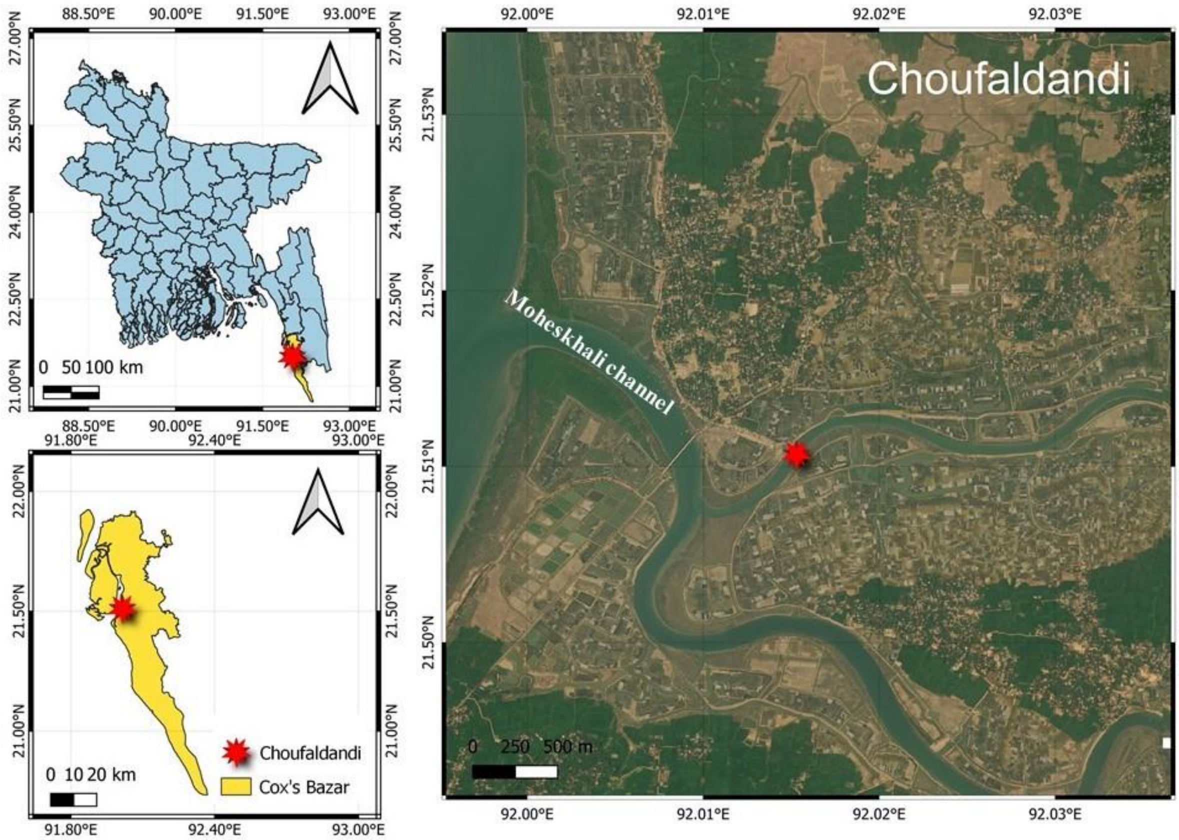


Figure 3: Study area in Chowfaldandi, near Moheshkhali channel, Cox's Bazar district, Bangladesh

3.2. Morphometric Data Collection

A total of 200 clams were sampled to assess the individual total weight spanning from December 2021 to November 2022. Clams were initially got rid of all encrusting organisms, and their byssus threads were eliminated. Biometric parameters including length (greatest dimension along the anterior-posterior axis), height (maximum dimension along the dorsal-ventral axis), and width (greatest dimension across both valves) were individually measured using a Vernier caliper accurate to 0.01 of its Vernier constant. Subsequently, the total weight of each clam was determined using an electronic weight meter (AS 220.R2, Radwag, Poland) subsequent to draining the inter-valval (mantle) fluid.

Each individual clam was then dissected, and the weights of the soft tissue and the shell were quantified using an electronic weight meter (AS 220.R2, Radwag, Poland).

3.3 Length-Weight Relationship:

The relationship of shell length (SL) to total weight (TW), meat weight (MW) and shell weight (SW) were calculated according to the allometric equation (Le Cren, 1951):

$$W = a * L^b$$

$$\log W = \log a + b \log L$$

As weight exhibits a power-function relationship with length, the logarithm is employed to transform the relationship into a linear equation that represents the exponential connection. This equation can then be presented in its linearized format is related to regression equation:

$$Y = a + bX$$

W is the shell weight (total weight, total unshelled meat, shell weight L is the shell length whilst ‘a’ is the intercept (initial growth coefficient) and ‘b’ is slope (relative growth rate of variables)

Figure 4: Morphometric data Collection: (a)Shell length measurement;(b) Shell height measurement;(c) Shell width measurement

3.4 Condition index (CI)

About 20 *M. meretrix* were used for calculating Condition Index in every month. After length, weight and width data collection, the clam's mantle was carefully opened using a scalpel to extract and bottled the soft tissue. CThen the sample tissue was dried at 70°C for 48 h in hot air oven (DIN-12880, German) and was cooled in desiccator. After drying, dry tissue weight was measured with electric weight meter (AS 220.R2, Radwag, Poland). Dry tissues were then determined for Condition Index (CI) value based on Marcado-Silva (2005) and Davenport and Chen (1987)

$$\mathbf{CI} = \frac{\textbf{total soft tissue dry weight (gm)}}{\textbf{Dry shell weight}} \times \mathbf{100}$$

(a) (b) (c)

Figure 5: Conditon Index data collection (a) Muscle collection b) Drying at hot air oven c) measuring dry weight

3.5 Gonadosomatic Index (GSI)

In every month, about 20 clams were selected for measuring Gonadosomatic Index. The clam mantle was opened using a knife. The undesirable filth, digestive organs, and intestine were then removed, and the clam's gonad was delicately extracted with scalpel and forceps. The GSI is the ratio of gonad weight to body weight in a sample, and it is especially useful for determining spawning seasons. The GSI was calculated by month-wise using the equation by Vladykov (1956).

$$\mathbf{GSI} = \frac{\textbf{Gonad weight}}{\textbf{total weight of clam}} \times \mathbf{100}$$

3.6 Histological process

In every month about 20 samples were selected for histology of the clam. The histological processes involve several steps as described in Table 2. The soft tissue of each clam was dissected within 24 hours of collection from the fresh individuals. Two incisions into the animal's body revealed a transverse slice of the visceral mass, which includes the gonad, renal gland, and digestive system, as well as pieces of the gill and mantle. the clam's gonad was delicately extracted with forceps. After being fixed in Bouin’s solution for 24 hours, the gonad samples were dehydrated in a series of alcohol solutions (70% to 100%), cleaned in two dips of 100% xylene, and finally embedded in paraffin. All procedures were followed manually following the procedure described in the table 2.

Table 2: Protocol of histological procedure of *M. meretrix* gonad (Hamli, 2015)

Steps	Chemicals	Time (Hours)
A. Fixation	Bouin’s fixative	24 hours
B. Dehydration	I. Ethanol 50%	2
	II. Ethanol 70%	2 or overnight
	III. Ethanol 80%	2
	IV. Ethanol 90%	2
	V. Ethanol 95%	2
	VI. Ethanol 100%	2
	VII. Ethanol 100%	2
C. Clearing	1. Alcohol (50%) +Xylene (50%)	2hours or overnight
	2. Xylene (100%)	2 hours
	3. Xylene (100%)	2 hours or overnight
D. Infiltration	A) Paraffin + xylene (2 drops)	2 hours or overnight
	B) Paraffin	2
	C) Paraffin	2

E. Embedding

After infiltration, the gonadal tissues were embedded in paraffin blocks. Following embedding the tissues, the paraffin blocks were cut to allow for precise sectioning.

F. Trimming:

Trimming is the technique of removing undesired wax layers from embedded blocks with a knife in order to get appropriate blocks. It facilitates sectioning. Trimming was performed after appropriate embedding to eliminate undesired wax layers.

G. Sectioning

Subsequently, the blocks were sliced into sections with a thickness of 5 μm using a microtome. These sections were then placed onto slides and subjected to drying in an incubator set at approximately 40°C for a duration of 24 hours.

H. Staining and mounting

The slices were dewaxed before to dyeing by dipping them in various bathes of xylene, alcohol, and water (Table-3). Hematoxylin (Harris Hematoxylin) and Eosin were stained using conventional techniques in accordance with Bancroft and Stevens, 1996. When the proper staining levels were achieved, the slides were permanently mounted using D. P. X. mounting medium.

Table 3: The staining schedule

Serial No	Solutions	Time	Process
1.	100% Xylene	10 minutes	**Clearing**
2.	100% Xylene	10 minutes	
3.	100% Xylene	10 minutes	
4.	100% alcohol	5 minutes	**Rehydration**
5.	100% alcohol	5 minutes	
6.	90% alcohol	3 minutes	
8.	80% alcohol	3 minutes	
9.	70% alcohol	3 minutes	
10.	50% ethyl alcohol	2 minutes	**Staining**
11.	Distilled water	15 dips	
12.	Hematoxylin	3 minutes	
13.	Wash in tap water	15 minutes	
14	1% Acid Alcohol	2-4 dips	

15	Wash in tap water	5 minutes	
16.	50% ethyl alcohol	10-15 dips	
17.	95% ethyl alcohol	30 seconds	
18.	Eosin Y	1 minute	
19.	95% ethyl alcohol	2 minutes	**Dehydration**
20.	100% ethyl alcohol	1 minute	
21.	100% ethyl alcohol	3 minutes	
22.	100% ethyl alcohol	1 minute	
23.	100% Xylene	20 minutes	**Clearing**
24.	100% Xylene	20 minutes	
25.	Drying at room temperature	Overnight	**Drying**

3.7 Microscopic observation:

The mounted slides were observed under a microscope (Fein Optic, FIO SN 532982) connected to computer and Digital camera. Several images were taken at 10X magnifications using software. Images were analyzed using Image J software.

3.8 Data Analysis

For data analysis and graphical representation, Microsoft Excel and SPSS software were employed. The Microsoft Excel tool was used to compute the percentages of gonad development stages and sex ratio as well as graphical presentation of both data. SPSS was utilized to conduct various statistical analyses on data related to GSI, and CI (dry). These analyses included descriptive statistics such as mean, standard deviation, and more such as correlations, regression analysis, and inferential tests (t-tests, ANOVA, etc.). The goal was to uncover patterns, trends, and associations within the GSI and CI (dry) data, providing a deeper understanding of their interrelationships and potential effects.

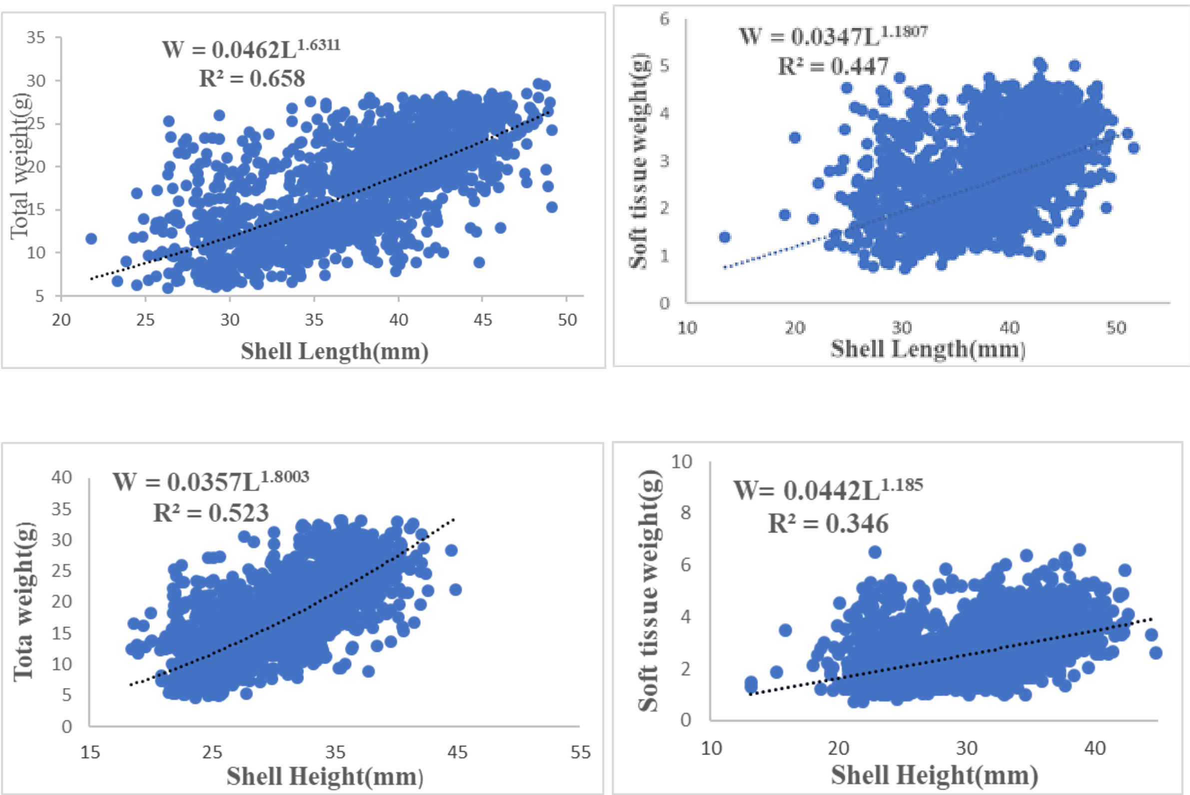
Figure 6: ***M. meretrix*** **gonad histological procedure**

CHAPTER FOUR
RESULTS

4.1 Length-Weight relationship

During the study period, a total of 2267 samples were collected from Chowfaldandi, canal near Moheskhali Channel to investigate the association of different shell dimensions (Total length, shell width, and shell height) and weight (Total weight, soft tissue weight). The shell length varied from 1.40 mm to 51.68 mm range, with mean of 37.649±0.12) mm. For shell height, the range was from 3.45 mm to 44.87 mm, with a mean height of 30.886± 5.12220) mm. Shell width was about 23.69±1.52 mm with a ranged from 2.16 mm to 15.44mm. Total weight was18.44±0.15 g along with a range of 1.81 g to 83.30 g. Mean wet weight (mw) ranged from 0.18 g to 12.10 g, with a mean of 2.76±0.02 g. The computed growth coefficient (b) for the length-weight relationship was 2.02 (± 0.04).

The morphometric correlations were identified using regression power, as shown in figure 4. The regression analysis "b" values were analyzed to determine if they differed significantly from 3.0. Highest correlation values were observed between shell lengths and weights in the SL-TW (r^2 = 0.658), TH-TW (r^2 = 0.523) and TW-TW (r^2=0.50) relationships (Table 5).The length weight relationship equation was found using this equation: $W=aL^b$



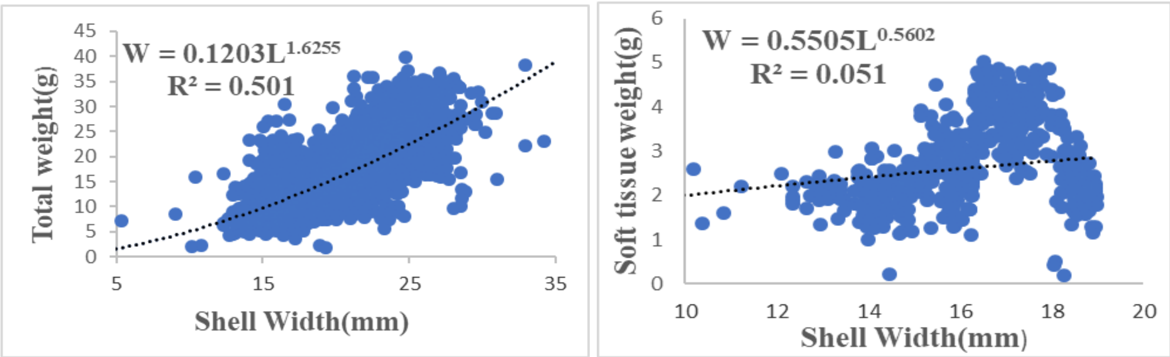


Figure 7: Power equation and correlation between length and weight dimensions of *M. meretrix* collected from Choufaldondi, Cox's Bazar, Bangladesh.

The analysis of various morphometric traits and their relationship with growth patterns in clams revealed significant findings. Shell length showed the strongest correlation with total weight ($r = 0.811$, $p < 0.001$). This finding conceived a significant positive linear relationship among shell length and the total weight of the clams. Moreover, total length showed a moderate positive correlation with soft tissue weight ($r = 0.442$, $p < 0.001$), suggesting that soft tissue weight increased whenever the total length of clams increased. Shell height and total weight showed a strong positive correlation among the ($r = 0.723$, $p < 0.001$) and soft tissue weight ($r = 0.582$, $p < 0.001$). Shell width also displayed a strong positive correlation with total weight ($r = 0.70$, $p < 0.001$). But this showed a weaker correlation with soft tissue weight ($r = 0.22$, $p < 0.001$). These findings individually revealed that the morphometric traits, particularly shell length, total length, shell height, and shell width, are gone along with the growth patterns of clams.

Table 4: Observational Relationship between different length dimensions and weight dimensions

Variables	R	R^2	Regression co-efficient (b)	Growth test	Growth pattern
Shell length –Total weight	0.8	0.65	1.63	b<3	Allometric
Total length -soft tissue weight	0.663	0.44	1.38	b<3	Allometric
Shell height – Total length	0.72	0.52	1.8	b<3	Allometric
Shell height – soft tissue weight	0.58	0.34	1.18	b<3	Allometric
Shell width- Total weight	0.70	0.50	1.62	b<3	Allometric
Shell width - soft tissue weight	0.223	0.05	0.56	b<3	Allometric

4.2 Sex Ratio

Among 227 individuals observed 73 were male, 127 were female, and 28 were undifferentiated. Hermaphrodite specimens were not detected in the study. The sex distribution was determined through histological analysis, revealing that males constituted 30% while females accounted for 50.6% (Figure 5). The cumulative male-to-female sex ratio was calculated to be 1:1.66. However, when examining monthly ratios, it was observed that in certain months, the number of males surpassed that of females, whereas in other months, the number of females exceeded that of males (as indicated in Table 5). Male dominancy was observed in September; female dominancy was seen December; January, August and October.

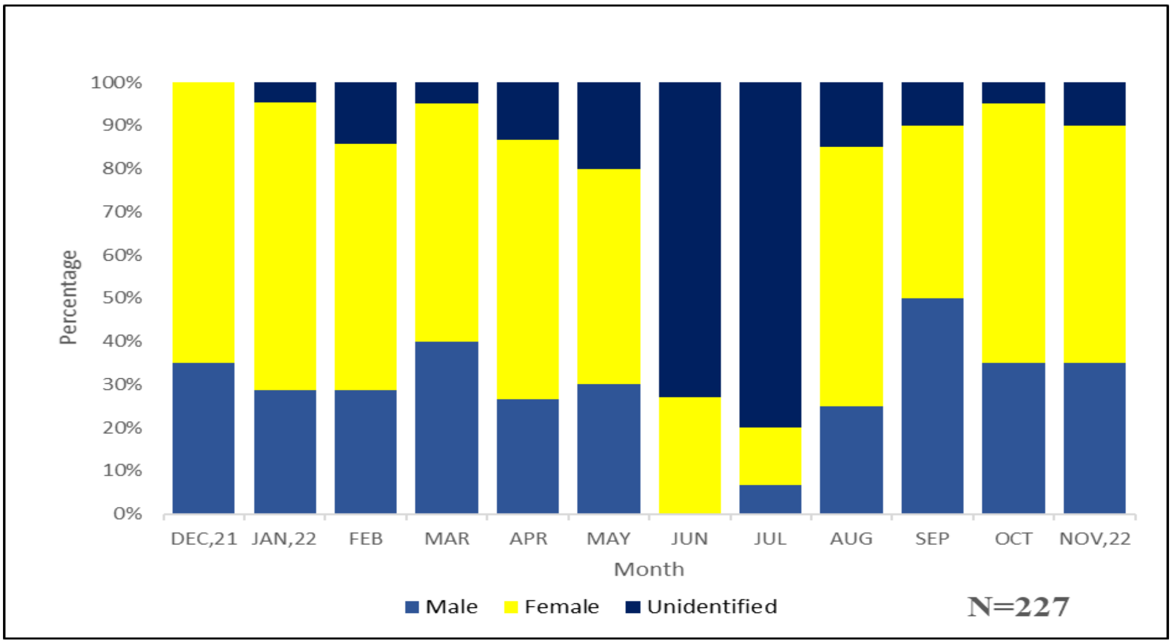


Figure 8: Proportion and monthly variations of male, female and undifferentiated individuals of *M. meretrix* collected from Moheskhali channel

Table 5: Monthly variations of the number of male and female and their sex ratio of *M. meretrix*

Month	Total	Ratio of male	Ratio of female	Male -Female Ratio
Dec	20	1	1.857	1:1.857
Jan	21	1	2.33	1:2.33
Feb	21	1	2	1:2
Mar	20	1	1.375	1:1.375
Apr	15	1	2.25	1:2.25
May	20	1	1.66	1:1.66
June	15	0	0	0
July	15	1	2	1:2
Aug	20	1	2.4	1:2.4
Sep	20	1	0.8	1:0.8
Oct	20	1	1.71	1:1.71
Nov	20	1	1.571	1:1.57
Total	227	1	1.66	1:1.66

4.3 Gametogenesis and Reproductive cycle

Histological examination of the gonads allows for the differentiation of sexes in *M. meretrix* The study revealed that *M. meretrix* undergoes gametogenesis in distinct stages, a process similar to other bivalves like cockles, short-necked clams, and manila clams. However, there are variations in the spawning duration; for instance, clams in temperate regions typically spawn during the summer months, while those in tropical areas spawn year-round.

Gametogenesis in both male and female *M. meretrix* can be divided into six stages. The stages of development were identified and characterized by examining the gonadal tissues using a light microscope.

Stage 0, Resting:

The gonad appears small and transparent Thus, the sexes cannot be differentiated at this stage, containing a substantial amount of connective tissue (Figure:6 A). No visible gametes are present.

Stage 1, Early Development:

At this particular stage, it becomes possible to differentiate the sexes, and the gonad begins to proliferate. In histology, gonadal lobules can be seen isolated from one another. In the female gonad, the number of observable early oocytes in the follicle walls is seen, while the oocytes remain small with no particular shape (Figure:6-B). Along the follicle walls, numerous oogonia (OG) and early oocytes (EO) are developing, while the follicle lumen (L) is empty and does not contain mature ova.

In the male gonad, dense accumulation spermatogonia in each lobule without specific column is seen. Numerous spermatic cells occupy each seminiferous tubule, with spermatogonia adhering to the walls of the tubules, and the lumen is not distinctly visible. (Figure:6-G).

Stage 2, Late Development:

During this stage, follicles become noticeable and increase both in size and quantity. In the female gonad growing oocytes are present attached with the follicle cells with egg. Attached oocytes are evenly abundant. Female gonads contain early oocytes (EO) and growing oocytes (GO) (Figure6-C). Growing oocytes are characterized by round nucleus in the center and size is about micrometer. Some follicles have ripe ova (RO) in their centers, but they are no longer tightly packed.

Within the male gonad, the presence of spermatogonia, spermatocytes, spermatids, and spermatozoa are observed within seminiferous tubules. In less mature gonads, a prevailing single cell type is not identifiable. As the gonads mature further, a notable observation is that the majority of the sperm are predominantly made up of spermatids and spermatozoa, as depicted in Figure 9-H.

Stage 3 Ripe or mature:

At this stage, in females, the gonad occupies a substantial surface area. The follicles are completely expanded and merged, with no interfollicular space present. The follicle lumens are almost entirely occupied by ripe ova, which are densely packed into a polygonal shape due to compression. Ripe ova are surrounded by an envelope. There is a significant decline in the early stages of oogenesis (Figure 9-D).

In general, male seminiferous tubules are formed of mature spermatozoa with their flagellum/spermatic column pointing towards the center of the lumen, producing concentric bands or plugs. There was a substantial decrement in the early stages of spermatogenesis**.** From the bottom of the follicle cells, spermatic columns stretched into the lumen and produce spermatocytes at the top (Figure 9-I)

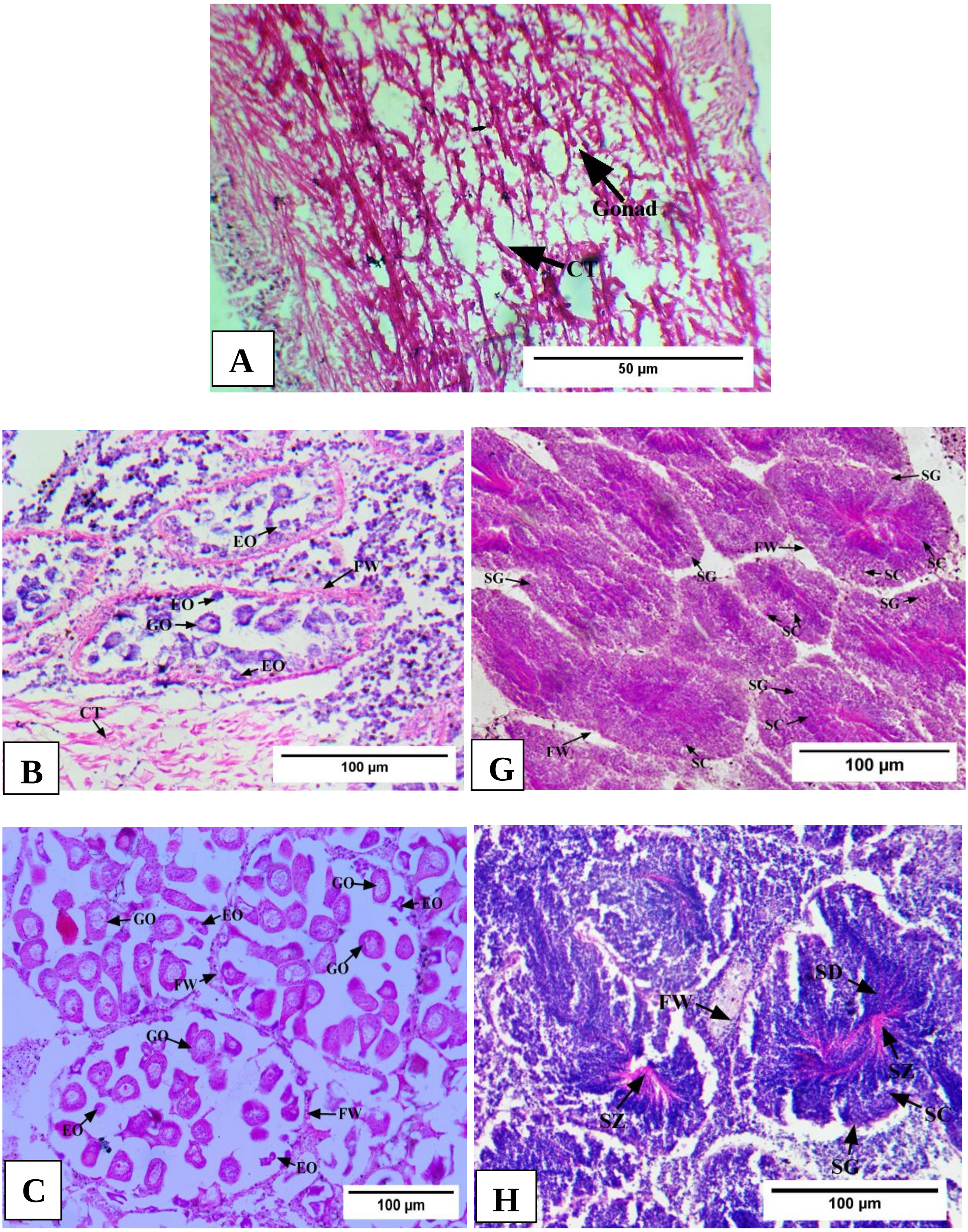
Stage 4 Partially spent or spawning:

At this point, the quantity of liberated oocytes in each follicle of the female gonad diminishes. Certain follicles are empty due to the discharge of gametes, and the follicle walls become fragmented (Fig 9-E)

In males, spermatozoa are observed in a spiraling configuration, constituting the majority of cells within the follicle. Empty areas appear in some follicles due to the discharge of mature spermatozoa (Fig 9-J)

Stage 5 Spent:

Following spawning, the follicle walls were fractured, dispersed and comparatively depleted in both males and females. In the female, only a small number of oocytes and spermatozoa remain (Fig.9-F), while in the male seminiferous tubules, a limited quantity of spermatozoa can be observed (Fig 9-K).


Gonad
CT
A
50 µm
EO
FW
EO
GO
EO
CT
B
100 µm
SG
FW
SG
SC
SC
SG
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SC
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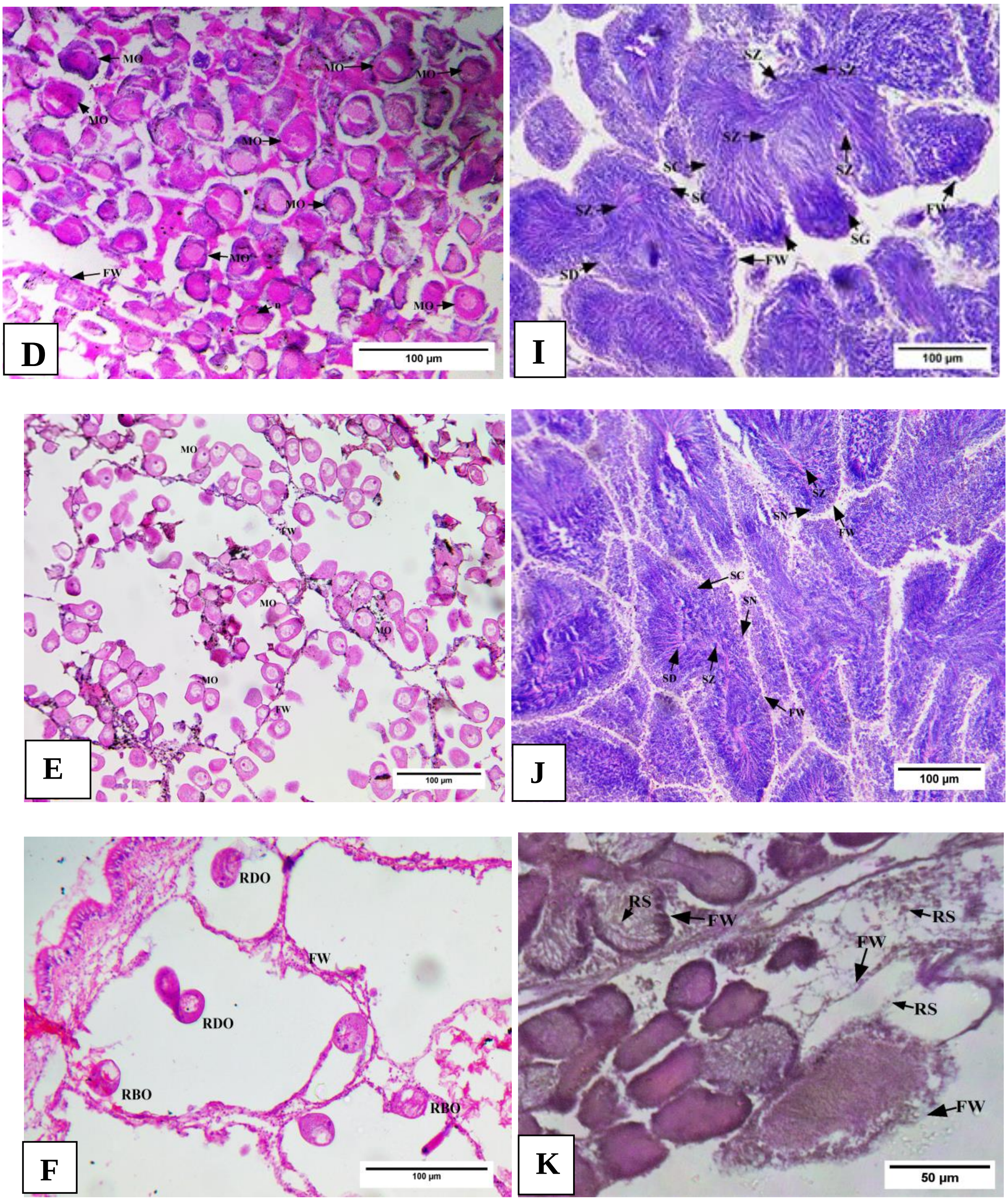

Figure 9: Histological section (H & E) of female and male of clam *M. meretrix* Gonads. Resting phase(A): CT=Connective tissue, muscle tissue at the both side.Female stages: Early development (B); Late development (C); Ripe(D); Partially spent (E);Spent(F); FW = follicle walls, EO = Early oocyte, GO= Growing oocyte, MO= Mature oocytes, RBO = resorbing oocyte; RDO = residual Oocyte. Male stages: Early development (G); Late development (H); Ripe(I); Partially spent (J)Spent(K); SC = spermatocyte; SG = spermatogonia, SD= Spermatide, SZ=Spermatozoa, RS = Residual Spermatozoa

4.3.1 Egg Diameter:

Only mature eggs were utilized for the measurement of egg diameter. Notably, the eggs of clam exhibited a polygonal shape rather than being perfectly round. To know the accurate measurement, a select number of lines were being drawn, and lately, an average value was measured. The observed mature eggs diameter were ranged from 21.02 to 40.02 micrometers, with a mean egg diameter of 31.26 micrometers

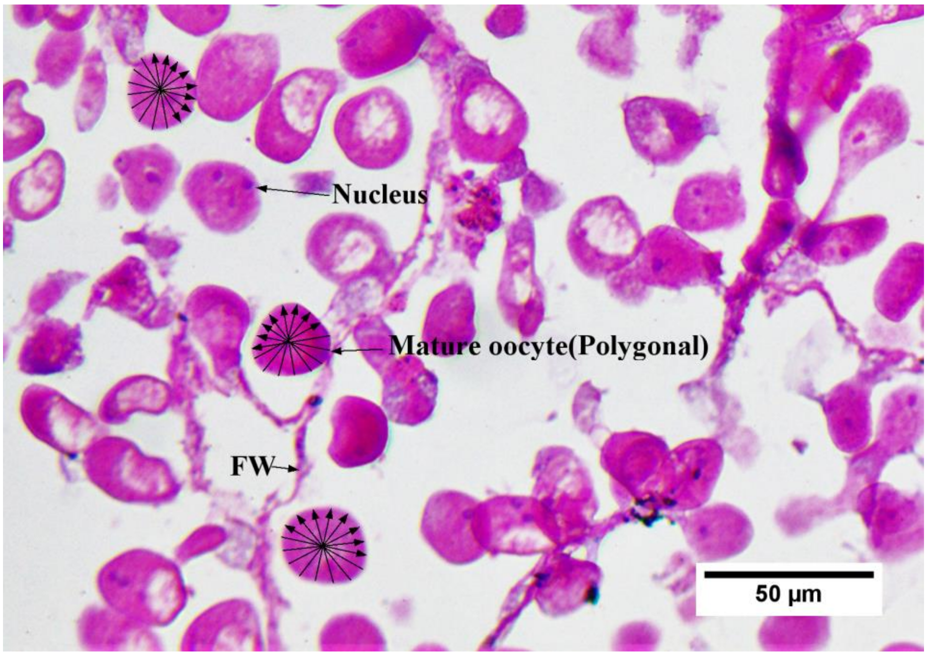

Figure 10: Egg diameter measurement: FW=Follicle wall

Table 6: Egg diameter at different stages of female *M. meretrix*

Stages	Egg diameter (um)	Sample size
Resting	Not defined	10
Early developing	4.75 ± 2.33	10
Late developing	23.91 ± 4.79	10
Ripe	31.26 ± 5.15	10
Spawning	27.03 ± 5.95	10
Spent	18.78 ± 6.58	10

4.4 Seasonal variation in gametogenesis

To explore the seasonal variations of gametogenesis, this study assessed two key indicators: monthly gonad development stages and gonadosomatic index (GSI). The description is given below-

4.4.1 Monthly gonad development stages

The gametogenesis cycles of male and female *M. meretrix*, commonly known as the Asian hard clam unfolds over a series of months, with distinct stages of development and maturation.

For instance, in December 2021, both males and females exhibited progress in late development (LD), though with differing proportions—males at 30% and females at 27%. A greater proportion of late development stages were evident in males compared to females, suggesting that males initiate the gametogenesis process prior to females. By January 2022, the trend continued, with a rise in late development in both sexes, reaching 33% in males and 43% in females. These suggest a coordinated synchronization in the progression of gametogenesis, preparing both male and female clams for reproduction. Males exhibited late spawning seasons spanning from to March 2022and female from January to April,2022. Additionally, an undifferentiated gonad stage became evident in May 2022, signifying a resting phase preceding redevelopment. The presence of spent gonads was observable in both sexes, with females expending their gametes earlier. Among males, the highest proportions of development observed in September (80%); maturation in October and January (33%), and spawning in April-May,2022. In contrast, females displayed peak development in October (63%) &January (64%), maturation in November (30%) & March (34%) and a major spawning observed in April (54%)-May (57%) with a minor spawning period in November (33%)- December (37%). A minor early development period was indicated by only 10% gonad spent in July 2022 for both sexes, suggesting a brief period of decreased reproductive activity.

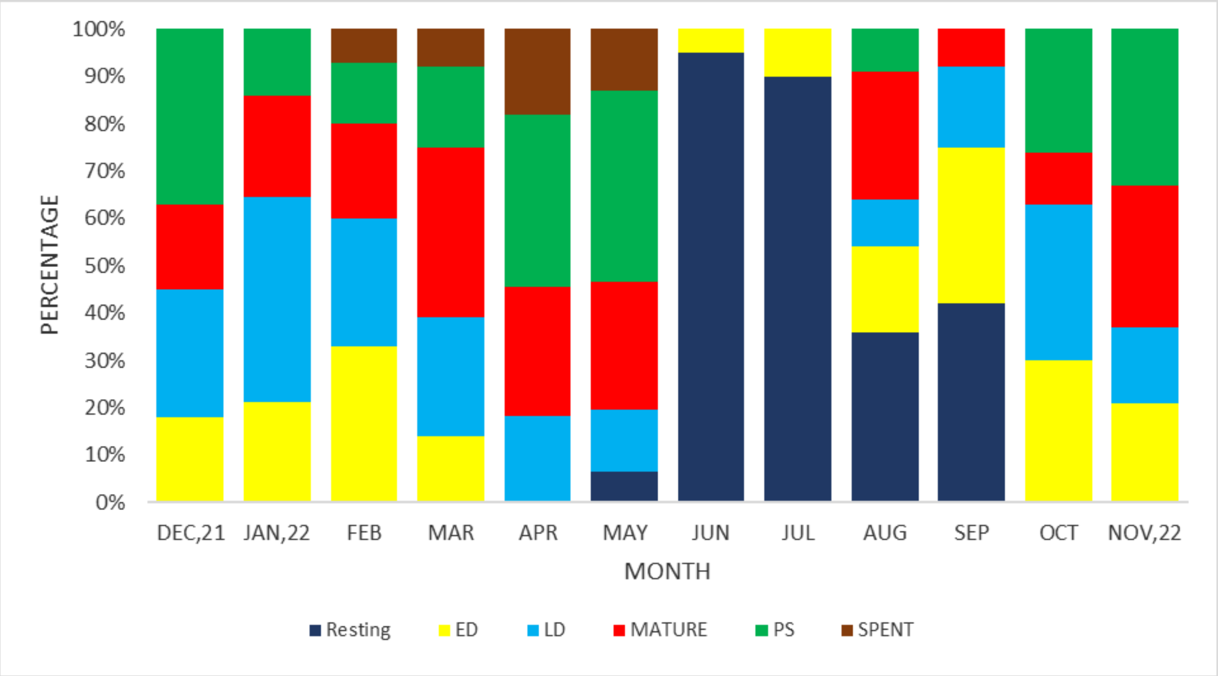

Figure 11: Percentage of gonad development stage of female *M. meretrix*

(ED=Early Development, LD=Late Development, PS=Partially spent/Spawning

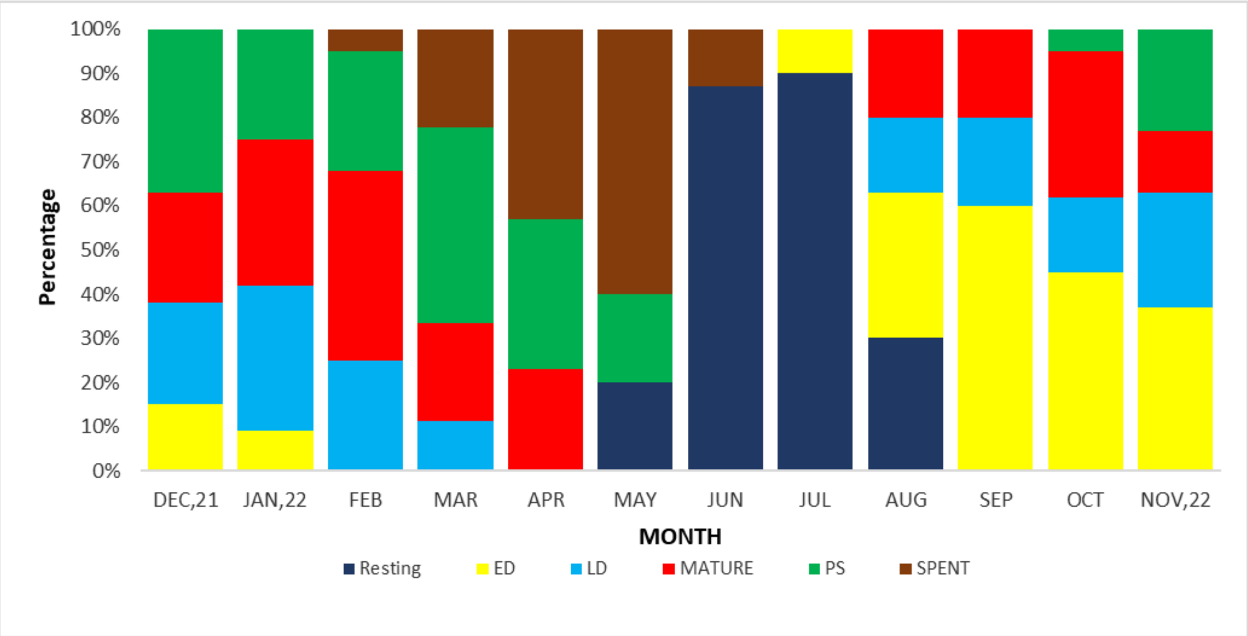

Figure 12: Percentage of gonad development stage of male *M. meretrix*

(ED=Early Development, LD=Late Development, PS=Partially spent/Spawning)

4.4.2 Gonadosomatic Index:

The GSI (Gonadosomatic Index) results were analyzed using ANOVA test. There were significant differences observed in GSI values among 12 months data which were being compared ($F(11, 215) = 21.316$, $p < .001$). The between-months analysis showed a significant effect ($F(11, 215) = 21.316$, $p < .001$) with a mean square value of 10.025.The Welch test also indicated significant differences in GSI values between months (*Welch's F*(11, 83.755) = 46.018, $p < .001$). These findings demonstrate that a year round data has a significant impact on GSI due to seasonal change, suggesting variations in reproductive cycle or gonad development among different months.

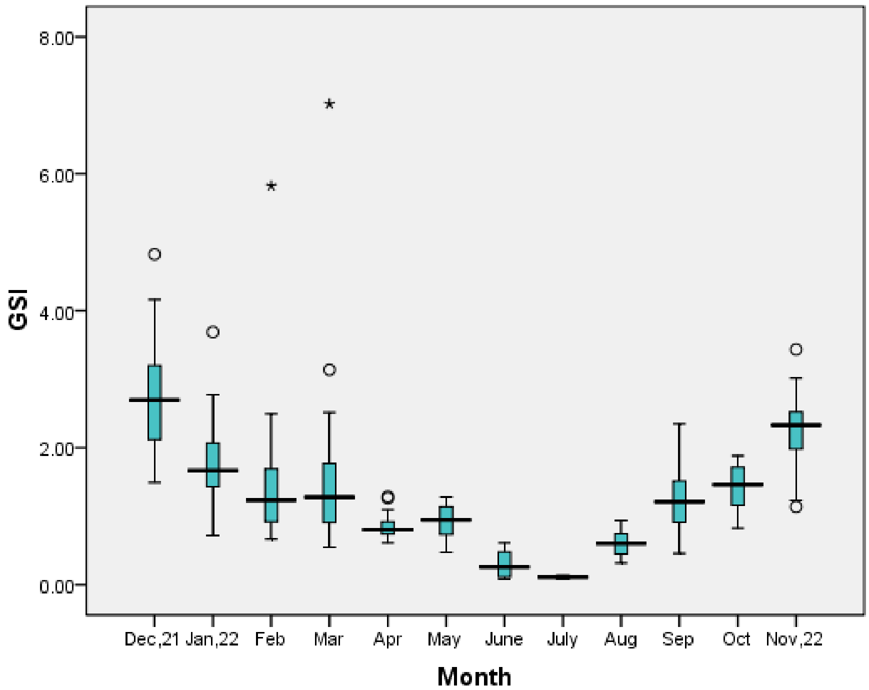

Figure 13: Monthly variation of GSI (%) variation of GSI in male and female

The comprehensive analysis revealed that the *M. meretrix* population within the Moheskhali Channel experienced a primary breeding peak during November-December, accompanied by resting phase during June-August throughout the entire duration of the study. The interpretation of the gametogenic cycle, as deduced from monthly gonad development and GSI values, follows the subsequent pattern

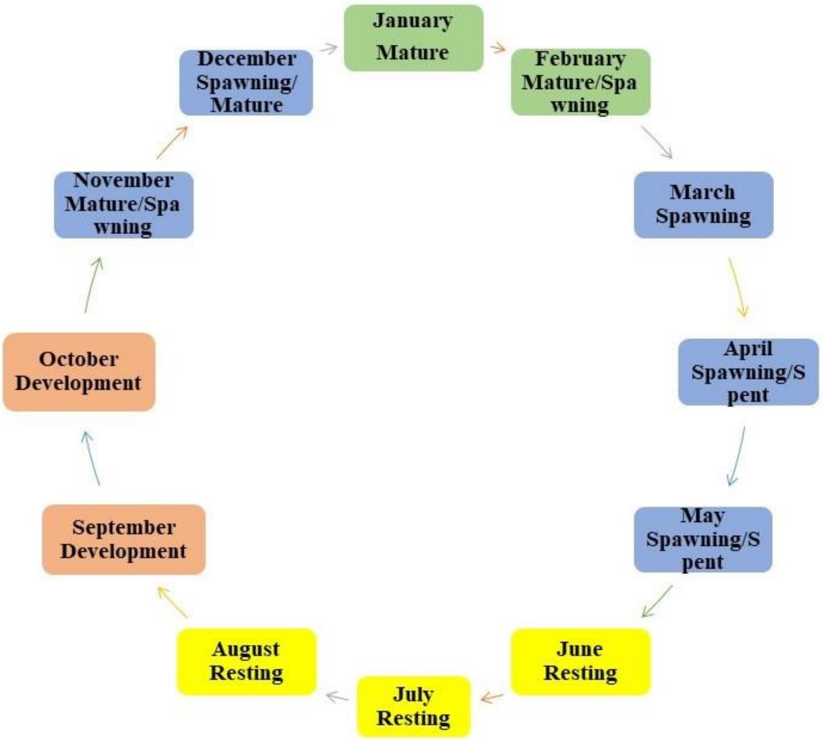

Figure 14: Gonad Development Cycle

4.5 Monthly variations in Condition Index:

The highest mean value of condition index was observed in March,2022(5.44±2.05) and the lowest value of condition index was noted in May,2022(2.16±1.31). One way ANOVA test of condition index showed significant differences in different months (F=8.192, df (11), p=0.000) and within groups (F=230, df=230, p=0.000). Significant differences in unequal variance was also found by *Welch F test* (F=11, df (90.132), p=0). Mean condition index of male (3.81±1.84) was higher than female (3.38±1.54) but resting stage possess moderate value (3.78 ±1.96).

The examination of Confidence Interval (CI) data for *M. meretrix* offers insights into the potential reproductive seasons within the species across the months of the year. Notably, various periods demonstrate distinct mean values, implying different levels of reproductive activity. Specifically, March presents the highest mean (5.4432±0.44), possibly signifying a spawning season. Additionally, November & December exhibit relatively elevated means

(4.8914±0.49 & (4.18 ±.26) suggestive of potential reproductive periods. the lowest value of condition index was noted in May,2022(2.16±1.31) potentially indicating intervals of diminished reproductive activity or spent. In contrast, January, February, September and October, showcase lower mean values, potentially indicating developing of gonads. Although there is variation, with July exhibiting a high CI value (4.19±0.29), it aligns with the resting phase based on histology and GSI values. This occurrence is attributed to the fact that the CI value is not exclusively indicative of reproductive changes; external factors like water parameters& environmental condition can also impact the flesh quality of *M. meretrix.*

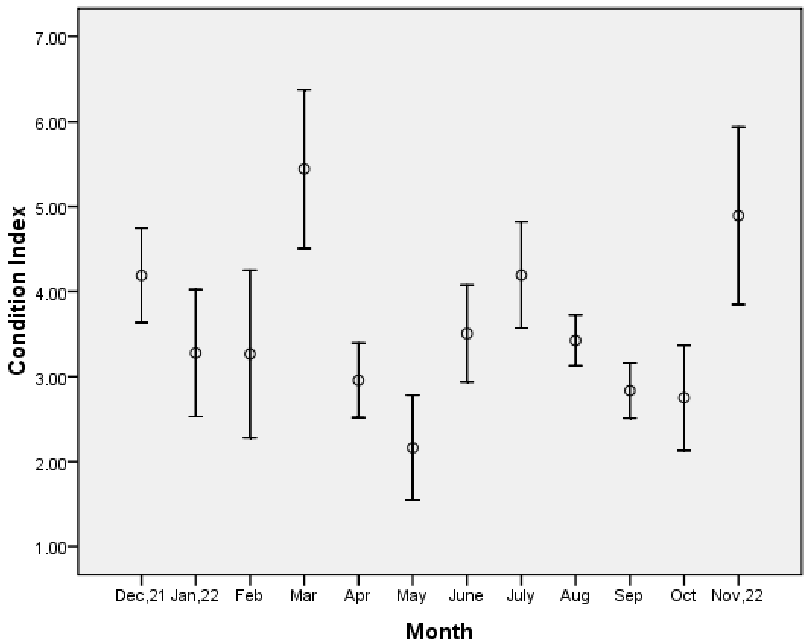

Figure 15: Monthly condition index of *M. meretrix* from Moheskhali Channel (data represented as mean, standard error)

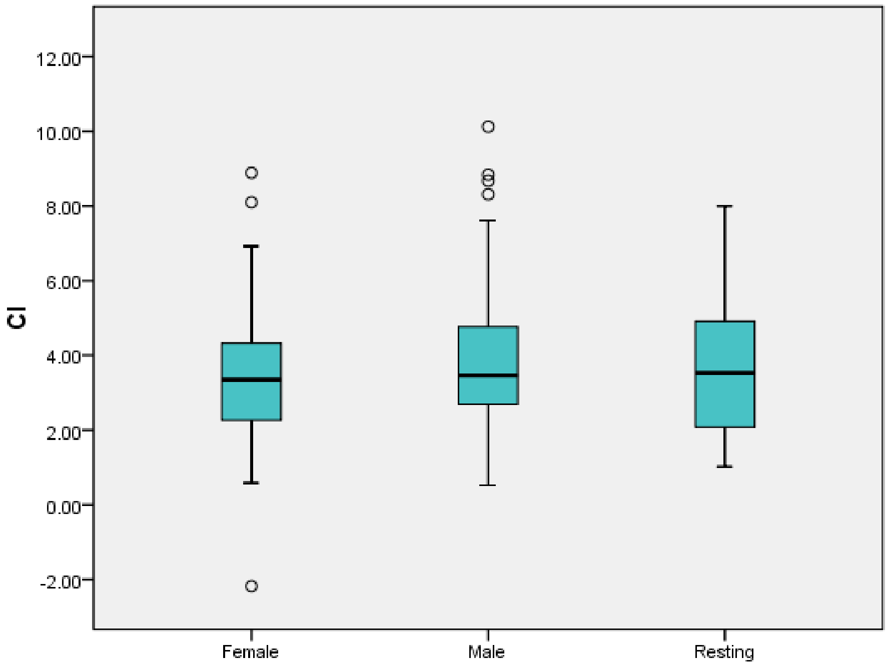

Figure 16: Dry condition index of *M. meretrix* from Moheskhali Channel (data represented as mean, standard error) between genders.

CHAPTER FIVE
DISCUSSION

5.1 Length - weight relationship

A total of 2267 clam samples were collected over a year from Chowfaldandi, a canal near Moheskhali Channel, for morphometric analysis. the study aimed to analyze the relationship between length and weight of *M. meretrix*. The "b" values in regression analysis were examined to see if they deviated from an expected value of 3.0. In this study, the calculated slopes for shell length, height, and width parameters coordinated with the other parameter such as total weight and soft tissue weight were found to be <3. These relationships are indicative of non-linear growth patterns where changes in one parameter do not proportionally correspond to changes in another. The "b" values for various parameter associations, such as total length (TL) and total weight (TW), TL and soft tissue weight (STW), shell height (SH) and TW, SH and STW, shell width (SW) and TW, and SW and STW, offer insights into growth dynamics. Notably, "b" values less than 3 indicate that growth rates are not uniform. For instance, the TL-TW relationship's "b" value of 1.63 signifies slower weight increase compared to total length growth, potentially indicating changing body composition. Similarly, the shell-related parameters display varying priorities, with shell height and width having higher "b" values (1.8 and 1.62, respectively), suggesting preferential energy allocation towards shell growth. Contrasting this, the SW-STW relationship's "b" value of 0.56 implies a relatively more rapid increase in soft tissue. The study carried out on *M. meretrix* from Korampallam Creek, Tuticorin discovered that the 'b' value concerning the correlation between shell length and total weight was 3.08, while for the connection between shell length and soft tissue weight, the 'b' value was 2.9 (Narasimhan, 1988). Neverthless, Orton (1935) recorded a 'b' value of 1.3, whereas Andreu (1968) observed a 'b' value of 3.5 for the English oyster *Ostrea edulis* in Britain and Spain, respectively. These findings collectively provide valuable insights into the species' growth strategies, adaptation, and energy allocation patterns throughout its lifecycle, contributing to a deeper understanding of its biology and ecology.

The strongest correlations were observed in the relationships between shell length (SL) and total weight (TW) ($r^2 = 0.65$), as well as between total length (TL) and shell height (SH) ($r^2 = 0.52$). Shell width (SW) and total weight (TW) also showed strong positive correlations ($r^2=0.50$). The presence of the highest "r" and "r^2" values in relation to shell length and weight dimensions suggests that shell length serves as the most optimal indicator for weight growth (refer to Table 5). Jahangir *et al.,* (2015) encountered a comparable "r^2" value (0.572) between shell length-total weight in *Meretrix casta* collected from the Sonmiani bay, Balochistan, Pakistan.

Ambarwati *et al.,* (2021) undertook a research study examining the morphometric characteristics of *Meretrix sp.* specimens collected from Bancaran, Madura. The investigation highlighted the shell dimensions of these specimens. In terms of shell length, notable variations were observed among the different *Meretrix sp. M. astricta* exhibited a range from 16 mm to 42 mm, *M. meretrix* ranged from 25 mm to 45 mm, M. casta displayed measurements between 6 mm to 42 mm, *and M. aurora* demonstrated a shell length spanning 19 mm to 39 mm (Ambarwati, 2021). Similarly, the shell height dimensions displayed variation: *M. astricta* ranged from 18 mm to 38 mm, *M. meretrix* ranged from 20 mm to 39 mm, *M. casta* showed measurements from 18 mm to 35 mm, *M. aurora* exhibited a range of 24 mm to 37 mm (Ambarwati, 2021) The present study showed the shell length varied from 14 mm to 51.7 mm range, and shell height, the range was from 3.5 mm to 31.80 mm which is comparatively lower than Ambarwati (2021).The computed growth coefficient (b) for the length-weight relationship was 2.021. The "b" values for the length-total weight relationships determined for *M. meretrix* collected from the Moheskhali channel, Bangladesh ranged from 1.93 to 2.11 (Amin *et al.,* 2009) which is similar to the present study. Nayar (1955) had observed a maximum length of 3.5- 27.3 mm in the case of *Donax cuneatu* where estimated b value was 2.8. Coe (1938) has found that the bean clam, *Donax gouldi* from the coast of Southern California attains the size of 12-20 mm with estimated b value 1.87. *M. meretrix* collected from Moheshkhali channel showed the length of individuals ranged from 22.5 mm to 82.5 mm and the weight from 8.89 to 107.32 g (Amin *et al.,* 2009) where present study showed lower values.

5.2 Sex ratio

A greater proportion of females compared to males are abundant in the wild, specifically in Choufaldondi canal near Moheshkhali channel, Bangladesh.The male-to-female sex ratio of the Asian hard clam, *M. meretrix* was 1:1.66 (Table 5), which indicates that *M. meretrix* is a dioecious organism with female dominancy.Though the typical sex ratio for dioecious bivalves approximates 1:1, with a slightly higher representation of females compared to males (Gosling, 2003; Nabuab & del Norte-Campos, 2006).But this study showed higher female percentage which may be occurred due to unequal distribution of clam in the channel beds or limitions during study period. Earlier investigations into bivalve reproductive patterns have mostly reported a sex ratio of 1:1. This has been observed in various species, including *Gafrarium tumidum* (Jagadis & Rajagopal, 2007), *Donax trunculus* (Gaspar *et al.*,1999; Silina, 2006),*Anadara granosa* (Thongchai, 2008), *Anadara inaequivalvis* (Sahin *et al.*,2006).

Sexual predominance has been documented in various bivalve species. Notably, instances of female dominance were recorded in certain species. For instance, *Tapes decussatus*, the carpet shell clams from Sufa Lagoon in Turkey, displayed female dominance with male-female ratio 1: 1.85 (Serdar & Lok, 2009). Additionally, the cockle species *Anadara granosa* exhibited female dominance (sex ratio-1: 1.74) (Suwanjarat & Parnrong, 1991). On the other hand, male predominance was reported in different bivalves. For instance, *Ruditapes philippinarum*, the manila clams collected from the North-West of Ireland showcased male dominance with male -female ratio 1:0.93 (Drummond *et al.*,2006), while the leather Donax clam, *Donax scortum*, exhibited the same pattern (Peerakeitkhachorn, 1995).

This study did not find any hermaphrodite individuals among the 243 specimen slides examined. Numerous researchers within India have not recorded any instances of hermaphrodite individuals within the *M. meretrix* population in their respective areas (Duisan *et al.*,2021; Jayabal *et al.*,1986; Jayabal *et al.*, 1987; Loosanoff,1936; Loosanoff, 1937; Narasimham *et al.*,1988; Thangavelu *et al.*,1985)

The information presented above highlights that the clam *M. meretrix* appears to be unisexual, at least during its adult stage. Loosanoff (1937) stated that in Venus clam *Mercenaria*, the primary gonads of animals measuring 4 to 6 mm. exhibit distinct bisexuality, displaying a tendency toward hermaphroditism. In the case of adults, they are clearly identifiable as either definitive males or females, with a few exceptions referred to as partial hermaphrodites. While there is a lack of information regarding the primary gonads of *M. meretrix* related species in Bangladesh, it remains an area of significant interest. Nevertheless, within the adult *M. meretrix* population, no individuals were observed to exhibit any type of hermaphroditism.

5.3 Gametogenesis and Reproductive Cycle *of M. meretrix*:

The gametogenesis process of *M. meretrix* involved multiple releases of gametes in each reproductive cycle, indicating the adoption of continuous reproduction strategy (Salih *et* *al.*,1973). This study involved a comprehensive analysis of gonad histology to determine the various stages of reproductive maturity within selected clam sample. Classification comprising five reproductive maturity stages, along with an undifferentiated stage, was employed for both male and female short-neck clams. This gonad histological approach aligns with that of several authors who have utilized similar methods for assessing the reproductive biology of different clam species (Suwanjarat, 1999; Drummond *et al.*,2006; Suja & Muthiah, 2007; Jagadis & Rajagopal, 2007).

Histological examinations provided evidence indicating that the initiation of gametogenesis took place in August, with the majority of gametes reaching maturation by December. Subsequent to the gametogenesis phase, spawning was observed to commence in February and extend through May. Notably, gametes that had reached maturation during February-May underwent a process of resorption. This statement also albeit with the GSI value. The highest GSI value was found in November-December through March to April data showed moderate value. The findings of this study reveal a clearly distinct biannual reproductive cycle (November-December; march-may). As per Hornell (1922), the reproductive pattern of *M. meretrix* is biannual, occurring in early September and subsequently in May along the eastern coast of India. Rai (1932) noted that the primary spawning season of this species along the Bombay coast spans from March to June, with the possibility of year-round spawning, barring the monsoon season, under favorable weather conditions. Findings from the Vellar estuary reveal that this species undergoes an eight-month spawning period, stretching from February to September (Jayabal & Kalyani, 1987). Krishnakumari *et al.*, (1977) noted ongoing spawning activities in October-December with a minor peak in March-April in the Goa region, India which broadly aligns with the outcomes of the current study.

Abraham (1953) conducted observations on *M. casta* in the Adyar estuary,India revealing pronounced spawning peaks in July-August, October-November, and March to April or May. Notably, the latter period exhibited the most intense and extended spawning activity. Nevertheless, Abraham suggests that the duration of active spawning varies from year to year within the same environment, and this pattern does not coincide across distinct environments. In a parallel context, Rao (1951) documented the spawning behavior of baby clam *Katelysia opima* in the Adyar estuary, noting that it initiates in late December and concludes in January. Parulekar *et al.,* (1973) indicated that this species engages in year-round breeding activities at Banastrim near Goa. Harkantra (1975) similarly reported continuous breeding behavior but proposed a potential cessation in spawning during winter in the Kali Estuary.On the other hand, Single spawning of *M. meretrix* have been recorded along the Mumbai coast of India (MPEDA,1997), Kalbadevi estuary, Ratnagiri, south-east coast of India and South Zhejiang, China (Zhihua,2004).

5.4 Condition Index:

In this study, The highest mean condition index value was documented in March 2022 (5.44 ± 2.05), while the lowest value was observed in May 2022 (2.16 ± 1.31). The month of May exhibited the lowest recorded condition index in the clams, a decline that may be attributed to the completion of spawning. Comparable findings have been reported by several researchers in the realm of Bivalves (Durve, 1964; Alagarswami, 1966; Narasimham, 1980, 1988).

In CI a decline was evident during April-May, a period when the majority of clams were either in the spent or resting phase. Conversely, *M. meretrix* exhibited its zenith in CI in March, indicative of its spawning stages. Notably, the current study observed an increase in the condition index (CI) during November-December, pointing toward ripe or initial spawning stages. Adjei-Boateng and Wilson (2013) revealed that the bivalve *Galatea paradoxa* exhibited elevated CI values during gametogenesis, particularly at the ripe and initial spawning stages. In contrast, the *M. lyrata* population displayed its lowest CI values in September, November, and April, suggesting that during these months, the population was in spent and resting stages. In this study, the clams exhibited their lowest condition index in May, possibly attributed to the conclusion of spawning. This pattern aligns with observations made by several researchers in the field of Bivalves (Durve, 1964 ; Alagarswami, 1966 and Narasimham, 1988).

Prior studies involving clams and their CI have established a positive correlation between gonad maturity and the condition factor (Durve, 1964; Alagarswami, 1966). However, the case of *M. meretrix* in Mumbai waters diverged from this pattern, revealing seasonal variations in the condition factor, an observation akin to that observed in *Perna viridis* (Mohan, 2018) as well. The condition index (CI) of *M. recens* does not provide a comprehensive depiction of gonadal development, as it indicates an increase during spawning and a decrease during gametogenesis. Drummond *et al.,* (2006) proposed that the highest CI values align with the peak ripeness of clams, representing an accumulation of resources in readiness for spawning. In contrast, Chavez-Villalba *et al.,* (2007) noted the absence of a connection between the condition index and reproduction in the oyster *Crassostrea gigas.*

CHAPTER SIX

CONCLUSION

The reproductive aspects of *M. meretrix* from Chowfaldandi, Moheshkhali channel, Cox's Bazar, were investigated, focusing on GSI, Condition Index, gametogenesis and the reproductive cycle through a year-round study along with morphometric traits of this species. The male-female sex ratio was found to be 1:1.66, with males dominating in early developmental stages and females taking the lead in mature stages. This high ratio of female happened due to unequal distribution or collection of clam in the sampling site. The reproductive cycles displayed two distinct spawning peaks (April-May; November-December) exhibiting similar patterns in both males and females, with little differences in the duration of the spawning period over the month of the study. For females, a mighty proportion of partially spent activities were observed during the middle of the hot season and the end of the rainy season, whereas in males, these activities were prominent during the monsoon season.The GSI value showed a resting phase during June-july which also align with the histology result. The condition indices were found to be linked to the gametogenic cycle, and this study suggests that the condition index could serve as a potential indicator for tracking the reproductive cycles of *M. meretrix*, albeit with minor adjustments. A positive correlation between length and weight dimensions was noticed,which indicated an allometric growth pattern. This fundamental study on reproductive biology serves as a foundational resource for aquaculture practitioners who are working towards advancements in artificial breeding technologies and the establishment of clam cultivation techniques.

CHAPTER SEVEN

FUTURE PERSPECTIVE & RECOMMENDATIONS

The study of the reproductive biology of *M. meretrix* holds significant importance in understanding the actual mechanisms and patterns that govern the reproductive processes of these organisms. Through a comprehensive investigation of gametogenesis, reproductive cycles, and sex ratios, this research provides valuable insights into the species' reproductive dynamics. The identification of two distinct spawning peaks, shared similarities in male and female reproductive patterns, and the influence of environmental factors on reproductive activities highlights the adaptability and resilience of *M. meretrix* populations. Futher exploration of the genetic, environmental and physiological factors may provide actual reproductive strategies .It also contributes to the culture and management of *M. meretrix*. In the field of aquaculture, the cultivation of *M. meretrix* holds promising avenues for future exploration and development. As global demand for seafood continues to rise, the sustainable production of bivalves like *M. meretrix* presents an opportunity to meet this demand while simultaneously addressing environmental concerns. Overall, a deeper comprehension of *M. meretrix* reproductive biology has the potential to not only advance our scientific knowledge but also inform sustainable aquaculture practices and conservation initiatives for these ecologically significant organisms.

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APENDIX 1

Monthly value of Condition Index:

Descriptives

ci

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	20	3.2768	1.59459	.35656	2.5305	4.0231	.83	8.10
2	21	3.2649	2.16874	.47326	2.2777	4.2521	-2.18	8.31
3	21	5.4432	2.05157	.44769	4.5094	6.3771	2.87	10.12
4	20	2.9571	.93382	.20881	2.5200	3.3941	.58	5.39
5	20	2.1626	1.31616	.29430	1.5466	2.7786	.71	5.37
6	20	3.5061	1.21344	.27133	2.9382	4.0740	1.68	5.72
7	20	4.1941	1.33549	.29862	3.5690	4.8191	1.75	6.00
8	20	3.4260	.64011	.14313	3.1264	3.7255	2.25	4.41
9	20	2.8337	.69018	.15433	2.5107	3.1568	1.65	4.09
10	20	2.7491	1.32006	.29518	2.1313	3.3669	1.05	5.69
11	20	4.8914	2.23378	.49949	3.8460	5.9369	1.02	8.84
12	20	4.1897	1.19135	.26639	3.6322	4.7473	1.04	5.76
Total	242	3.5810	1.71556	.11028	3.3638	3.7982	-2.18	10.12

APENDIX 2

Monthly value of GSI

Descriptives

gsi

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	21	1.7720	.66073	.14418	1.4713	2.0728	.72	3.69
2	21	1.5016	1.09493	.23893	1.0032	2.0000	.66	5.82
3	20	1.6450	1.43014	.31979	.9756	2.3143	.55	7.02
4	15	.8609	.20678	.05339	.7464	.9754	.61	1.29
5	20	.9120	.25624	.05730	.7921	1.0320	.48	1.28
6	15	.2900	.19625	.05067	.1813	.3987	.08	.61
7	2	.1101	.03521	.02490	-.2063	.4265	.09	.14
8	19	.5998	.19841	.04552	.5042	.6954	.31	.93
9	20	1.2623	.50074	.11197	1.0280	1.4967	.46	2.35
10	20	1.4019	.34773	.07775	1.2391	1.5646	.83	1.88
11	20	2.2980	.55254	.12355	2.0395	2.5566	1.14	3.43
12	20	2.7944	.82626	.18476	2.4077	3.1811	1.49	4.82
Total	213	1.4268	.97893	.06708	1.2945	1.5590	.08	7.02