2016

Reproductive Biology and Biochemical Composition of Cyprinus Carpio Var. Specularis (Lacepede, 1803) in Atrai River of Northwestern Bangladesh

Manon, Md. Rashed

University of Rajshahi

Ph. D.
Thesis

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The Thesis submitted for the Doctor of Philosophy at the Department of Fisheries in the Faculty of Agriculture of the University of Rajshahi, Bangladesh.

By

MD. RASHED MANON
B. Sc. (Hons.), M. Sc., M. Phil.

AUGUST 2016

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Dedicated

To my beloved

Parents

Wife and Children

Whose sacrifice, love and inspiration encouraged me to complete the research work.

DECLARATION

I declare that the thesis entitled "**REPRODUCTIVE BIOLOGY AND BIOCHEMICAL COMPOSITION OF *CYPRINUS CARPIO* VAR. *SPECULARIS* (LACEPEDE, 1803) IN ATRAI RIVER OF NORTHWESTERN BANGLADESH"** submitted by me for the degree of Doctor of Philosophy in the Department of Fisheries, Faculty of Agriculture, University of Rajshahi, Bangladesh, is the record of work carried out by me during the period from July, 2013 to June, 2015 under the supervision of Dr. Md. Delwer Hossain, Professor, Department of Fisheries, University of Rajshahi, Bangladesh and this has not formed the basis for the award of any degree for this University or any other University or any other similar institution of higher learning or prize.

August 2016

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CERTIFICATE

This is to certify that **Md. Rashed Manon**, Ph.D. Fellow, Department of Fisheries, Faculty of Agriculture, University of Rajshahi, Bangladesh carried out the investigation on "**REPRODUCTIVE BIOLOGY AND BIOCHEMICAL COMPOSITION OF *CYPRINUS CARPIO* VAR. *SPECULARIS* (LACEPEDE, 1803) IN ATRAI RIVER OF NORTHWESTERN BANGLADESH**" under my supervision. He fulfilled all the requirements and regulations relating to the nature and period of research. It is further certified that the entire work presented as a thesis for the degree of **Doctor of Philosophy** is based on the results of author's own investigation.

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ACKNOWLEDGEMENTS

All the admiration goes to almighty Allah, the most merciful and beneficent who has enabled me to submit this humble work to the degree.

I wish to express my deepest sense of respect and gratitude and the best regards to my honorable supervisor Dr. Md. Delwer Hossain, Professor and Ex-chairman, Department of Fisheries, Faculty of Agriculture, University of Rajshahi, Rajshahi, Bangladesh.

I am grateful to the Chairman of the Department of Fisheries, University of Rajshahi for providing me with library and laboratory facilities during the progress of the research work.

I would like to extend my due respect, sincere thanks to all of the respectable teachers of the Department of Fisheries, University of Rajshahi for the moral support, assistance and care throughout the research work. I also thank the research students and office staffs, Department of Fisheries, University of Rajshahi for their generous cooperation.

I am very much grateful to Dr. Mahammad Amirul Islam, Professor, Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh and Dr. Md. Sazzad Hossain, Professor, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh who support me to analyse the biochemical composition by providing necessary laboratory facilities.

My sincere thanks and gratefulness go to my respected teachers, colleagues, staffs, present and ex-principle and honorable members of the governing body, Niamatpur Degree College, Naogaon, Bangladesh for there inspiration and active help for sanctioning study leave to procured this research work.

My profound gratitude and thank must go to the fishermen of the research areas, fish traders, members of the meteorology department and other associated persons for their kind cooperation during the course of investigation, collecting data and specimens.

Special thanks are also due to my all family members, relatives and friends, for their moral support and inspiration always encouraged me to carry out this work.

I am very grateful to my respected parents Md. Abdus Samad Mondal and Most. Rowshan Ara Begam for their constant encouragement and inspiration. Thanks are also due to my wife Dr. Mst. Atia Sultana (Gynecologist & Obstetrician), Children Md. Adittyo Manon Tamim and Mst. Ruqaiya Sultana Tasmia without whose sacrifices and constant encouragement it would not have been possible for me to complete this research work. Their generous attitude, untiring patience and continued inspiration always motivated me to carry out this research work.

Last but not the least I am grateful to those who directly or indirectly helped me in completion of this work.

(Md. Rashed Manon)

The Author

ABSTRACT

The study focused on physico-chemical conditions, reproductive biology and biochemical composition of *Cyprinus carpio* var. *specularis* (Mirror carp) for a period of 24 months (July, 2013 to June, 2015) in the Atrai river of northwestern (Naogaon district) Bangladesh. The physico-chemical conditions of the river Atrai exhibited more or less variations according to the change of months and seasons. In the study area four physical and three chemical parameters have been considered. The two years mean values of these parameters were recorded as air temperature 28.24°C, water temperature 26.81°C, water transparency 0.45 m, rainfall 122.24 mm, pH 7.91, dissolved oxygen 6.21 mg/l and free carbon dioxide 7.00 mg/l. So, the water body was suitable condition for fishes in the study area. The male and female reproductive systems were also observed. The ovaries were classified in ten stages as Immature, maturing-I (early developing), maturing-II (late developing), mature, ripe, pre-spawning, spawning, post spawning, spent and resting stages. The ova were also divided into four stages on the basis of ova diameter and coloration as immature stage (0.22 to 0.32 mm, whitish), maturing stage (0.28 to 0.69 mm, light yellow), mature stage (0.64 to 1.15 mm, yellow) and ripe stage (0.99 to 1.98 mm, Deep yellow). The mean ova diameter ranged from 0.26±0.03 to 1.26±0.15 mm. The minimum and maximum GLI and GSI were obtained from 20.89±3.83 to 38.32±5.62 and 0.36±0.14 to 8.34±6.18 respectively. The spawning time extended from November to April with the peak in February. The fecundity ranged from 5928.91 to 209120.18 with the mean of 87377.98±79544.17 which revealed that the species was high fecund. A comparison of correlation co-efficient relation between fecundity with total length ($r = 0.9129$), standard length ($r = 0.9319$), total weight ($r = 0.9519$) gonadal length ($r = 0.9121$) and gonadal weight ($r = 0.9948$) were established. All the relationships were highly significant. The sex ratio of 1084 specimens collected during 24 months of study has been determined. The total male (512) and female (572) ratio was 1:1.12. Females were prominent in natural population of the fish. In the present investigation of biochemical composition was carried out for 12 months (January, 2014 to December, 2014). The percentage of moisture (M 72.54±0.551 to 74.53±1.078 and F 73.32±0.778 to 75.42±0.538%), ash (M 3.02±0.637 to 3.44±0.536 and F 2.64±0.393 to 3.77±0.766%), lipid (M 3.36±0.400 to 4.19±0.231 and F 2.95±0.349 to 4.08±0.400%), protein (M 18.25±1.189 to 20.07±0.190 and F 17.03±0.925 to 19.56±0.538%) and carbohydrate (M 0.035±0.002 to 0.042±0.005 and F 0.037±0.004 to 0.042±0.003%) respectively. An increasing trend of moisture, ash and carbohydrate were found in both males and females in winter season. Whereas lipid and protein content showed a decreasing trend in both sexes in winter season (winter season was spawning period of this fish). In case of male protein and lipid content was higher than female fishes in all seasons. The results indicate variation in biochemical composition on the basis of sex and season.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

CHAPTER-1
GENERAL INTRODUCTION

Background of the study

Bangladesh is a small populous country. It is a south Asian country is located between latitude 20°34′ to 26°38′ North and longitude 88°01′ to 92°40′ East. This country is bordered by India to the west, north and the northeast Myanmar to the southeast and the Bay of Bengal to the south. It is a small riverine developing country which covers an area of 144000 km^2 with a population of 140 million (Hossain *et al.*, 2006). It mostly flat terrain is criss crossed by rivers which together with ox-bow lakes (known as beels and haors in Bangla), floodplains, marshes and deltas constitute the inland water bodies and associated wetlands that are home to a wide variety of aquatic plants and animals (Craig *et al.*, 2004, Hoggarth *et al.*, 1999).

Inland open water fishery resources play a significant role in the economy, culture, tradition and food habits of the people of Bangladesh (Kibria and Ahmed, 2005). Fish and fisheries have been an integral part of the life of the people of Bangladesh from time immemorial and play a major role in employment, nutrition, foreign exchange earnings and other aspects of the economy (Alam and Thompson, 2001). Fish is a natural implement to rice in the national diet giving rise to the adage Mase-Bhate Bangali (A Bengali is made of fish and rice). Fish alone supplies about 60% of animal protein intake (Ahmed *et al.*, 1997).

Fisheries is the second crop in the overall agro-based economy of Bangladesh. This sector is playing a very vital role regarding employment generation, animal protein supply, foreign currency earning and poverty alleviation. Fish production in ponds, lakes, borrow pits, floodplains, oxbow lakes, open and closed water bodies are increasing day by day through transfer of modern technology. Fish production has been increased to 36,84,245 metric tons in 2014-2015 which was contribute about 60% to the nation's animal protein intake. Fish production in rivers increased from 4.75% and 205 kg/ha. Fisheries contributing to GDP in 2014-2015 was 3.69% and more than 4,660.60 million TK of foreign currencies had been earned by exporting fish and fishery products according to the report of DoF (2016). So on the basis of contribution towards national economy fish is rightly regarded as the "silver crop" of Bangladesh. About 7% of total population is involved in fish transport, processing and marketing. Unfortunately fish production in the country is very low as compared to other developing countries of the world. The low level of fish production is the result of many factors of which socio-economic and technical problems are the most important ones (Rahman *et al.*, 1989).

Justification of species selection

The mirror carp (*Cyprinus carpio* var. *specularis*) is a thermophilic, gregarious fish, dwelling mostly at the bottom of ponds. It is an omnivorous bottom feeder on larvae of insects, worms, molluscs, stalks and leaves of submerged plants and occasionally on zooplankton (Santhanam *et al.,* 1999). The growth of the carp is very rapid, particularly in favourable environments. Its rapid growth, tasty flesh, good reproductive ability and modest requirements have led to the carp's becoming the stable fish of warm water fisheries (Talwar and Jhingran, 1991).

According to Karim (1975), unlike the major native carps, mirror carp breeds in confined water of the pond and almost throughout the year, with the peak period from December to April. The eggs being adhesive are found sticky to the leaves of submerged aquatic plants. The fry are very hardy and can be transported under a wide range conditions.

Mirror carp is a native carp of Europe. In 1979, this carp was introduced to Bangladesh from Nepal for aquaculture purpose (Rahman, 2005).

Cyprinus carpio var. *specularis* (Lacepede) is a native of the temperate region in Asia, especially China. The Directorate of Fisheries first introduced the species in Bangladesh in 1960. Evidence shows that interested fish farmers of Comilla and Jessore introduced the species in their ponds from India where it was transplanted in ponds of Raipur Fish Hatchery in 1979.

Cyprinus carpio species is highly recommended as a source of protein, which contain 16.6g fat, 2.6g iron, 1.1g Ca, 514g Phosphorous and water 76.7% (Siddique and Choudhury, 1996). In a country having population suffering from malnutrition and protein deficiency such fish species may have positive steps regarding the increment of the National health.

Therefore, the research work is aimed to solve the present day's need.

Objectives of the study

The present study has been conducted with a view to gaining some information on the following aspects such as:

- To know the physico-chemical conditions of the study area.
- To find out the reproductive period and peak period of breeding.
- To estimate the fecundity and its relationship with different body measurements.
- To know the gonadal length index, gonado somatic index.
- To determine the sex ratio, and
- To study the biochemical composition of the species.

Geographical distribution

C. carpio is considered the most abundant exotic species and also an important fishery resource (Norbis *et al.,* 2006), the fish is used in aquaculture worldwide, and has already been introduced to 120 countries and established in at least 91 (Casal, 2006). Introduced throughout the world. Wild stocks are only present naturally in rivers draining to the Black, Caspian and Aral Sea (Kottelat and Freyhof, 2007). A reophilic wild population in the Danube is assumed to be the origin of the European species; this population is now under threat (Kottelat, 1997).

Geomorphology of the study area

The geomorphology and effects of environmental parameters greatly influence the nature and pattern of the population of a locality. Such in formation is of immense importance in the study of biological aspects of any species. The Atrai river is situated of northwestern Bangladesh in Naogaon district the latitude between 24°25′ and 20°50′ N and the longitudes between 88°30′ and 88°50′ E (Source: http://enwikipedia.org/wikinaogaondistrict). The district is bounded by west bengla of India and Joypurhat district on the north, Rajshahi and Natore district on the south, Bogra district on the east and west Bangal of India and Nawabganj district on the west (Fig. 1.2).

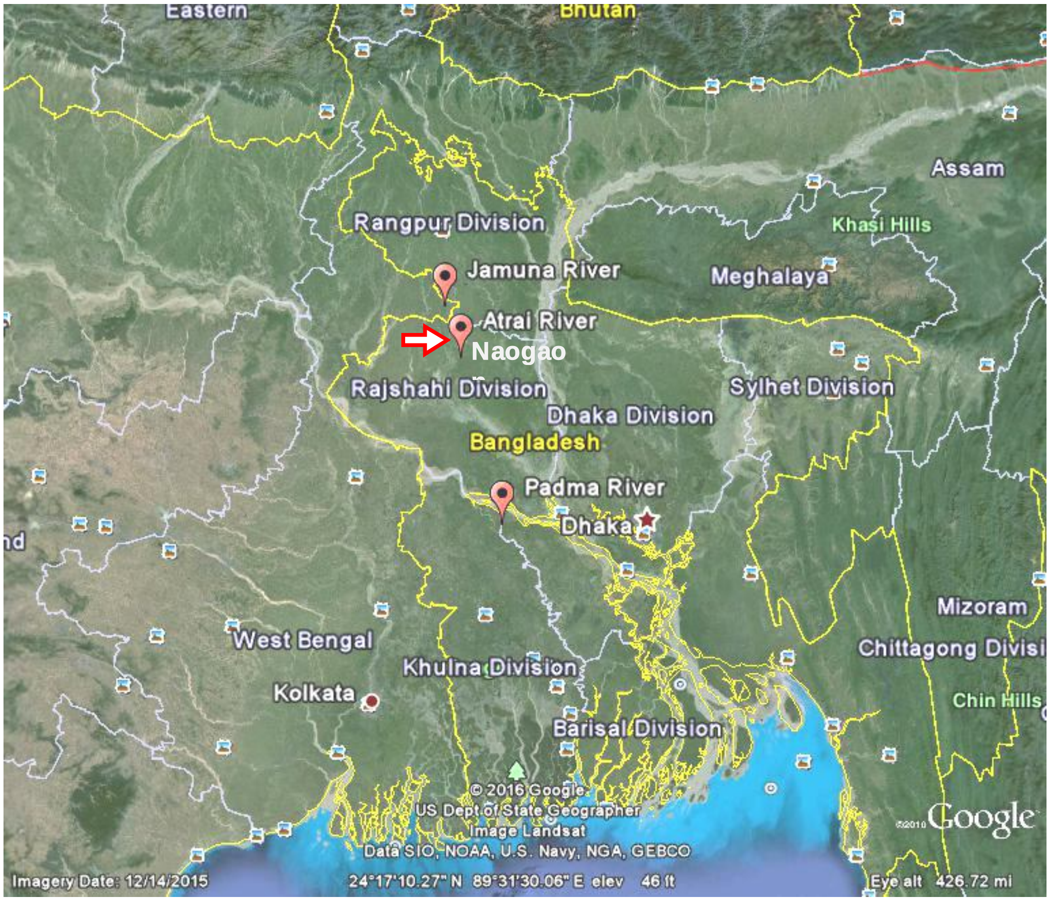


Fig. 1.1: The Geomorphological Map of Atrai river (Source: Google earth).

Physical and climatic features of the study area

This district is described as having tropical monsoon weather with relatively high temperature fluctuations, moderate rainfall is less than 400 mm and it considered as a dry zone of Bangladesh. The relative humidity is at the maximum during monsoon. The air temperature fluctuates in summer around 26.5° to 43.8°C and in the winter 4.2° to 21.5°C (Source: Rajshahi Meteorological Station, 2015).

Water resources of the study area

The Atrai river of northwestern Bangladesh. In ancient times the river was called Atrai and finds a mention in the Mahabharata. It is linked with Karatoya river. It originates in west Bengal of India and then after flowing through Dinajpur district of Bangladesh, it enters India again. It passes through Kumarganj and Balurghat community divelopment blocks in Dakshin Dinajpur district. The river then renters Bangladesh. It splits into two rivers-the Gabura and the Kankra in Dinajpur district. It crosses the Barind Tract and flows in to Chalan beel. The river serves as a perennial source of Fishing even though it is often the cause of flooding in many areas during monsoons. Total length of the river is approximately 390 km and the maximum depth of the river is 30m. [Ref. Banglapedia: National Encyclopedia of Bangladesh 2nd.ed.2012. Asiatic Society of Bangladesh.]

a

b

Plate 1.1(a-b): The Atrai river.

Fig. 1.2: Location map of the study area (➡)

A few words about *Cyprinus carpio* var. *specularis*

Cyprinus carpio var. *specularis* is commonly known as mirror carp (Plate 1.2). This fish is found in ponds, beels, haors, baors, inundated floodplains and rivers throughout Bangladesh. But it is a culturable fish mostly found in ponds.

Key to the species

Dorsal spines (total): 3 - 4; Dorsal soft rays (total): 17-23; Anal spines: 2 - 3; Anal soft rays: 5 - 6; Vertebrae: 36 - 37. Two pairs of barbels; dorsal fin with 15-20½ branched rays; caudal fin deeply emarginate (Kottelat and Freyhof, 2007). Pharyngeal teeth 1, 1, 3:3, 1,1, robust, molar-like with crown flattened or somewhat furrowed. Scales large and thick. `Wild carp ' is generally distinguished by its less stocky build with height of body 1:3.2-4.8 in standard length. Very variable in form, proportions, squamation, development of fins, and color. Caudal fin with 3 spines and 17-19 rays (Spillman, 1961). Last simple anal ray bony and serrated posteriorly; 4 barbels; 17-20 branched dorsal rays; body grey to bronze (Kottelat, 2001).

Taxonomic position

Phylum : Chordata

Sub phylum : Vertebrata

Super Class : Pisces

Class : Ostiechthyes

Sub Class : Actinopterygii

Super Order : Teleostei

Order : Cypriniformes

Sub Order : Cyprinoidae

Family : Cyprinidae

Genus : *Cyprinus*

Species : *Cyprinus carpio* var. *specularis* (Lacepede, 1803)

Plate 1.2: *Cyprinus carpio* var. *specularis* (Mirror carp)

Local name

Mirror carp, Miner carp.

Variety

The species has four recognized phenotypes differentiated according to the patterns of scaling.

Scale carp/common carp- *Cyprinus carpio* var. *communis* (Linnaeus)

Mirror carp- *Cyprinus carpio* var. *specularis* (Lacepede, 1803)

Leather carp- *Cyprinus carpio* var. *nudus* (Bloch)

Orange coloured carp- *Cyprinus carpio* var. *flavipinnis* (Ali *et al.*, 1995)

Habit

Mirror carp is an omnivorous fish. Bottom feeder and non-predatory in nature. It eats phytoplankton, zooplankton, algae, aquatic plant parts, debris and detritus etc.

Habitat

Mirror carp lives in the ponds, haors, baors, beels, floodplains and rivers.

Distinguishing characters

Shape: Body snout, slightly compressed.

Head: Moderate, triangular

Snout: Obtusely rounded.

Colour: Its colour is olivaceous, with silvery or golden sides. Fins yellowish, reddish or golden; anal fin becomes bright red in breeding season.

Mouth: Mouth small and oblique, protrusible; lips thick and fleshy. Barbels two pairs; maxillary barbels twice as long as rostral pair.

Gillrakers: Gillrakers 21 to 29 on first arch.

Fin: Dorsal fin inserted midway between snout-tip and base of caudal fin, dorsal spine stout, serrated behind. Anal fin trapezodial. Pectoral fins large and rounded. Caudal fin deeply emarginate (Plate 1.2).

CHAPTER-2
REVIEW OF LITERATURE

Physcio-chemical conditions

Physico-chemical conditions means the combined effort of physical and chemical factors. These factors are water depth, water temperature, water transparency, dissolved oxygen, free carbon dioxide, ammonia, total hardness, alkalinity, pH etc. These factors are very much important for the total environment of a particular water body. In this field some works which have been done are discussed below in brief.

Ganapati (1955) noticed diurnal variation of physico-chemical parameters in rock pools on stream bed of Mettur dam in India. He found that the surface layer was supersaturated and the bottom layer was under saturated.

Das and Srivastava (1956) worked on the quantitative study of the freshwater plankton of a fish tank in Lucknow, India. He observed monthly fluctuations in plankton volume and percentage composition differ in different month.

Chakraborty *et al.,* (1959) observed that the abundance, seasonal fluctuations and diurnal variation of plankton in relation to physico-chemical conditions of water in the river Jamuna at Alahabad.

Vyas and Kumar (1968) studied the water conditions of Indrasagar Tank, India. They observed that the periods of high temperature nearly coincided with those of low oxygen content.

Dhawan (1970) studied the hydrobiological factors at Kandla in the gulf of Kutch. He noticed seasonal variation in the temperature, salinity, pH, dissolved oxygen, inorganic phosphate, nitrate and silicate and their influence on plankton.

Michael (1970) observed the diurnal variation in physico-chemical factors and zooplankton in the surface layer in freshwater. He said, many aquatic organisms exhibit diurnal rhythms in their activities.

Islam *et al.,* (1974) worked on the physico-chemical effects of the river Buriganga in Bangladesh. They observed water temperature was always less than the air temperature and it varied within 2°C. The maximum dissolved oxygen content value (6.57 mg/l) was in August.

Khalaf and MacDonald (1975) conducted a hydrobiological investigation in the temporary ponds in New Forest. They observed that the pH of the ponds fluctuated with the change in dissolved oxygen concentration and heavy rainfall which produced an immediate decrease in the pH of the ponds.

Islam and Mendes (1976) studied on the limnology of a jheel in Sher-E-Bangla Nagar, Dhaka. They said that water temperature was always less than the air temperature and varied winter 2°C. Dissolved oxygen content was maximum 9.83 ppm in November and minimum 4.48ppm in August. The maximum free carbon dioxide values were 29.30 ppm in September and minimum 2.00ppm in December.

Islam *et al.,* (1978) worked on some physico-chemical parameters and growth of major carps in two ponds of village Boyra, Mymensingh. They noticed that the water temperature was closely related with air temperature. The water transparency value increased with the increase in water volume and consequent decrease in concentration of microscopic organisms. They studied indicated an inverse correlation of CO_2 with dissolved oxygen and direct correlation with temperature.

Ali *et al.,* (1980) made an observation on the ecology and seasonal abundance of zooplankton in an artificial fish pond. The role of temperature, pH, hardness of water as $CaCO_3$ and total alkalinity and the abundance of different of genera were discussed.

Chowdhury and Mazumder (1981) made a limnological survey of the Kaptai lake-a tropical impoundment. Emphasis was given on the seasonal vertical, horizontal and diurnal fluctuations in some physico-chemical parameters of the lake water. This lake was found to be of oligotrophic nature in acidic to near neutral pH and low nitrate and phosphate content.

Rahman *et al.,* (1982) studied the physico-chemical conditions of four ponds. The physico-chemical aspects investigated were average depth, temperature, dissolved oxygen, free carbon dioxide, pH, carbonate etc. They stated that the water temperature, dissolved oxygen and pH values were higher at the surface water than those at the bottom waters and free carbon dioxide contents in vertical variation were low at the bottom waters that those at the surface waters.

Ali and Islam (1983) worked on the monthly variations of abundance and distribution of plankton in a lake of Bangladesh Agricultural University Campus. They found the minimum zooplankton was in the month of April.

Habib *et al.,* (1983) made a comparative study on monthly fluctuation of physico-chemical parameters like transparency, hardness, free carbon dioxide, dissolved oxygen, phosphate, phosphorus, magnesium and calcium in seasonal ponds of Bangladesh.

Miah *et al.,* (1983) studied some ecological parameters and growth of major carps in two ponds of Bangladesh Agricultural University Campus, Mymensingh. They recorded water temperature, highest and lowest water transparency, dissolved oxygen and carbon dioxide. They stated that dissolved oxygen and pH were higher in the surface whereas hardness, free carbon dioxide, phosphate and nitrate were higher at the bottom water.

Rahmatullah *et al.,* (1983) studied the qualitative and quantitative nature of phytoplankton in a pond in Bangladesh Agricultural University campus, Mymensingh. The maximum and the minimum average number of phytoplankton were recorded.

Habib *et al.,* (1984) studied the monthly fluctuation in zooplankton population in relation to physico-chemical factors of water. The successful fisheries management basically depends upon limnological aspect of bottom soil and water bodies.

Alam *et al.,* (1985) investigated the correlations of some physico-chemical properties of water with zooplankton in nursery ponds. During the study they observed that water temperature, pH, dissolved oxygen, free carbon dioxide, nitrate, phosphate and exchangeable potassium, calcium were the influencing factors for the various genera of zooplankton.

Ali *et al.,* (1985) studied the meteorological factors and temperature, transparency and pH of water had combined effect upon the fluctuation of various species of phytoplankton. They observed that various species of phytoplankton were inversely correlated with rainfall. The total hours of sunshine influenced the growth and abundance of various species.

Patra and Azadi (1985) observed the physical and chemical parameters of water of Halda river, Chittagong, Bangladesh at a spawning site for the three major carps. High water level, strong water current and high turbidity, comparative low water temperature, low conductivity, low dissolved oxygen content and pH influenced spawning of the major carps in the Halda river.

Alam *et al.,* (1987) investigated the abundance of zooplankton of four newly constructed ponds with reference to some meteorological and limnological factors. Zooplankton populations were found to be correlated directly to dissolved oxygen and pH while inversely to free carbon dioxide.

Begum and Alam (1987) studied the relationship between the different physico-chemical variables and abundance of plankton of two ponds in Maijdee Court, Noakhali. He stated that on the pond phytoplankton dominated over zooplankton.

Ameen *et al.,* (1988) worked on the light penetration temperature, dissolved oxygen and free carbon dioxide at the surface and bottom water of a pond in the southern part of Bangladesh.

Khondoker *et al.,* (1988) studied the gross primary productivity in Dhanmondi lake at Dhaka. The water temperature, secchi disc visibility, pH, DO, free CO_2 and alkalinity were determined and their spatial and temporal variations were shown.

Ali *et al.,* (1989) investigated the seasonal variation of physico-chemical condition of water, plankton and benthic macro invertebrates in a perennial pond. There were considerable variations in water quantities in different seasons. The temperature of water varied from 20.5 to 36°C, pH 6.7 to 8.4, the highest value of CO_2 42ppm, carbonate 3.2 to 24ppm and higher dissolved oxygen content value in winter and early summer.

Begum *et al.,* (1989) studied the limnology in a minipond and growth of Tilapia. They discussed seasonal variations and interrelationship of physico-chemical parameters and plankton. An inverse relationship was noted between phytoplankton and zooplankton. They also stated the possible reasons for higher yield in the minipond.

Chowdhury *et al.,* (1989) observed the occurrence of seasonal variation of zooplankton in a fish pond in relation to some physico-chemical factors. The coefficient of correlation between temperature and occurrence of zooplankton showed an inverse relationship.

Khan *et al.,* (1990) made a detailed description on the physico-chemical factors of Dhanmondi lake in Bangladesh. The seasonal variations in chemical factors are also described, obtained on inverse correlation between temperature and dissolved oxygen during the study period.

Munjurul *et al.,* (1990) studied the seasonal variation in physico-chemical conditions of Dhanmondi lake water. In summer, the water was chemically richest because of the mixing of surface and bottom water. An inverse correlation between temperature and dissolved oxygen was observed throughout the year.

Khondoker and Parveen (1992) showed depth wise distribution of temperature and DO in Dhanmondi lake throughout the year. Average value of nutrients, dissolved gases conductivity and alkalinity were seen higher when compared with some other typical eutrophic ecosystem.

Rahman (1992) carried out an experiment on pond ecology and found that the values of water temperature from 26.2 to 34.5°C, transparency from 12 to 46.5 cm, total alkalinity from 71 to 175 mg/l, pH from 6.5 to 8.8 and dissolved Oxygen from 6 to 8 mg/l.

Begum *et al.,* (1994) observed the physico-chemical parameters of a semi-intensively managed fish pond and noticed fluctuations. The pH value of pond was found to have the lowest variability while carbonate had the highest variability.

Banik (1995) studied the zooplankton abundance in a freshwater fish farming pond in West Bengal in relation to some environmental factors. Zooplankton showed the highest numbers in the winter and lowest in the summer.

Wahab *et al.*, (1995) measured water temperature from 27.5 to 32.4°C, Secchi depth reading between 26 to 50 cm, pH around 6.0, total hardness of water from 45 to 108 mg/l, DO level from 2.2 to 7.1 mg/l in ponds of Mymensingh.

Bhuiyan and Nahar (1996) studied some physico-chemical parameters of a fish pond. They stated that free CO_2 and HCO_3 were inversely related with DO. The relationship between HCO_3 and free CO_2 were positive and negative with pH. They indicated the pond to be suitable for pisciculture.

Bhuiyan and Nessa (1996) made observation on the physico-chemical parameters of a pond in Rajshahi. They observed, there is a coefficient of correlation between water temperature and water turbidity and inverse relationship was found with free carbon dioxide and bicarbonate alkalinity.

Chowdhury *et al.*, (1996) investigated the impact of industrial effluents on the physico-chemical and biological conditions in the polluted area of Padma river. High pH, conductivity, COD, BOD and chloride value of lower DO content followed by a lower variety of plankton indicate an approach towards a higher polluted condition.

Das *et al.*, (1997) studied the ecological parameter such as water temperature, salinity, dissolved oxygen, pH etc. which greatly influence the ovary maturation embryonic development and spawning capability of shrimp.

Ehsan *et al.*, (1997) made an observation on limnological conditions of the floodplain Halti Beel. The noticed that the pH values remained slightly alkaline whereas turbidity was found to be higher in the canal.

Hossain *et al.*, (1997) studied some physical and chemical parameters of the BSKB beel, a completely closed system floodplain. Emphasis was given on temporal and spatial fluctuation in respect of turbidity values. They observed dissolved oxygen which showed an unusual event and maintained an inverse relationship with temperature.

Pavel *et al.*, (1997) analyzed some aspects of physico-chemical properties of a pond bottom soil of Jahangir Nagar University campus. They found that the soil was clay to loamy and indicated that the experimental pond was a favourable condition for fish culture.

Singha *et al.,* (1997) observed physico-chemical parameters and fish species composition of Harinchora beel in Dhubri district in Assam were studied by in the study growth rate of eight commercially important fish species was examined using the length-weight relationship. With the exception of *Channa punctatus* (3.63), all other species showed negative allometric growth (< 3.0). Primary productivity of the beel was also examined to ascertain the status of the producers with respect to fish yield.

Chowdhury and Zaman (1999) investigated zooplankton content and physico-chemical conditions of the river Padma near Rajshahi. They stated that the abundance of zooplankton was found to be affected by the physico-chemical condition of water, current and turbidity during the study period.

Ahmed *et al.,* (2000) observed the physico-chemical characteristics of water bodies in Bangladesh Agricultural University under two different treatments. Mean value of temperature, secchi depth, dissolved oxygen, pH, chlorophyla, alkalinity, phosphorus were found significantly different among the ponds.

Bhuiyan and Nahar (2000) made an observation on the migration of some zooplankton in a fish pond for light, dissolved oxygen and availability of food. The zooplankton varied in different hours of the day. They also observed that pH, DO value were higher at evening and CO_2 was high in the morning.

Alam *et al.,* (2002) investigated seasonal changes of physico-chemical parameters of freshwater wetlands of greater Dhaka district. They stated that water quality of any water body depended on the interactions of various physico-chemical factors which influence its biotic communities.

Haroon *et al.,* (2002) studied water chemistry of two representative *beels* of Sylhet sub-basin and made comment that water temperature fluctuated between 18.5 and 33.8°C, water transparency between 60.0 and 157.0 cm, pH between 6.1 and 7.2, total alkalinity between 50.0 and 89.0 mg/l and NO_3-N between 0.1 and 0.22.

Hussain *et al.,* (2003) worked on some physico-chemical parameters in river Padma, adjacent flood plains area (beel) and ponds. They reported that the water temperature varied from 13.5 to 29.8°C, transparency 0.05 to 1.24 m, DO 3.15 to 5.95 mg/l, CO_2 2.62 to 8.41 mg/l and pH 6.75 to 8.1 in Padma river. The water temperature ranged from 16.7 to 28.9°C, transparency 0.31 to 0.43 m, DO 4.23 to 8.33 mg/l, CO_2 2.69 to 8.66 mg/l and pH 6.97 to 7.63 in flood plains (beel) whereas the water temperature ranged from 28 to 31.4°C, transparency 0.35 to 0.46 m, DO 3.19 to 6.54 mg/l, CO_2 mg/l to 9.83 mg/l and pH 6.83 to 8.39 in pond.

Bhaumik *et al.,* (2003) investigated the ecology of Barnoo reservoir, Madhya Pradesh for augmenting fish production. Their study revealed that water and soil quality of the reservoir with season and location. They stated that temperature showed negative correlation with dissolved oxygen.

Chakraborty and Chattopadhyaya (2003) study on the trophic status of Mathura beel in West Bengal, India. The study investigated the water and sediment quality of the Mathura beel receiving effluents from Haringhata Dairy Farm and domestic sewage from Kancharpara town in West Bengal, India during February to May 2002. They found a significant variation in the physico-chemical characteristics of water and in sediment characteristics.

Rao *et al.,* (2003) studied limnology of Markanohalli reservoir across river Shima. They noticed that seasonal variation of physical and chemical parameters influenced fish production.

Rout *et al.,* (2003) conducted research on impact assessment of the surroundings on water quality of Kulia beel in Nadia district of West Bengal, India. The water body was found to be moderately polluted, which can be improved for fish production by eco-friendly management norms of the surrounding users.

Sultan *et al.,* (2003) investigated physico-chemical features and productivity status of Pahunj reservoir located at Jhansi in Uttar Pradesh. They stated that higher primary productivity indicated congenial environment for biological production.

Alam *et al.,* (2007) examined the water quality of Posna *beel* during 2003-2005. They found surface water temperature, transparency, pH, DO, CO_2, alkalinity, total suspended solids (TSS), biological oxygen demand (BOD), chemical oxygen demand (COD), total hardness, NH_3 and nitrate nitrogen (NO_3-N) to vary from 16 to 33°C, from 25 cm to 189 cm, from 7.2 to 8.0, from 6.6 to 9.6 mg/l, from 5.0 to 13.0 mg/l, from 28 to 51 mg/l, from 7.0 to 123 mg/l, from 2.70 to 5.43 mg/l, from 5.20 to 8.15 mg/l, from 51.0 to 68.0 mg/l, from 0.01 to 0.07 mg/l and from 0.15 to 0.48 mg/l respectively.

Khan *et al.,* (2007) studied the limnological parameters of Kalian and Haily *beel* of Mymensingh. They reported the water temperature, pH, alkalinity, DO and ammonia vary from 19.5 to 30.5°C, 7.0 to 7.5, 83 to 201 mg/l, 2.5 to 3.8 mg/l and 0.1 to 0.3 mg/l respectively in Kalian *beel* whereas in Haily beel the parameters varied from 20.0 to 30.5°C, 7.0 to 7.5, 70 to 139 mg/l, 2.5 to 4.6 mg/l and 0.2 to 0.4 mg/l respectively.

Rahman *et al.,* (2007) studied the limnological properties of Nandinar *beel*, Chapundaha *beel* and Dariar *beel* and reported the mean values of water temperature as 28.81±0.51°C, 29.33±0.51°C and 28.40±0.51°C, water transparency as 31.97±2.00cm, 35.22±1.51cm and 59.26±1.72cm, total alkalinity as 158.93±19.99 mg/l, 21.13±0.57 mg/l and 25.56 ±0.58 mg/l, pH as 8.11, 7.08 and 7.55, DO as 3.36±0.23 mg/l, 6.75±0.09 mg/l and 9.08±0.11 mg/l, NH_3-N as 2.73±0.33 mg/l, 0.24±0.029 mg/l and 0.06±0.004 mg/l respectively.

Zaman *et al.,* (2009) studied water chemistry of farmer managed waste fed fish ponds in Rajshahi City Corporation. They described the values of water temperature, transparency, pH, DO, CO_2, NH_3 and alkalinity varied from 30±0.50 to 30.87±0.70°C, 25.00±0.50 to 29.60±0.20 cm, 7.55±0.08 to 7.75±0.15, 1.37±0.10 to 1.83±0.24 mg/l, 4.61±0.34 to 4.95±0.00 mg/l, 5.50±0.50 to 6.03±1.00 mg/l, 0.110±0.012 to 0.215±0.007 mg/l and 220.00±6.00 to 242.58±5.50 mg/l respectively.

Alam *et al.,* (2011) studied water quality in Hilna beel of Rajshahi district in Bangladesh. They found the value of water transparency 27.33±5.09 cm, water temperature 26.66±4.54°C, pH 7.88±0.22, DO 8.45±0.98 mg/l, Free CO_2 10.62±1.83 mg/l, Nitrite-Nitrogen 0.0196±0.0092 mg/l, Chloride 16.0±5.26 mg/l, Total alkalinity 57.85±18.87 mg/l, Hardness 72.68±12.89 mg/l, Conductivity 162.72±67.81 µs/cm, TDS 93.48±41.62 mg/l and Turbidity 139.53±112.24 ppm respectively.

Siddique *et al.,* (2011) recorded the mean values of water temperature, transparency, pH, DO, CO_2, NH_3-N and alkalinity of some fish culture ponds of Rajshahi as 22.68±5.53°C, 31.50±3.60cm, 7.54±0.23, 3.04±0.29 mg/l, 7.75±1.95 mg/l, 0.012±0.01 mg/l and 97.55±12.79mg/l respectively.

Masaddequr *et al.,* (2012) observed the Padma River is one of the longest rivers and it is believed to be an important spawning and feeding ground for riverine fish species of Bangladesh. This study analyzed the fish biodiversity and main threats to biodiversity to provide recommendations for conservation in the Padma River. Sampling was conducted fortnightly in the Padma River during March 2009 to February 2010 from fishermen catch landed at different fish landing centers between Binodpur and Godagari, Rajshahi, northwestern Bangladesh. A total of 80 species of fish under 9 orders and 24 families were recorded. Cypriniformes were most dominant order constituting 35% of the total fish population followed by Siluriformes (32.50%), Perciformes (17.50%), Clupeiformes (5.00%), Synbranchiformes (3.75%), Osteoglossiformes (2.50%), Beloniformes (1.25%), Mugiliformes (1.25%) and Tetraodontiformes (1.25%). Among the species found during the present study, 12.50% were vulnerable, 21.25% were endangered and 8.75% were critically endangered. Major threats to fish biodiversity include habitat destruction and defragmentation, water pollution, indiscriminate harvesting of fry and fingerlings, construction of barrages, construction of embankments by the Ganges-Kobadak project, sedimentation.

Saidur *et al.,* (2012) fount the status and diversity of animals are the important indicators for a healthy habitat of animals. We conducted a survey on ecology and status of herpeto-mammalian fauna in the Padma river and its adjacent areas, Rajshahi. A total of 50 species of herpeto-mammalian fauna was recorded from the study area. Of these, 5(10%) were amphibians, 20 (40%) reptiles and 25 (50%) mammals. Group discussion with the local people indicated that species diversity of herpetomammalian fauna has been decreased day by day in the study area. This might be due to the results of highly disturbance by human. Meanwhile, increase of human population, destruction of habitat, expansion of agricultural activities, illegal hunting and trade are the main causes for declining herpeto-mammalian fauna in the study area.

Reproductive biology

There is abundant information in the literature of reproductive biology of fishes. Some important works that related with the present work are mentioned below:

Harry (1959) studied the time of spawning, length of maturity and fecundity of the English and Dover soles and noted that the spawning season of these fishes is from November to February, with peak in December and January. The fecundity of English sole was 498,000 in the left ovary and 684,000 in the right ovary.

Chokder (1970) studied the recruitment of the total number of eggs produced by a fish *(Catla catla)*. He estimated the ova production of *Catla catla* which ranged from 8,83,963 to 10,89,600 and of *Cirrhina mrigala* from 1,06,230 to 2,48,400 and *Labeo rohita* 5,24,470. On estimating the production of ova per pound of body weight *Catla catla* it was found to produce the highest number of ova by body weight of fish among the three species studied.

Beacham (1982) studied the variability in the length and age at which 50% of individuals were mature was investigated for argentine *(Argentina silus)* in the Northwest Atlantic. Median length at maturity generally declined with time, the trend more pronounced in females than in males. Males and females matured at similar length but males tended to mature at older ages. From 1965-1969 median length at maturity for males ranged from 35.4 cm in NAFO subdiv. 4Vs to 26.4 cm in Div. 4W, while that for females ranged from 28.0 cm in subdiv. 4Vs to 24.9 cm in subdiv. 5Ze. from 1965-1969, median age at maturity was about 5.3 yr for males and 4.6 yr for females.

Islam and Hossain (1984) worked on the fecundity of Chela, *Oxygaster bacaila* and found that the fecundity of the species ranged from 7,146 to 33,997. The mean number of eggs was calculated as 14,014±7347.09 with the fecundity of 1404/g body weight of fish.

Takur and Das (1985) observed that the fecundity of *Clarias batrachus* (Linnaeus) ranged from 1,060 eggs to 21,790 eggs from the fish of 150 m to 350 mm.

Davies *et al.*, (1986) made a study on the effect of temperature and photoperiod on sexual maturation and spawning of the common carp. They observed that this group was closer to final maturation and did not need the simulative effect of a long photoperiod. They reported that when maintained at 16°C carp completed rematuration within 3 to 4 months, including an initial stay of 2 to 3 weeks at 24°C, and could stay mature indefinitely.

Uktolseja and Purwasasmita (1987) reported that the average fecundity of skipjack tuna, *Katsuwonus pelamis* was 5,63,393, ranging from 2,85,384 to 1,141,869, the average diameter of ovum was 0.4252 mm (range 0.3596 to 0.4921 mm) corresponding to an average fork length of 494 mm, 2432 g body weight and 65.55 g ovary weight. The ova diameter (frequency from ovarium stage III in late maturing and IV in mature) showed three and two distinct modes.

Mollah (1988) studied the cyclic changes in the testes of freshwater *Clarias mancocephalus* histologically. Six histologically stages were identified in the testis maturity stages. He observed spawning that take place twice a year; in late September to early November and again in March till early June.

Islam and Azadi (1989) made observation of fecundity of *Mystus cavasius*. They studied the length of the specimen ranging from 23.2 cm to 29.2 cm and their fecundity was found to be from 39,405 to 1 ,34,285 eggs.

Bhuiyan and Islam (1990) made an observation on the fecundity of *Xenentodon cancila* which ranged from 750 to 2,852 eggs per female varying in length and weight 165 mm and 15g to 255 mm and 43.5 g respectively. The mean fecundity recorded was 1 ,432 egg for a fish with a mean total length of 21 1 .8 mm and mean body weight 24.05 g.

Islam and Hossain (1990) studied on the fecundity and sex ratio of the common punti, *Puntius stigma* (Cuvier and Valenciennes) from the river Padma near Rajshahi (in Bangladesh). The fecundity varied from 2475 (total length 74mm and body weight 8.85g) to 14461 (total length 98mm and body weight 14923g). The fecundity offish per g body weight was 849.

Norberg *et al.,* (1991) made a study on ovulatory rhythms and egg viability in the Atlantic halibut. They reported that careful monitoring of the individual spawning cycles resulted in a 120% increase in total egg yield and a 220% increase in the amount of eggs showing high (>80%) fertilization rates. Handling stress had no apparent effect on the yield of eggs. The results show the importance of careful surveillance of individual, female halibut during the spawning season, in order to establish ovulatory rhythms and optimize the yield of eggs.

Hye and Alamgir (1992) carried out investigation to establish natural spawning of carps in the lake Kaptai and to determine the location of spawning area. Large scale spawning activity was recorded in Kassalong channel and 2.5 million hatchling developed in happas and earthen pits from the eggs and release back to the lake.

Khan *et al.,* (1992) studied the size frequency distribution and fecundity of *Mystus tengara* during its peak breeding season. The highest peaks observed were in size group of 5.4 to 5.6 cm in October. Fecundity varied from minimum 720 to maximum 5,233 eggs.

Mustafa (1992) studied the monthly changes in the mean ova diameter, gonado somatic index and relative condition factor of *Puntius sarana* which indicated that the peak gonadal development occurred in April to May followed by spawning in June. The fish spawned once a year.

Rahman *et al.,* (1992) worked on the sex ratio of the *Lepidocephalus guntea* and found that the females dominated over the males in all the months excepting July, September, October, November, April and June which concluding that the females are predominant in the month of August, December, January, February, March and May in the natural population of the species. The male and female ratio was obtained as 1:1.61.

Haque and Hossain (1993) studied the maturation of females by observing the color of the ovary and with diameter of the ova of *Mystus vittatus*. They further observed that the fecundity of the freshwater cat fish *Mystus vittatus* ranged from 2,534 to 60,746 ova in specimens varying in length from 92 to 116mm.

Opuszynski and Shireman, (1995) in the Pampanga River Basin of the Philippines, for example, where natural spawning occurs, the temperature does not change appreciably during the year. The average range in monthly air temperature is from 25.9 to 19.6°C.

Mazzoni *et al.,* (1995) studied the length-frequency analyses showed that male *Hypostomus affinis* are bigger than females, but *H. luetkeni* showed no such differences.

The sex ratio was estimated at 1:1 for both species. The temporal variation of sex ratio indicated a decrease in male population when GSI was at its maximum value, suggesting that males are less prone to capture during the spawning season. According to the decrease of males during the reproductive period, paternal behavior is proposed for both species. *H. affinis* reached sexual maturity at a smaller size than *H. luetkeni*.

Faruq (1995) worked on fecundity of four species of catfish viz. *Heteropmustes fossilis* (bloch), *Clarius batrachus* (Hamilton), *Mystus cavasius* (Hamilton) and *Mystus vitatus* (bloch) have been studied during April to July 1995. The fecundity of *Heteropneutes fossilis* was from 9308 eggs (total length 19.3 cm) to 22210 egg (total length 26.0 cm), *Clarius batrachus* from 3028 eggs (total length 18.1 cm) to 9064 eggs (total length 26.2 cm), *Mystus cavasius* was from 12262 eggs (total length 13.6 cm) to 20626 eggs (total length 18.5 cm), *Mystus vitatus* was from 3842 eggs (total length 7.0 cm) to 9866 eggs (total length 13.1 cm).

Alam *et al.,* (1996) observed the maturation and spawning of *Setipinna phasa* and found that the fish shows four maturation stages for male and six for female with a prolonged breeding season from October to April. The average sex ratio was observed 1t1.14 which was varied significantly in different months.

Bhuiyan and Afrose (1996) stated that the fecundity of *Oreochromis nilotica* ranged from 290 to 1265 with a mean of 575.91±296.75. The number of eggs increased linearly with the increase of body length, body weight, ovary length, ovary weight and ovary breadth. The total male and female ratio was 1t1.31 *i e.*, the females were predominating throughout the year.

Kamilov and Salikhov (1996) in Silver Carp, egg production per females varies with location and body size, ranging from 50,000 to 5,000,000.

Kuwamura *et al.,* (1996) studied that the number and maximum body size of the eobioid fish, *Paragobiodon echinocephalus*, increase with the size of its obligated host coral, Stylophora. Only the largest two individual breeds monogamously in each coral head and the reproductive success of each spawning are positively correlated with body size. In this study, the plasticity in size and age at maturity in *P. echinocephalus* was examined. The size at maturity ranged widely from 17.2 to 36.0 mm TL.

The estimated lifetime reproductive success did not differ between the gobies inhabiting corals of different size. Thus the plasticity in size and age at maturity in this species may be maintained by frequency-dependent selection in choosing a host coral size that affects an individual's social status.

Laird and Page, (1996) Because Silver Carp eggs, like those of Bighead Carp, are semi-buoyant, spawning typically occurs in water of sufficient flow to keep the eggs from sinking to the bottom and dying.

Ellis and Shackley (1997) stated that the overall sex ratio (males t females) of *Scyliorhinus canicula* is 1t1·23. Males matured between 490 and 540 mm LT with 50% maturity at 520 mm. Females matured between 520 and 640 mm LT with 50% maturity at 550 mm. Female gonadosomatic index peaked in May. Fecundity increased with fish length. The egg laying season lasted 10 months with a peak in June and July.

Bhuiyan and Parveen (1998) worked on the fecundity of *Puntius sophore* and observed that the fecundity of this fish ranged from 1,221 to 3,432 eggs per fish of total length and total weight were 103mm and 16.58 g respectively. The mean fecundity was 1824.6 egg for a fish with a mean total length 86.773 mm. Same sized fish had different number of eggs in their ovaries.

Kabir *et al.*, (1998) estimated the fecundity of pond raised Chapila *(Gudusia chapra)* in the range 25220 to 154528 with the average of 72383. They also found that fecundity increased linearly with the increased in length weight of the fish.

Bhatt *et al.*, (1998) studied the maturity stages and sex ratio in the golden mahaseer. *Tor putitora* (Hamilton) in the foothill section of the river Ganga. They identified three maturity stages; immature virgins, maturing virgins and ripening females. No mature fishes were found and it was suggested that golden mahaseer do not breed in the Ganges River.

Sultana *et al.*, (1998) found that the fish *Pangasius pangasius* is bispawner and spawn once in the winter (January-March) and second time in the monsoon and post monsoon (June to November). The fish is highly fecund and produce 85,832.50 eggs per kg of body weight. The fecundity varied from 1,02,600 to 27,50,000 with the mean of 10,69,473±3,39,323.90.

Bhuiyan *et al.,* (1999) observed that *Clupisoma atherinoides* which spawn from May to September with a peak in July. Five methods were used to estimate the spawning periodicity.

Bhuiyan *et al.,* (2000) published a paper on the fecundity and sex ratio of *Barbodes gonionotus*. They observed that the matured fish had 18,001 to 42,038 eggs in varying weight of 72 to 159 g and male, female ratio was 1 -0 90 It was observed that males were predominant during the months of February, April to June, October, December while the females were predominant in the rest of the months.

Everson *et al.,* (2000) worked on the inter-annual variation in the gonad cycle of *Champsocephalus gunnari* and found that the gonadal maturation is closely associated with the time of year. Spawning appears to occur at the same time each year, but the timing of gonadal development is subject to a considerable inter-annual variation.

Caputo *et al.,* (2001) found that the paedomorphic goby *Aphia minuta* does not show semelparity. In spring, the mature ovary contains several batches of eggs at different stages of vitellogenesis. Which indicates the breeding season of *A. minuta* is quite long and that spawning takes place at least twice during its short life span.

Olney *et al.,* (2001) studied the criteria for determining maturity stages in female American shad, *Alosa sapidissima* and a proposed reproductive cycle. They found no differences in scoring of maturation stages were observed in comparisons of samples from different regions of the ovary. American shad in both semelparous (Edisto River) and iteroparous populations (York and Connecticut rivers) exhibit indeterminate fecundity and group-synchronous oocyte development.

Schrank and Guy (2002) conducted a study on fecundity of Bighead Carp from the lower Missouri River collected in 1998-1999 and they have found that the fecundity ranged from 11,588 to 769,964, with an average of 226,213 eggs.

Bhuiyan and Akhter (2002) studied the number of eggs of *Cypnnus carpio* varying from 2,22,222 of total length 16.2 cm to 2,55,942 of total length 30.8 cm. The total male and female sex ratio was 1:1.241.

Keys and Ortega (2002) studied the initial sexual maturity and fecundity of two anabantids under laboratory conditions. They concluded the sixty-day-old juvenile fish receive a prophylactic treatment of brackish-water (15 per ml) and methylene blue (1%) baths and were fed live cladocerans, Daphnia magna. Later induced spawning techniques were applied at initial sexual maturity in 110 and 140 days old fish. Mean absolute fecundity was 2023 and 8021 ova respectively and mean relative fecundity was 860 and 1035 eggs/g fish weight.

Latifa *et al.,* (2002) studied the fecundity and sexual maturity of the gonad of *Channa striatus.* They observed the fecundity which ranged between 3,454 for a fish of length of 21.9 cm and weight of 122 g and 20,568 eggs for a fish of length of 38.3 cm and weight of 421 g. The average number of eggs was 11,720.

Barros and Regidor (2002) analysed the spawning period of *Odontesthes bonariensis* using gonadosomatic index (GSI) curves and monthly percentage distributions of maturation stages and found that the fish show a single spawning season with two peaks (late winter and early autumn) within 1 year.

Hossain *et al.,* (2002) studied the reproductive periodicity of *Gonialosa manminna* and observed that the reproductive periodicity of the species starts from January and continues upto June with a peak in April.

Mabragana *et al.,* (2002) studied the reproductive ecology of *S. bonapartii* and found that the right ovary of mature females is larger than the left. Gonadosomatic index and diameter of ovarian follicles of mature females peaked in late spring and was at a minimum from late summer and through the winter.

Caputo *et al.,* (2003) found that the pelagic crystal goby *Crystallogobius linearis* is semelparous; and after the first spawning the mature gonads of both males and females appeared to have lost the capability for further spawning. Mature females had very low fecundity (200-700 mature oocytes) and small egg size (0.2-0.55mm).

Alp *et al.,* (2003) observed the reproductive biology of *Salmo trutta macrostigma* and found that spawning of the fish lasted from November to January. Some 27.7% of the females and 62.5% of the males attained sexual maturity in their second year. The mean fecundity of the sampled population was 554 eggs per fish.

The diameter of mature eggs in the spawning season ranged from 3.250 to 5.930 mm, with a 4.146 mm average.

Abou and Dadzie (2003) studied the histological of ovarian development and maturity stages in the yellow fin sea bream, *Acanthopagrus latus* (Teleostei : Sparidae) (Hottuyn, 1782) reared in cage. Observations on seasonal maturity stages indicated that the species has a prolonged spawning period extending from December to April. However, an earlier report based on quantitative study indicates that level of spawning in December is too low to be of significance in terms of management. Data on the relationship or maturity stages to season and age of individuals revealed a lack of synchronization in the rhythm of ovarian development in fish of the same generation.

Morley *et al.,* (2004) studied the biology of *Macrourus holotrachys* and found that the fish is a bentho-pelagic predator and scavenger which feeds on a wide range of fishes and invertebrates. The ovaries of mature fish had oocytes at all developmental stages. Absolute fecundity ranged from 22,000 to 260,000.

Cudmore and Mandrak (2004) According to age at maturity of Grass Carp ranges from 2-10 years (50-86 cm) and is largely a function of water temperature and diet. Males generally mature one year earlier than females. Spawning activity is associated with high spring flows, and spawning areas have high water velocity, turbid water, and a temperature in the range of 15-30°C. Grass carp eggs are non-adhesive and semi-buoyant, requiring flowing water for incubation.

Sindhe and Kulkarni (2004) stated that the reproductive cycle of *N. notopterus* can be categorised into immature, developing, maturing, mature, ripe and spent stages on the basis of gonadosomatic index. Liver forms important organ of the body, which has a role in the ovarian development. They found that on exposure to heavy metals at sublethal concentration both gonadosomatic and hepatosomatic index are reduced.

Kolar *et al.,* (2005) studied on the biological synopsis of Bighead Carp *(Aristichthys nobilis)* and Silver Carp *(Hypophthalmichthys molitrix).* Their study described the description and identification characters, temperature and salinity tolerance, reproductive biology (fecundity, sexual maturity and mating behavior, spawning, development, feeding habits etc. of the mentioned fish species

Dos *et al.*, (2005) stated that the reproductive period of *P. brasiliensis* extends from August to March, presenting greater activity from October to February. Females reach first maturation at 15.4 cm total length (TL).

Martín *et al.*, (2005) observed that the relationship between total body mass and total length (TL) of *Psammobatis bergi* was not significantly different between sexes, but females were heavier than males. The reproductive cycle was found to be continuous with a maximum number of females carrying egg cases in warmer seasons.

Sindhe and Kulkarni (2005) studied on the fecundity of the freshwater fish, *Notopterus notopterus* in natural and heavy metal contamination; and found that the fish has bigger oocytes and are few in number. They also found that the fecundity has straight line relationship with total length, body weight, ovary length and ovary weight in control fish which did not alter after heavy metal exposure. The result provides the viability of species in only specific environment.

Narejo *et al.,* (2006) studied the fecundity and gonadosomatic index of a carp, *Cirrhinus reba,* during the period March 2004 to February 2005. It was observed that both males and females mature simultaneously. The gonads attain the maximum weight 5.05±0.88 and 12.5±1.55 for males and females respectively in July and minimum in November. The fish has only one spawning season of short duration, running from June to August as indicated by the peaks of gonadosomatic index and ova diameter. The fecundity of *C. reba* varied from 20,722 eggs in fish "of 150 mm (TL) to 211,200 in fish of 290 mm total length. The fecundity increased with increasing total length, gonad length, gonad weight and body weight. The relationship of fecundity was curvilinear with total length and gonad length and linear with body weight and gonad weight.

Erisman and Allen (2006) studied the reproductive behaviour of a temperate serranid fish, *Paralabrax clathratus* and found that spawning of the fish occurred 32 min before sunset to 120 min after sunset, and both males and females were capable of spawning multiple times during a single evening. Behavioural observations of adults and estimates of spawning periodicity from the collection of females with hydrated oocytes suggested that spawning occurred continuously throughout the summer months and showed no significant relationship with the lunar cycle.

Kennedy *et al.*, (2006) stated that relative fecundity of Shovelnose sturgeon ranged from 11220 to 23956 eggs/kg (mean=18156 eggs/kg). Absolute fecundity ranged from 14294 to 65490 eggs/female (mean=30397 eggs/female). GSI values ranged from 9.4 to 27.2 (mean=19.3) and were positively correlated to FL (r^2=0.18).

Pajuelo *et al.,* (2006a) stated that the Male t female ratio of coastal fish, *Diplodus vulgaris* is slightly unbalanced in favour of females (1t1.17), although no significant difference of the relation 1t1 was found. A protracted winter spawning season has been identified from November to March, with a peak in spawning activity in December–January. Size of maturity for males and females is attained in the second year of life.

Pajuelo *et al.,* (2006b) stated that the total length of *Pagrus auriga* ranged from 120 to 780 mm. The species was characterized by protogynous hermaphroditism. The malet female ratio was in favour of females (1t8.2). The reproductive season extended from September to February, with a peak in spawning activity in October–November.

Kolar *et al.,* (2007) Female bighead carp reach sexual maturity at three years of age with a body weight of 7-10 kg, while males can reach sexual maturity in two years with a body weight of 5-8 kg; however, this varies significantly with changing environmental conditions.

Azzurro *et al.,* (2007) investigated the aspects of gonad morphology, fecundity, atresia and oocyte dynamics of *Siganus luridus* and found that the ovarian development was consistent with the group-synchronous type, and testicular organization was of the unrestricted spermatogonial testis type, with cystic spermatogenesis. Absolute fecundity ranged from 115739 to 740433 oocytes per female. Relative fecundity ranged from 1239 to 3162 oocytes/g, with a mean of 1885 ± 868 oocytes/g.

Colombo *et al.,* (2007) identified seven developmental stages of ovary of shovelnose sturgeon. They also stated that males reached maturity at a smaller size than females. The sex ratio was not different from 1t1. The fecundity was positively related to body weight.

Ferriz *et al.,* (2007) stated that the reproductive period of *Pseudocorynopoma doriai* is seasonal, occurring from late winter to mid-summer, with another reproductive peak of smaller magnitude in early autumn. The mean monthly GSI in males has a significant correlation with rainfall. First maturity in females was reached within the 42–43 mm standard length class. The mean absolute fecundity was 1286.42.

Glamuzina *et al.,* (2007) found the maximum length 28.04 cm and maximum weight 206.8g of *Chondrostoma knerii* while estimating the length-weight relationship. The correlation between egg number and length and weight was extremely low. The gonadosomatic index distribution showed an inverse proportionality with total length. The fecundity ranged between 2000 and 16000 eggs per female.

Knight *et al.,* (2007) studied the reproductive biology of the Oxleyan pygmy perch *Nannoperca oxleyana* and observed that the species displays sexual dichromatism during the spawning season, with males developing more intense red and brown fin and body colouration, and black pelvic fins. Gonado-somatic indices averaged from 4.1–4.2% for ripe females with the total fecundities of 1323 eggs per fish.

Koc *et al.,* (2007) observed that the sex ratio (MtF) of Turkish chub, *Leuciscus cephalus* was 1t1.4, corresponding to 58.4% males and 41.6% females. Significant statistical differences in condition factors between age classes and sexes were not found. Spawning period of this species in the lake was between April and May.

Hossain *et al.,* (2008) described some biological parameters, including sex ratio, length-frequency distribution, size at sexual maturity, fecundity as well as length-weight (LWR) and length-length (LLR) relationship of the Ganges River sprat *Corica soborna* (Pieces: Clupidae), and important target species for small scale fisheries in the Mathabhanga River in Bangladesh. A total of 135 specimens ranging from 30.6-48.9 mm TL (total length) and 0.22-1.20 g BW (body weight) were analyzed in the present study. Sampling was done using traditional basket traps and funnel bag nets between January and December 2004. The sex ratio showed no significant differences from expected value of 1:1 (χ^2 0.07, p>0.05). The size at sexual maturity (TL_{50}) for *C. soborna* females was estimated to be 44.4 mm TL and the mean fecundity of the sampled population was 1280+-870 eggs, ranging from 420 to 3240. The allometric coefficient b values of the LWR indicated isometric growth (-3.0) for both male and female (2.946 and 2.948 respectively). The LLR analysis between TL and fork length (FL) showed a highly significant correlation in both sexes (r > 0.911, p<0.001). The data presented in this study would be useful for the sustainable management of the Ganged River sprat fishery in the Mathabhanga River in Bangladesh and neighboring country.

Dadzie and Abou-Seedo (2008) found four major phases of gonadal development in the black pomfret, *Parastromateus niger* as primary growth phase; secondary growth phase; maturation phase; and spawning phase, followed by the regressed phase. *P. niger* is capable of spawning multiple times throughout the reproductive season lasting from February to September. Batch fecundity was calculated as 2132–2001648 with the mean of 406010.

Fazli *et al.*, (2008) found that the overall male and female sex ratio of golden grey mullet (*Liza aurata* Risso, 1810) in Iranian waters is 1t1.42. They also observed that the reproductive season extended from October to December. Mature gonads were present in 20% of fish at age 3, 63% at age 4, 88% at age 5, and 97% at age 6. Individual absolute fecundity of the golden grey mullet ranged from 113386 to 1470000 million eggs, with a mean (±SD) of 451963 (±274,114.2).

Filer and Sedberry (2008) found that the females of *Hyperoglyphe perciformis* spawns from September to May with a peak from November to January and the males spawns year round, but had a peak from September to April. The sex ratio (MtF) for this population was 1t1.34.

Gibbs *et al.*, (2008) found that the invasive loricariid catfish, *Pterygoplichthys disjunctivus* has cystovarian ovaries, a left ovary that is significantly larger than the right ovary, and is highly fecund. Ovaries contained multiple oocyte size classes and completely spent females were not found, both of which indicate that this species is a multiple spawner.

Kahraman *et al.,* (2008) observed that the little tunny, *Euthynnus alletteratus* spawns generally between May and September with the most intensive spawning period between July and August. The sex ratio between male and female was calculated as 1t1.7.

Kendall and Gray (2008) studied the reproductive biology of *Liza argentea* and *Myxus elongatus* and found variation in spawning periodicity for each. They observed that the peak spawning period of *L. argentea* occurred between March and November in Lake Macquarie and January and April in St Georges Basin. In contrast, peak spawning of *M. elongatus* was concentrated between January and March in both estuaries.

Hossain *et al.,* (2010) presented the new methodology on how to estimate the size at first sexual maturity and fecundity for female *Gudusia chapra* from the lower part of Ganges River, northwester Bangladesh. A total of 250 female specimens, 3.60 to 13.70 cm standard length (SL) and 1.00 to 43.60 g body weight (BW) were collected during March to August 2006. The gonadosomatic index (GSI) for females was calculated by the equation, GSI (%) = (GW/BW) x 100. The size at first sexual maturity of females was estimated by the relationship between Gonadosomatic index and its standard length. The specimen larger (>) than first size at sexual maturity was used for the estimation of fecundity. The size at first size at sexual maturity for female *G. chapra* was considered to be 8.00 cm SL in the Ganges River. The mean total fecundity was 20160±6545 abd ranged from 10800 to 36200. This study should be useful for fisheries biologist/managers to impose adequate regulations for sustainable fishery management in the Ganges River and nearby areas of Bangladesh.

Solomon *et al.,* (2011) presented fundamental information on the reproductive biology of *Puntius denisonii,* an endemic and threatened aquarium fish of the Western Ghats Hotspot. Results are based on the observations from three river systems, Chandragiri, Valapattannam and Chaliyar. Sex ratio deviated significantly from 1:1 and was skewed in favour of males.

Ghailen *et al.,* (2011) studied some biological parameters of the little tuna *Euthynnus alletteratus* (Rafmesque, 1810) in the Tunisian waters. Between

January 2008 and December 2009, 592 specimens of Scombrid fish species *Euthynnus alletteratus* (Rafmesque, 18100 were captured in the coastal areas of Tunisian waters. Sex-ratio was 57.77% of females. They were found to outnumber males during all year except in June and July. The calculated length-weight relationships were $Wt = 0.0329FL^{2\ 8101}$ for females, $Wt = 0.0368FL^{2.7832}$, for $Wt = 0.0349FL^{2.7962}$ for pooled data.

Hossain *et al.*, (2012) described the size at first sexual maturity, length-weight relationships (LWR) in relation to size at first sexual maturity, and Fulton's condition factor (Kp) of *Eutropiichthys vacha* in the Ganges River, northwestern Bangladesh during January, April and July to December 2010. The analysis of covariance (ANCOVA) revealed significant differences in slope and intercept between early and late phases for males (F=4.532, PO.001) and females (F=21.984, PO.001). The K_F was not significantly correlated with TL for males (r_s= 0.052; P=0.378), but highly correlated for females (r_s= -0.165; P=0.005).

Debora *et al.*, (2012) observed the common carp (*Cyprinus carpio*) can live in a wide range of biotic and abiotic conditions. The combination of these features contributes to their high invasiveness potential allowing its rapid spread and increased biomass. The species has already established in 91 out of 120 countries where it has been introduced, especially due to aquaculture and ornamental activities. The species can adapt to regional environmental conditions.

Ahmed *et al.*, (2012a) described the length-weight relationship (LWR), length-length relationship (LLR) and the condition of the silver hatchet Chela, *Chela cachius* (Hamilton, 1822) collected 2400 specimens from the .Old Brahmaputra River in Bangladesh during the month of November 2004 to October 2005. He found no significant difference significant difference from the expected value of 1:1 and the analysis of covariance (ANCOVA) revealed no significant difference between LWRs of male and female for the pooled data over a year. Parameters of LWRs of combined gender varied monthly with high coefficients of determination (r^2>0.751; p<0.001). All the LLRs (SL vs. FL, FL vs. TL and SL vs. TL) exhibited strong correlation (r^2 >0.886; p<0.001) and ANCOVA analyses further indicated that LLRs did not differ between males and females.

Hossain *et al.*, (2013) described the first complete and inclusive description of life-history traits including sex ratio, length-frequency distributions (LFD), length-weight relationships (LWR), condition factors (Allometric, K_A; Fulton's, K_F; Relative condition, K_R; Relative weight, WR), form factor ($a_{3.0}$) and size at first sexual maturity of *Cirrhinus reba* in the Ganges River, NW Bangladesh. Sampling was done using traditional fishing gears including cast net, square lift net and conical trap from April 2011 to March 2012. The total length (TL), fork length (FL) and standard length (SL) were measured to the nearest 0.01 cm using digital slide calipers and total body weight (BW) was measured using an electronic balance with 0.01 g accuracy. The overall sex ratio did not differ significantly from the expected value of 1:1 (χ^2 =3.38, p<0.05), but there was significant differences in the TL-frequency distributions (Mann-Whitney U-test, p< 0.001) between male (median= 12.00 cm) and female (median=15.80cm). The calculated b for the LWR indicated positive allometric growth (>3.00) in male and female and there was significant differences in the intercepts (ANCOVA, p<0.001) and in the slopes (ANCOVA, p<0.001) between the sexes. In addition, the Mann-Whitney U-test showed significant differences in the Fulton's condition factor between male and female (p<0.00l). Moreover, the size at sexual maturity of male and female *C. reba* were estimated as 11.50 cm TL and 3.50 cm TL, respectively.

Mathewos H. (2013) observed There were no significant differences between the sex ratio (χ^2 = 2.33; P= 0.126). The mean ± SD of Fulton’s condition factor was 1.22 ± 0.14 for both sexes. The size at first sexual maturity (L50) for male *Cyprinus carpio* was 27.2 cm fork length (FL) while the females attained L50 at 28.3 cm FL. Absolute fecundity (F) varied between 36955 and 318584 with a mean ± SD of 170937 ± 13084 for the length group (270-470 mm FL). The relationship between F and FL were significant (F=0.224*FL3.708, R^2 = 0.933; p<0.001).

Hossain *et al.*, (2014) furnished the length-weight relationships (LWRs), length-length relationships (LLRs) and form factor (03.0) of these three threatened fishes from the Ganges River, northwestern Bangladesh. A total of 773 specimens from three species under two families used for this study were caught by traditional fishing gear between April 2011 and March 2012.The analysis of covariance (ANCOVA) revealed significant differences between the sexes in LWRs for *L. boga* (P<0.001), but not with rest of the species (P>0.05). Furthermore, the LLRs were highly correlated (r^2>0.983; P<0.001), and ANCOVA analyses additionally indicated that LLRs did not differ between sexes (P>0.05). The calculated form factor ($a_{3.0}$) was 0.0111, 0.0159 and 0.0129 for *L. boga, N.nandus* and *P. ticto,* respectively. This study presents the first reference on LWRs, LLRs and form factor for these threatened species in Bangladesh. The results would be useful for further studies on the population assessment and sustainable conservation of the limited stocks in the Ganges River ecosystem.

Jan *et al.,* (2014) found, the mean value of fecundity was estimated as 14599 (SD 9219.7) eggs with a mean total length of 34.340 (SD 6.86) and a mean total body weight of 440.60 (SD267.62). The relationship of fecundity with other parameters such as total length, total weight, ovary length and ovary weight were found to be linear and the value of correlation coefficient (r) was 0.965, 0.961, 0.933 and 0.972 respectively.

Bhattacharya P. *et al.,* (2015) found the absolute fecundity of *Ompok pabo* as determined in the present study varied from 2500 to 19636.71 in the specimens measuring 133 mm to 192 mm. The average fecundity was determined to be 9857.315. Number of eggs per gram body weight was found to be 207.41 (L=145 mm and W=12.18 g) to 545.71 (L=193 mm and W=44.81 g). The ova diameter observed minimum 0.55 (mm) to maximum 1.25 (mm).

Biochemical composition

Literature on the landing statistics of *C. carpio* var. *specularis* is scanty. Some fragmentary observation on some aspects of biochemical composition is more or less available. In the present study some related observations provided by different authors are as follows:

Milory (1908) estimated the amount of fat and protein contents in the reproductive period of herring and he derived the values as 3.50% fat and 18.29% protein.

Johnstone (1918) worked out the amount of fat in halibut the values of which ranged from 0.5% to 18%.

Lovern and Wood (1937) stressed out the amount of moisture, fat and protein contents from the flesh of herrings which were 73.5%, 2.9% and 19.2% respectively.

Nilson (1946) stipulated that about 85% to 95% of fish protein were digestible and all ditary essential amino acids were present in fish.

Del Riego (1948) pointed out the value of protein content in Atlantic sardine which were 16% in March and 20.6% in July.

Jacquot and Creak (1950) noted that fat was generally acquired at the cost of water of the tissue.

Venkatarman and Chari (1951) made a thorough study on seasonal variation in the bio-chemical composition of mackerel and found that ash and protein remained constant. Moisture and fat were subjected to seasonal variation and there was reciprocal relationship between them.

Stansby (1954) worked out the macro nutrient content from the edible flesh of certain freshwater fishes and he observed that these fishes contained 76.8% moisture, 1.2% ash, 5% fat and 19% protein.

Thurston (1958) reported that Alaska pink salmon maintained inverse relationship between fat and moisture and positive correlation between protein and moisture.

Jacobs (1958) concluded that fat in the fish flesh replaced equal amount of moisture.

Sesa (1959) stated that smaller fish contained more moisture but less fat.

Borgstrom (1961) observed that the fat and protein contents in fish depended on. some factors such as size, age species, sex, seasonal changes and season of capture.

Khuda *et al.*, (1962) worked out that the amount of moisture and ash content in *Puntius sophora* and they observed that the fresh fish contained 77.14% moisture and 1.48% ash. They also estimated the amount of protein on the two stages of growth of *Labeo rohita*, *Cirrhina mrigala* and *Labeo calbasu* and found that in the case of fishes ranging from 55.5-78.5g body weight, the crude protein varied from 17.18% to 19.56% but for higher body weight from 10.9-16.80%. They again reported that the decrease in moisture content and increase in fat content were related with the increase of age in case of carps.

Stansby *et al.,* (1963) investigated that the nutritive food value depended on proximate composition of fish which varied widely from species to species and from one individual to another.

Malek *et al.,* (1966) determined the moisture and ash contents in *Puntius stigma* and results were 72.65% and 2% respectively.

Rao (1967) observed that the muscle of *Johnius carata* contained 70.05 to 80.75% of water and 1.5 to 12% of fat.

Adhikari and Noor (1967) studied the seasonal variation in oil, moisture contents and solid matter of *Puntius puntius* and observed higher oil contents in winter.

Desnosier (1977) studied the amount of moisture, fat and protein contents present in fish and he observed that in general fish contained 70% to 80% moisture, 105% to 21% fat and 13% to 22% protein.

Kamaluddin *et al.,* (1977) investigated that the fish *Heteropnustes fossilis* contained 68% moisture. They also reported that 1% fat arid 15% protein were present in freshwater cat fish. *Clarius batrachus.* They (1986) again reported the same result.

Gopalan *et al.,* (1978) determined the moisture content in *Mystus vittatus, Labeo rohita, Catla catla, Puntius* and they obtained the results as 70%, 76.2%, 72% and 75% respectively. Kamaluddin *et al.,* (1977) reported almost the same result in case of freshwater tengra fish. They again quoted the values of fat and protein content in the species *Mystus vittatus, Mystus bleker, Amblypharyngodon mola, Catla catla and Puntius.* They ovserved that *Mystus vittatus* contained 6.4% fat and 19.2% protein. *Mystus blelker* contained 2.73% fat and 18.85% protein. *Amblypharyngodon mola* contained 2.4% fat and 19.5% protein and *Puntius* contained 2.4% fat and 18.1% protein.

Govindan (1985) analysed the mount of protein content that was present in different fishes form both the freshwater and the marine environment and he obtained the result as fish contained 9% to 25% protein and on most cases the limit was 16% to 19%

Molla *et al.,* (1987) reported about the biochemical and nutritional composition of freshwater eel *Anguilla bengalensis* (Bao Baim). The fish afforded 12.06% oil, 20.83% protein, 63.65% moisture and 24.29% solid matter in the month of November. The compositions of oil, protein, moisture and solid matter in May were 6.62%, 22.17%, 67.21% and 26.17% respectively.

Rubbi *et al.,* (1987) found that the fat and protein content in case of *Heteropneustes fossilis* were 1.73% and 18.25% respectively. She determined the moisture and ash content in case of Cypriniformes and these were 77.28+0.11g and 1.83+0.07g respectively.

Soldevilla (1989) determined the moisture, ash and fat contents is fish from the water of the canary Island and couluded that the average amounts of moisture, ash and fat contents in fresh species were 76.5%, 24% and 17.8% respectively.

Al-Habib (1990) estimated the protein contents of six freshwater fishes and he observed that these fishes contained 11%-16.75% protein.

Banu *et al.,* (1991) compared the mineral contents of edible portion of 19 species of freshwater fishes of Bangladesh.

Molla *et al.,* (1998) found that the nutritional status and chemical composition of *Rita rita* fish along with the seasonal variation of lipid, protein, mineral, vitamin and related substances. The highest amount of moisture, dry matter, mineral, crude fibre, protein and lipid of *Rita rita* was found to be 69.13%, 30.87%, 1.41%, 6.25%, 17.84% and 13.92% respectively.

Bhuiyan *et al.,* (1999) stated that protein content is directly related to fat, but the moisture content is inversely related to both protein and fat. Protein and fat content was higher in male (protein 20.30%, fat 9.72%) than female (protein 19.89%, fat 2.36%) in grey mullet *Mugil cephalus* (L.).

Azam *et al.,* (2003) found the biochemical composition of fourteen dried fish in dry weight basis. They reported the value of moisture, ash, protein and fat content as 19.93%, 7.45%, 68.09% and 4.87% in *Mugil cephalus*, 23.49%, 11.32%, 58.35% and 7.84% in *Scoliodon sarrakowah*, 24.46%, 9.51%, 62.36% and 3.67% in *Setipinna phasa*, 21.26%, 15.02%, 61.25% and 3.5% in *Harpodon nehereus*, 19.22%, 5.08%, 66.52% and 9.03% in *Arius caelatus*, 23.9%, 9.11%, 40.69% and 26.13% in *Hilsa ilisha*, 21.65%, 12.14%, 57.25% and 8.95% in *Polynemus paradiseus*, 23.61%, 10.78%, 53.85% and 11.71% in *Trichuirus haumella*, 18.23%, 10.91%, 63.49% and 7.1% in *Pampus chinenchis*, 21.08%, 11.01%, 54.19% and 25.3% in *Himantura walga*, 20.98%, 9.98%, 56.77% and 11.19% in *Muraenesox bagio*, 23.19%, 6.32%, 61.24% and 7.94% in *Epinephelus lanceolatus*, 21.7%, 11.85%, 54.86% and 11.44% in *Cynoglossus benglalensis* and 23.31%, 7.22%, 57.51% and 9.69% in *Tetraodon patoka*.

Islam and Joadder (2005) conducted an experiment on seasonal variation of the biochemical composition of freshwater Gobi, *Glossobius giuris* (Hamilton) from the River Padma. The reported that the biochemical composition of the fish depends on season but also to a great extent in reaction to six and reproductive cycle. The results also showed that *G. giuris* is a ‘low fat-high protein’ fish. The highest amount was moisture content followed by protein, ash, lipid and carbohydrate. The female fish contained more moisture and lipid than those of the male fish (female>male) except protein and carbohydrate in male fish (male>female).

Nargis (2006) examined the protein, carbohydrate, fat, ash and moisture contents in the body muscle of *Anabas testudineus* and reported that the composition varied seasonally in relation to reproductive cycle of the fish and significant correlation existed between moisture and carbohydrate, moisture and fat, moisture and protein, moisture and ash, protein and ash, fat and carbohydrate, fat and ash, protein and carbohydrate. The protein content found to be higher in medium sized fishes and gradually decreased with the increase of age. Fat content was higher in large sized male than of the female. Carbohydrate content was slightly higher in male than the female.

Osibona *et al.,* (2006) investigated the biochemical composition and fatty acids profile of the African catfish, *Clarias gariepinus,* from Lekki Lagoon fishing grounds in Lagos, south-western coast Nigeria and reported mean values for the biochemical composition as protein 19.64%, lipid 1.15%, moisture 76.71% and ash 1.23%. There were no seasonal changes (P>0.05) in the mean monthly biochemical composition of the fish over two year period.

Kamal *et al.*, (2007) evaluated the biochemical composition of seven small indigenous freshwater fishes namely Magur (*Clarias butrachus*), Shingi (*Heteropneustes fossilis*), Koi (*Anabas tesudineus*), Foli (*Notopterus notopterus*), Royna (*Nandus nandus*), Taki (*Channa punctatus*) and Tangra (*Mystus vitatus*) from the Mouri river, Khulna, Bangladesh of their nutritional value. They reported the mean value of protein, fat, moisture and ash content as 14.87±0.63%, 7.90±1.91%, 73.49±0.69% and 3.74±0.46% in *C. batrachus*, 17.34±0.51%, 3.45±0.92%, 76.06±2.24% and 3.15±0.25% in *H. fossilis*, 19.63±0.5%, 7.79±2.73%, 69.27±1.04% and 3.31±0.83% in *A. testudineus*, 18.30±0.79%, 4.98±1.71%, 72.68±1.08% and 5.82±0.82% in *N. notopterus*, 16.09±2.66%, 7.34±0.49, 75.75±0.78 and 5.19±0.29% in *N. nandus*, 19.13±2.40%, 4.55±1.18%, 70.55±1.89% and 6.81±0.94% in *C. carpio* var. *specularis* and 15.62±0.32%, 7.53±1.10%, 73.99±3.13% and 6.50±0.63% in *M. vittatus*.

Mazumder *et al.*, (2008) observed the biochemical composition of small indigenous species (SIS) in Bangladesh. They found protein in *A. mola* (18.46%), *G. chapra* (15.23%), *P. chola* (14.08%), *C. nama* (18.26%), *P. atherinoides* (15.84%) and *A. coila* (16.99%). Fat content was recorded as 4.1%, 5.41%, 3.05%, 1.53%, 2.24% and 3.53% respectively. The overall nutrient contents of studied small indigenous fishes were observed as higher or equal to those of larger fish species.

Musa (2009) worked on biochemical compositions of body muscles of *Puntius stigma* (male and female) and observed the moisture content 75.60% to be higher in female, while protein 21.50%, fat 2.70%, ash 1.90% and carbohydrate contents of 1.55% were higher in male.

Aberoumad and Pourshafi (2010) measured biochemical analysis of three fishes such as *Skip Jack Tuna*, *Yelowfin tuna* and *longtail tuna*. They found value of protein, fat, moisture and ash content as 3.85, 1.5, 25.2 and 318.2 in *Skip Jack Tuna*, 4.8, 1.6, 25.2 and 17.5 in *Yellowfin tuna* and 0.69±0.16%, 0.75±0.06%, 23.11±0.15% and 16.8±0.11% in *Longtail tuna*.

Yousaf *et al*., (2011) conducted an experiment on body composition of freshwater *Wallago attu* in relation to body size, condition factor and sex from southern Punjab, Pakistan. They observed that body size had a positive influence on percentage of ash, fat and protein contents (wet weight) but there was no significant effect on percent water content.

Mohajera *et al.,* (2012) found that to estimate and compare the body comparisons six small indigenous species (SIS) *(Ailiichthys punctata, Clupisoma psendeutropius atherinoides, Puntius sarana, Gudusia chapra, Corica soborna),* One anadromous fish *(Tenualosa ilishd)* and two shell fishes *(Macrobrachium and Penaeus spp)* were collected and subjected to biochemical composition through moisture, protein, ash and fat determination. Considerable variations were observed in the proximate composition of different fish species. A classification based on the protein and oil content of the fish indicated that most of the fishes of Bangladesh are subjected to high protein and low or medium group (15% protein and 5-10% oil). Present study demonstrates that all the experimented fish contain higher nutriept value that are not less than the larger fish and helps to decrease the nutrient deficiency of our country people.

Ali, *et al.,* (2014) observed the proximate biochemical contents of some fish species i.e. Thunnus alalunga, Evynnis japonica, Caulerpa lentillifera, Orcynopsis unicolor and Euthynnus affinis were analyzed. Protein contents was determined in *T. alalunga (22%), E. japonica (13.02%), C. lentillifera (26.9%), O. unicolor (22%)* and in *E. affinis (24%)* respectively in the five species of fish. The ash content was highest in *C. lentillifera (8.8%).* The preent findings revealed that the highest protein content was recorded as in *C. lentillifera (26.9%)*, but the fat was highest in *T. alalunga (23.3%)*. The overall nutrient contents of studied medium indigenous fishes were observed as higher or equal to those of larger fish species.

CHAPTER-3

PHYSICO-CHEMICAL CONDITIONS IN THE HABITAT OF *CYPRINUS CARPIO* VAR. *SPECULARIS*

INTRODUCTION

Productivity and availability of fish depend on the physico-chemical conditions of the water bodies. Different environmental factors, which determine the characters of water, have a great role on the growth, maturity, reproduction and development of fish. Fishes are more depend on water temperature, turbidity, pH, dissolved oxygen, free carbon dioxide, alkalinity, total hardness and ammonia for growth and development. Any changes of these parameters may affect the growth, development and maturity of fish. Proper management for obtaining optimum fish yield depends upon water quality parameters, because these parameters influence the aquatic production that is why fish culturists are more conscious about the maintenance of optimum condition of water quality parameters. But in case of the beel physico-chemical condition depend on natural process.

According to Rahman (1992), pond should not be shallower than one meter and deeper than five meter and the optimum depth should be two meter. Fish is poikilothermous. So, growth, reproduction and other biological activities of fish are controlled by temperature. For 1°C rise of water temperature metabolic rate of fish increases 10%. Water temperature is directly and closely related with air temperature but sometimes exceptions may occur when water temperature may be slightly higher than air temperature. Temperature has different relations with other factors especially dissolved oxygen content. Turbidity is a very important factor to be considered in fish culture. 26,000 ppm or more is considered lethal to fishes. In hot summer in a shallow water body (depth less than 1 meter) reduction of dissolved oxygen due to high temperature makes critical condition for fish. With the rise of temperature, bacterial decomposition increases.

High amount of free carbon dioxide is associated with low concentration of dissolved oxygen. Free CO_2 more than 20 ppm may be harmful to fishes and even lower concentrations may be equally harmful when dissolved oxygen concentrations are less than 3 to 5 ppm (Lagler, 1956). The circumstance neutral pH or slightly alkaline pH is most suitable for fish culture. Ammonia as NH_4OH is toxic to fishes but ionic ammonia (NH_4^+) is not toxic. The toxicity of NH_4OH varies with pH, temperature, dissolved oxygen, etc. Total hardness and alkalinity are expressed as $CaCO_3$ but they are not same thing, total hardness means the cation concentration and alkalinity means the anion concentration. Hardness mainly means the concentration of calcium and magnesium ions.

In this study it has been observed that the open water are polluted due to the run off water carrying residues of agricultural chemicals like pesticides, herbicides, insecticides and other domestic wastes as well as fertilizers used. Water quality is the suitability of water for the survival and growth of fish and it is normally governed by only a few variables (Boyd, 1998). Temperature, dissolved oxygen, CO_2, pH, hardness and ammonia are quite different their previous environment it can causes severe stress. Environmental stress is associated with opportunistic infections such as fungal, bacterial and ectoparasitic protozoa, which take advantage of the stressed host fish. So the water quality parameter is the most important factor for the aquatic environment.

The present investigation is aimed on the following objectives

- To know the climatic condition of the study area
- To find out the mean value of some important physical parameters viz. air and water temperature, rainfall and water transparency in the study area.
- To find out the mean values of qualitative estimation of some chemical parameters like dissolved oxygen (DO), free carbon dioxide (CO_2) and pH of water.

MATERIALS AND METHODS

The study on the physico-chemical conditions in the Atrai river of northwestern (Naogaon District) Bangladesh which is the habitat of *Cyprinus carpio* var. *specularis* was carried out for a period of two years (24 months) from July, 2013 to June, 2015.

Water samples were collected twice in a month during the study period. Samples were also collected from the depth of 20-35 cm below the surface at the time of 10:00 A.M. – 12:00 P.M (Plate 3.1). Water samples were collected with the help of a glass-stoppered bottle wrapping with black paper. After collection samples were brought to the Fisheries Research Laboratory, Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh. Chemical analyses were also done immediate after arrival.

Physical parameters

Nearest Bangladesh Meteorological Department of the study area is in Rajshahi. So data on physical factors i.e. maximum and minimum air temperature, rainfall, and rainy day were obtained from Bangladesh Meteorological Department, Regional Station, Rajshahi. The researcher physically visited the Meteorological Regional Station, Rajshahi and collected the data from the authority. The raw data were recorded daily basis and were supplied to the researcher.

Water temperature: A centigrade thermometer with a range of 0°C to 110°C was used to measure the water temperature at the time of sample collection.

Water transparency: Transparency was the measurement of limit of visibility. Transparency was measured by Secchi disc. It was expressed in m.

Chemical parameters

The chemical parameters of the river water viz. dissolved oxygen (DO), free carbon dioxide (CO_2) were analyzed by HACH Test Kit (Model: FF-2) in the Fisheries Research Laboratory, Department of Fisheries, University of Rajshahi, Rajshahi, Bangladesh.

Hydrogen ion concentration (pH): The pH value of river water was measured by digital pocket pH meter and titration method.

Statistical analysis

The statistical analysis of different meteorological and physico-chemical parameters was carried out. Only simple analysis viz. mean, standard deviation, simple line graph by using both MS Excel and SPPS 20 version computer based software.

Plate 3.1: Showing the water body of the study area (Atrai river)

RESULTS AND OBSERVATIONS

The physico-chemical conditions of the river Atrai exhibited more or less variation according to the change of months and seasons. The result based on the direct observation and calculated monthly two years mean values of different physical and chemical parameters are shown in Fig. 3.1-3.2 and App. Table 1. Besides the mean value and standard deviation of different physico-chemical parameters as recorded from sampling area is shown in App. Table 1.

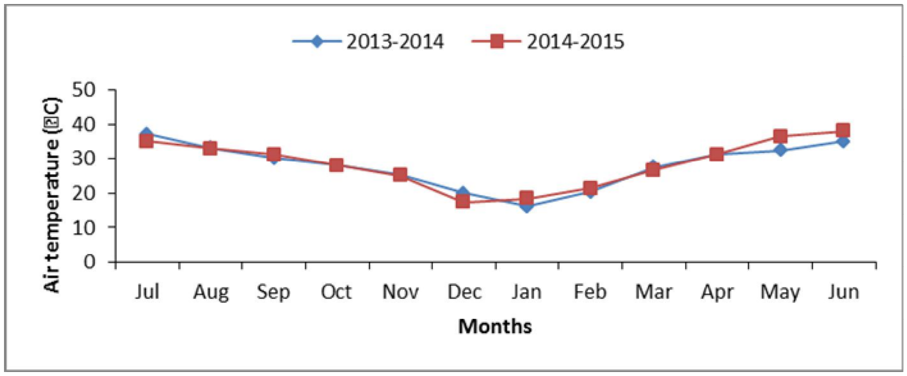
Physical parameters

Air temperature

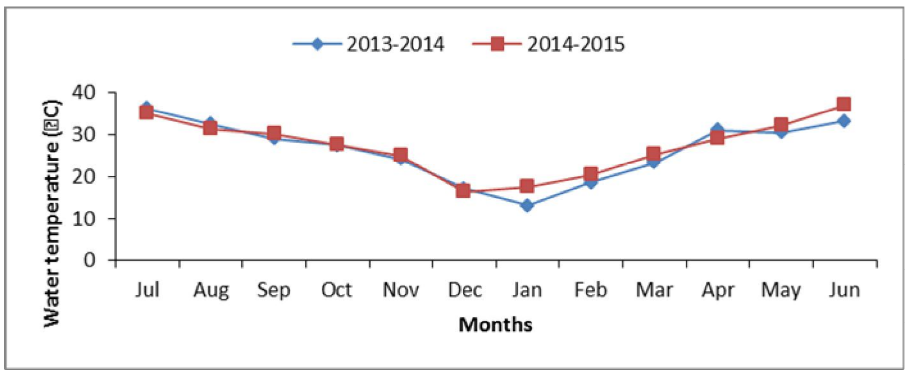
Air temperature varied considerably throughout the year. The mean values of average day night maximum and minimum air temperature of the river Atrai were recorded as 37.11°C in July, 2013 to 16.13°C in January, 2014 in the first year observation and 38.10°C in June, 2015 to 17.50°C in December, 2014 in the second year observation. The mean value two years of air temperature was recorded as 28.24°C (Fig. 3.1a, App. Table 1).

Water temperature

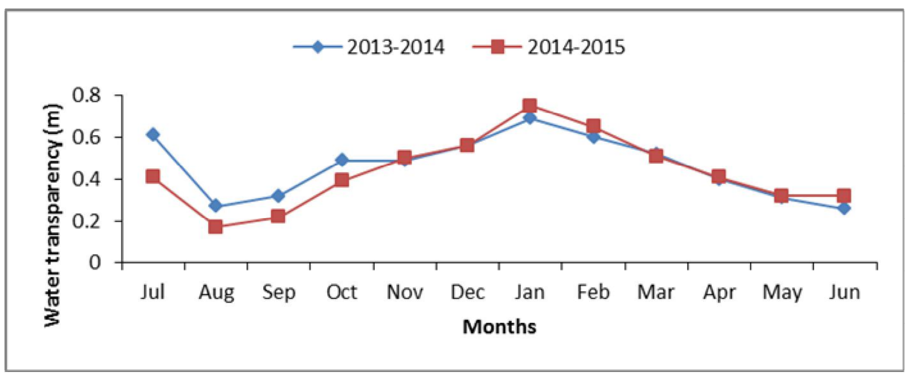
The water temperature of the sampling area showed considerable variation throughout the year. The water temperature fluctuated due to the cause of more or less sunny or rainfall condition. The mean values of monthly maximum and minimum water temperature of the river Atrai were recorded as 36.22°C in July, 2013 to 13.17°C in January, 2014 in the first year observation and 37.15°C in June, 2015 to 16.38°C in December, 2014 in the second year observation. The mean value two years of water temperature was recorded as 26.81°C (Fig. 3.1b, App. Table 1).



(a)



(b)



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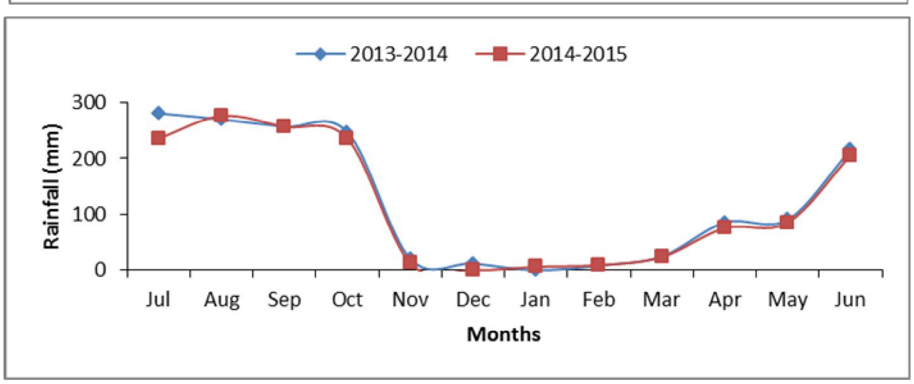
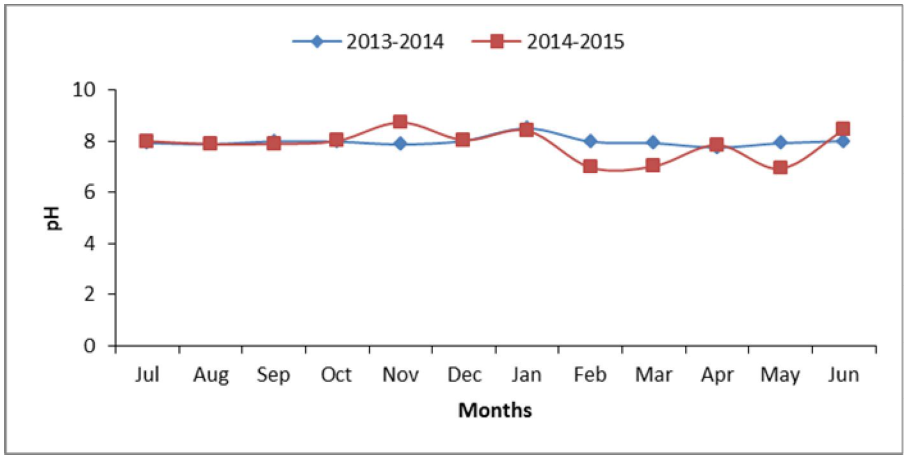
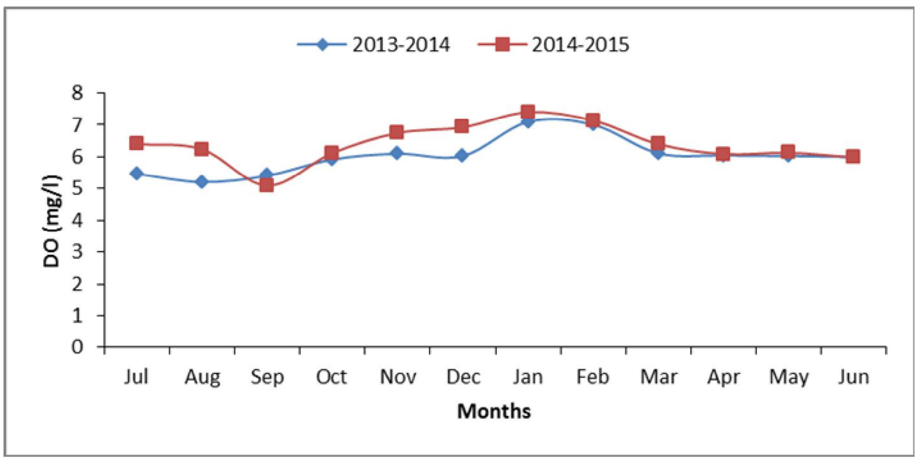


(d)

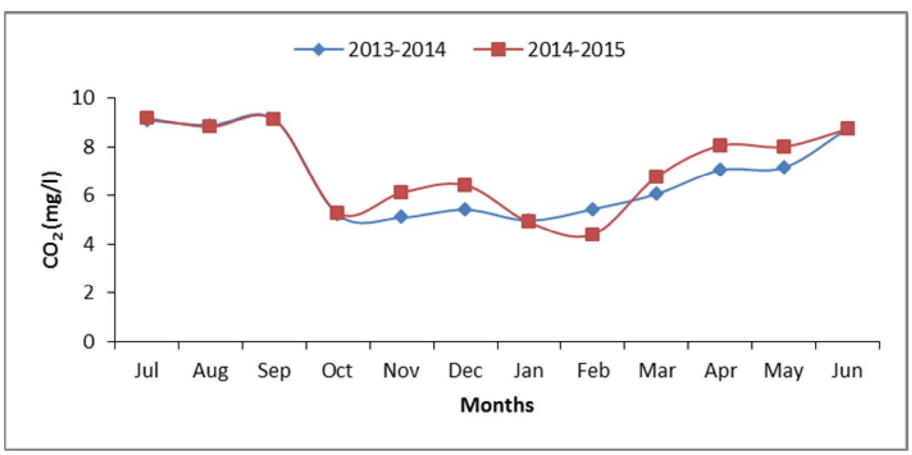
Fig. 3.1(a-d): Monthly fluctuation of physical parameters (a. Air temperature, b. Water temperature, c. water transparency and d. rainfall) of the Atrai river of northwestern (Naogaon District) Bangladesh during July, 2013 to June, 2015.



(a)



(b)



(c)

Fig. 3.2(a-c): Monthly fluctuation of chemical parameters (a. pH, b. DO and c. CO_2) of the Atrai river of northwestern (Naogaon District) Bangladesh during July, 2013 to June, 2015.

Water transparency

Limit of secchi disc visibility in the study area showed a marked variation. In the river Atrai the monthly mean values of water transparency exhibited the maximum and minimum were recorded as 0.69m in January, 2014 to 0.26m in June, 2014 in the first year observation and 0.75m in January, 2015 to 0.17m in August, 2014 in the second year observation. The mean value two years of water transparency was recorded as 0.45m (Fig. 3.1c, App. Table 1).

Rainfall

In the study area there was rainfall more or less all the year round. Usually in the winter season rainfall is occasional but in the monsoon or summer season there were frequent and heavy rainfall with gusty wind. In Naogaon the monthly maximum and minimum rainfall was recorded as 280.82mm in July, 2013 to 0.00 in January, 2014 in the first year observation and 275.97mm in August, 2014 to 0.00 in December, 2014 in the second year observation. The mean value two years of rainfall was recorded as 122.24mm (Fig. 3.1d, App. Table 1).

Chemical parameters

Hydrogen ion concentration (pH)

In determining the hydrogen ion concentration of the river Atrai it revealed that there was no marked variation in the values of pH. The maximum and minimum mean values of pH were recorded as 8.49 in January, 2014 to 7.75 in April, 2014 first year observations and 8.72 in November, 2014 to 6.94 in May, 2015 in second year observations. The mean value of pH was recorded as 7.91 (Fig. 3.2a, App. Table 1).

Dissolved oxygen (DO)

The dissolved oxygen content of the lotic and lentic water system was normally affected by some factors such as water current, turbidity, temperature, aquatic plants and sunlight as observed in the study area.

In the river Atrai the maximum and minimum monthly mean values of DO content were recorded as 7.10 mg/l in January, 2014 to 5.21 mg/l in August, 2013 in the first year observations and 7.40 mg/l in January, 2015 to 5.11 mg/l in September, 2014 in the second year observations. The two years mean value of DO was recorded as 6.21 mg/l (Fig. 3.2b, App. Table 1).

Free carbon dioxide (CO_2)

In the river Atrai the maximum and minimum monthly mean values of CO_2 were recorded as 9.12 mg/l in September, 2013 to 4.98 mg/l in January, 2014 in the first year observations and 9.18 mg/l July, 2014 to 4.40 mg/l in February, 2015 in the second year observations. The two years mean value of CO_2 was recorded as 7.00 mg/l (Fig. 3.2c, App. Table 1).

DISCUSSION

The ecological conditions such as the physical and chemical parameters of the river Atrai have been determined. The values of different parameters were calculated through the statistical analysis which can be treated as the useful indicator for the occurrence and abundance of *C. carpio* var. *specularis*.

The present observation reveals that the annual air temperature cycle maintained a close parallel relationship with annual cycle of water temperature. The temperature of the studied water body usually declined from November and reached the minimum in January and there after increased steadily reaching the maximum during April to July in the river Atrai. Differences between air temperature and water temperature are higher during summer and lower in winter, the occurrence of the temperature curve of air and water coincided each other during monsoon / flood season due to sudden fall of air temperature in comparison to the water temperature followed by the heavy rainfall. On the other hand both the curves differ before and after monsoon period due to absence of cooling effect by rainfall and the presence of windless and hot days which prevail in the area. Similar ideas were also stated by Geisler *et al.,* (1975); Islam and Mendes (1976).

There is more or less rainfall all the year round. Like other hydrological features rainfall is governed by the monsoon (Mahmood, 1986; Rahman *et al.,* 1989). The rainfall is also an important ecological factor for aquatic environment. Sahai and Sinha (1969) observed that the chemical factors like nitrate and phosphate were high in rainy season. Rao (1955) reported that the nitrate of water increased with the increase of rainfall. Again Lakshminarayana (1965) observed that the higher values of nitrate and phosphate during monsoon were due to rainfall and flood.

Rainfall also influences the breeding of fishes. Temperature has no specificity on spawning but cloudy days accompanied by thunder storm and rain, exercise some influence on spawning (Saha *et al.,* 1957). Success in majority of fishes have been induced on cloudy and rainy days, specially after heavy showers (Chowdhury, 1960).

In Naogaon, heavy rainfall was very common during June to October in the Atrai river area. Water transparency of the studied water body show low value due to greatest turbidity. The turbidity of water is generally due to the suspended inorganic substances and planktonic organisms present in water. Bamforth (1958) reported that the turbidity of water was mainly affected by heavy plankton bloom, non-living organisms, suspended organic matter, rains, floods and inflowing sediments. According to Jhingran (1983), turbidity due to profusion of plankton is an indication of high fertility but that caused by silt or mud beyond a limit, is harmful to fish and other organisms.

In the river Atrai the minimum values of transparency were found in the monsoon and post monsoon months like June to September due to strong current of water which washed away huge silt in water including many other suspended matters. From October onwards up to May water becomes slowly clear with the maximum values of transparency due to absence of such disturbing matters. Lakshminarayana (1965) and Hickman (1979) also made such type of observation.

The water of natural sources was not chemically pure. It contains different substances in solution giving an acid, neutral or alkaline reaction. The importance of pH value in fish culture was vast. Michael (1969) by an investigation reported that the pH range between 7.3 and 8.4 was considered suitable for fish culture. Swingle (1967) observed the relationship of pH of water to their suitability for fish culture and satisfactory results were obtained from water with pH ranging from 6.5 to 9.0. He reported that the water having pH values more than 9.5 were unproductive and above 11.0 marked the death point of fish.

The calculated mean values of pH of water of the river Atrai ranged from 8.49 to 7.75 and 8.72 to 6.94 in the first and second years observations respectively

Ehshan *et al.*, (1997) recorded the values of pH of water of Halti beel ranged between 7.1 to 8.03. In our Bangladesh pH values were recorded in some running water system by Islam *et al.*, (1974) as maximum 7.8 (July) and minimum 6.9 (March) in the river Buriganga and by Patra and Azadi (1987) as maximum 8.15 in October and minimum 6.96 in May in the river Halda.

Among the dissolved oxygen was the most important factor for the aquatic life. Dissolved oxygen contents of the river Atrai increase gradually from October to February and then decrease being lowest in July to September. Chakraborty *et al.*, (1959) observed minimum amount of dissolved oxygen during monsoon in the Jamuna river.

Carbon dioxide was essential ingredient of photosynthetic reaction. According to Chow (1958), carbon dioxide concentration of 30 to 40 ppm were liable to make the fish breathe with difficulty and often die when the excess of 30 ppm. Mean values of free carbon dioxide (CO_2) show inverse relationship to the oxygen.

In the river Atrai, the values of carbon dioxide were maximum in the month of June to September and minimum in January to February.

The free CO_2 content of the river, showed seasonal changes which increased during summer and autumn and decreased during winter and spring. Islam and Mendes (1976); Ismail *et al.*, (1984); Patra and Azadi (1987) observed similar results in Bangladesh. Vyas and Kumar (1968) also noted same observation in India.

The high free CO_2 content during summer was possibly due to the high temperature and heavy rainfall with heavy land drainage which speeded up the decomposition of organic matters, low photosynthetic activity which consumed CO_2, low precipitation of free CO_2 as carbonates which agree with Ali and Islam (1983), Chowdhury and Mazumder (1981) and Bhuiyan *et al.,* (1997). In summer the factors responsible for the absorption of oxygen is greater than those discharge CO_2, with the result that organic matters are decomposed by bacteria and free CO_2 which is liberated in large amount in these season.

The low free CO_2 content during winter season was possibly due to low temperature and low or no rainfall which caused low decomposition of organic matters and addition of CO_2, high photosynthesis which consumed CO_2, high precipitation of CO_2 as bicarbonate in divalent bonds agreeing Patra and Azadi (1987) and Bhuiyan *et al.*, (1997) in Bangladesh.

The occurrence of different fish in various water body varied in different seasons. Same result was observed in *C. carpio* var. *specularis*. The abundance of *C. carpio* var. *specularis* in Atrai river is almost continuous throughout the year. However the peak period in Atrai river observed during the monsoon (June to September). During the monsoon the highest value of water temperature was observed in July (36.22°C) to June (37.15°C) and lowest in January (13.17°C) to December (16.38°C) in the first and second years respectively. The highest and lowest values of water transparency were recorded in January (0.69m) to

June (0.26m) and January (0.75m) to August (0.17m) in the first and second years observations respectively. The highest and lowest values of pH was observed in January (8.49) to August (7.75) and November (8.72) to May (6.94) in the first and second years observations respectively. The highest and lowest values of dissolved oxygen was observed in January (7.10 mg/l) to August (5.21 mg/l) and January (7.40 mg/l) to September (5.11 mg/l) in the first and second years observations respectively. The highest and lowest value of free carbon dioxide was observed in September (9.12 mg/l) to January (4.98 mg/l) and July (9.18 mg/l) to February (4.40 mg/l) in the first and second years observations respectively. The reproductive cycle and pattern of gonad development of *C. carpio* in natural ecosystems greatly depends on the ambient water temperature (Smith and Walker, 2004; Tempero *et al.*, 2006). Spawning in *C. carpio* occurs at a water temperature of around 18°C (Femandez-Delgado, 1990).

Finding of the present study of the water body in the Atrai river was thought to be in suitable condition in the habitat of *C. carpio* var. *specularis.* From November to March, water temperature and free carbon dioxide (CO_2) were decreasing trend whereas water transparency and dissolved oxygen (DO) were increasing trend in that months. So, a unique fresh environment of the river area became calm.

CHAPTER-4
REPRODUCTIVE BIOLOGY

INTRODUCTION

In this very universe all living creatures continue their survival by a process which is called as "reproduction". This important physiological process is performed by all organisms for the maintenance of the continuity of their generation. So it is necessary to say that reproduction is a must to continue the generation of a species.

Reproduction is the process by which species are perpetuated and by which, in combination with genetic changes, characteristics for new species first appear (Lagler *et al.,* 1977). Most aquatic organisms spend much of their lives and energies for reproduction. After a brief juvenile stage, they develop sperms or eggs, spawn, recover, and repeat the process in a cycle that continues until senility or death (Royce, 1972). Continuity of this biological race is maintained by the process of reproduction and failing which they may lead to extinction. So, knowledge about reproduction and reproductive activities are vital and takes a place of paramount importance in studying the biology and life history of an organism.

Reproduction is a physiological phenomenon and it is influenced by several factors such as, genetic, environmental and hormonal factors, either singly or in combination (Marshal, 1965). Before reproduction, several developmental changes occur which affect mainly the development and maturation of the gonads and associated structures concerned with reproduction. These changes are termed as maturation changes or the process of maturation. According to Adiyodi and Adiyodi (1974), sex differentiation is a slow and stepwise process. Sexual development involves the maturation of structural, physiological and behavioural machineries concerned with mating and reproduction, controlled by the sex hormones (Berreur-Bonnenfant and Charniaux-Cotton, 1970) or abiotic and biotic factors (Nikolsky, 1963; Wootton, 1990). Changes in these factors are known to affect spawning success and gonadal development which in turn determines the size or age at which the species attains first sexual maturity (Quaatey and Maravelias, 1999).

Environmental parameters, such as day light, temperature, turbidity, rainfall, availability and nature of food, interspecies and intraspecies social relation may influence the nature and pattern of reproduction and developmental behaviour (Waterman, 1961). The above factors do not appear suitable for an organism throughout the year and, as such, the reproductive activities in aquatic organisms are time oriented and sometimes periodical. Hossain (1989) observed that the critical period of population replenishment is usually so timed by environmental variables or certain condition of water that the young are produced at periods favourable for their survival.

According to Stephenson (1934), the habitants of tropical waters can be classified as under:

1) Continuous breeder throughout the year.
2) Discontinuous breeder.
3) Annual breeder.
4) Those bred more than once a year during their reproductive cycle.

The time or season when the species are normally breeds termed as the breeding or spawning season. The breeding season repeats in cyclic order, in which the organism undergoes maturation changes and they get ready to breed again. The repeated phenomenon is known as the reproductive cycle or sexual periodicity. The reproductive cycle in an organism can be determined by several methods and many workers have studied it in different fishes such as Hossain *et al.,* (1989), Afroze and Hossain (1990), Hossain *et al.,* (1991a), Nargis and Hossain (1992), Hoque and Hossain (1993), Parween *et al.,* (1993), Azadi *et al.,* (1995), Nabi and Hossain (1996), Alam *et al.,* (1997), Fatema *et al.,* (1997), Yoneda *et al.,* (1998), Sultana *et al.,* (2001), Lenhardt and Cakic (2002), Rahman *et al.,* (2002), Alp *et al.,* (2003), Caputo *et al.,* (2003), Grabowska, (2005), Martín *et al.,* (2005), Kennedy *et al.,* (2006), Zorica *et al.,* (2006), Ferriz *et al.,* (2007), Sulikowski *et al.,* (2007), Kahraman *et al.,* (2008) and Kendall and Gray (2008).

A precise knowledge of fecundity of a fish is also important to evaluate its life history, commercial catch, artificial propagation, culture practice and proper management of the fishery (Shafi *et al.,* 1978; Afroze and Hossain, 1983;

Hossain *et al.,* 1992b; Rahman *et al.,* 2002). The term fecundity can be expressed as the number of eggs present in the ovary that should be laid in a single spawning season. The fecundity of a species is not a constant number which fluctuates within a certain range and species specific. Accurate estimation of fecundity is a must for studying the population dynamics (Hossain, 1989).

The reproductive capacity of fish population is a function of the fecundity of females (Shafi and Mustafa, 1976). Variation in fecundity is very common among the same species of fish depending on their size, age and environmental conditions (Lagler *et al.,* 1977). Fecundity is also related to length, somatic and gonad weight. In many fishes the relationship of fecundity to length, somatic and gonad weight is curvilinear than linear (Bagenal, 1978). Fecundity is one of the important aspects of the biology and population dynamics of a fish.

Many workers have studied the fecundity of different species of fishes, *viz*., Afroze and Hossain (1983), Banu *et al.,* (1985), Nargis and Hossain (1988), Islam and Azadi (1989), Islam and Hossain (1990), Hoque and Hossain (1993), Alam *et al.,* (1994b), Bhuiyan and Afrose (1996), Ellis and Shackley (1997), Hossain *et al.,* (1997), Mustafa *et al.,* (1998), Afroze *et al.,* (1999), Kovacic (2001), Lenhardt and Cakic (2002), Morley *et al.,* (2004), Grabowska (2005), Sindhe and Kulkarni (2005), Kennedy *et al.,* (2006), Azzurro *et al.,* (2007), Ferriz *et al.,* (2007), Glamuzina *et al.,* (2007), Knight *et al.,* (2007), Dadzie *et al.,* (2008), Dadzie and Abou-Seedo (2008), Fazli *et al.,* (2008) and Oker *et al.,* (2008).

Studies on the sex-ratio on different fishes have been done by Afroze and Hossain (1983), Hossain and Islam (1983), Nargis and Hossain (1988), Islam and Hossain (1990), Rahman *et al.,* (1992), Bhuiyan *et al.,* (1993), Bhuiyan and Afrose (1996), Alam *et al.,* (1996), Lorenzo *et al.,* (2002), Oliva-Paterna *et al.,* (2002), Pajuelo *et al.,* (2006a), Pinheiro *et al.,* (2006), Colombo *et al.,* (2007), Koc *et al.,* (2007), Fazli *et al.,* (2008), Filer and Sedberry (2008), Kahraman *et al.,* (2008), Mazlan and Rohaya (2008).

An understanding of breeding cycles as they occur in nature is an essential pre-requisite for the initiation of breeding program of the species concerned (Mollah and Tan, 1982). The foregoing literature clearly shows that though there is considerable information on the different aspects of reproductive habits and cycles of some fishes.

A study of reproductive biology of *C. carpio* var. *specularis* is essential in the sense that it may provide information and clues for a tactful and skillful nature of this fish. Knowledge on reproductive biology of a fish is of great importance in its rational exploitation through proper management of fishery resources, development of selective breeding, brood stock development, domestication and genetic improvement. A study on the various aspects of reproduction is essential in the determination of population stock size, periodicity of the strength of brood fishes, spawning time and place and sex composition of the exploited stock.

C. carpio var. *specularis* accounts for an important freshwater capture fishery. No published work on the breeding biology of this species is available, Considering the economic importance of this fish an investigation on the reproduction of this fish was undertaken.

The aims and objectives of the present study:

- To study the male and female reproductive systems.
- To determine the reproductive period and peak period of breeding season.
- To know the developmental stages of ovary and stages of ova in different months.
- To estimate the fecundity and its relationship with different body measurements.
- To know the gonado somatic index, gonadal length index, and
- To determine the sex ratio.

(a)

(b)

Plate 4.1(a-b): Fishing in the Atrai river area.

MATERIALS AND METHODS

Sampling and laboratory analysis

Random sampling was done to collect adult *C. carpio* var. *specularis* at a regular interval of one week from different fish landing centres in Atrai river of Northwestern (Naogaon district) Bangladesh since July, 2013 to June, 2015 (24 Months). A total of 1084 specimens were collected during day time and precautions were taken to save from spoilage or any damage. After collection, the specimens were washed well, confirmed to the species level and then tagged and preserve by date in plastic jars with 10% formalin.

Different lengths were measured with a precision of 0.01 cm with the help of measuring board fitted with a scale. A fine pointed divider allowed measuring the smaller parts of the body. Weight of the fishes and other parameters were taken with the help of Electronic precision balance Model: ED300KC to an accuracy of 0.5g and pen balance (capacity 20g - 5kg, Made in China) in grams. The specimens were sexed either by visual observation or by dissecting out the gonads and the numbers of gravid females were recorded.

The gonads of fishes were removed intact and placed in 5% formalin which not only preserved the ovary but made it much easier to separate the eggs from the ovarian wall (Shafi and Quddus, 1974). The length of the ovary was measured with the help of a fine point divider. The weight of gonads was taken with the help of an Electronic balance Model: EK300H and HL-400 to an accuracy of 0.01g. Excess moisture of the ovaries was removed by using blotting papers before weighing. The changes of color of the testes and ovary were noted depending on the degree of maturation. During preservation the gonads were properly labelled for subsequent studies.

The mean gonadal length index (GLI), gonado somatic index (GSI) and ova diameter was calculated for each month to find out the spawning season. Gonadal length index (GLI) and Gonado somatic index (GSI) were calculated according to the formula described by Welcomme (1985) and Wootton (1990) as follows:

$$GLI = \frac{\text{Length of the gonad}}{\text{Length of the fish}} \times 100, \qquad GSI = \frac{\text{Weight of the gonad}}{\text{Weight of the fish}} \times 100$$

Ten to fifteen ova were collected at random from the anterior, central and posterior regions of each ovarian lobe by separating them out from the tissue by a fine needle and a brush. These were arranged in several rows on a glass slide and the diameter of individual ova was measured with the help of an ocular micrometer fitted on a compound microscope. The ova diameter was recorded twice in every month.

A total of 572 females were studied for detecting the reproductive cycle. The following methods were applied to study the reproductive cycle of *C. carpio* var. *specularis:*

1) Percentage of gravid females against time
2) Gonadal length index (GLI)
3) Gonado somatic index (GSI)
4) Variation in ova diameter
5) Colouration of the ovary

A total of 273 gravid females were studied for the estimation of fecundity. The gravimetric method was followed to determine the fecundity according to Lagler (1966), Hossain *et al.*, (1997) and Latifa *et al.*, (2002). The relationship of fecundity with total length (TL), standard length (SL), total weight (TW), gonadal length (GL), and gonadal weight (GW) were estimated by the least square method.

To estimate the fecundity of ripe ova, first the pattern of distribution of ripe ova in the two lobes of the ovary were determined. A portion of the ovary weighing approximately about 0.2 to 0.6 g were taken from the middle, anterior and posterior regions of the left ovary and the number of ova was counted. The grand total of ova in the ovary was calculated from the known weight of sample, the number of ova in the sample and the total weight of the ovary was determined as follows:

$$\text{Estimated fecundity} = \frac{\text{Number of ova in the sample}}{\text{Weight of the sample}} \times \text{gonadal weight}$$

The sex ratio from the collected fishes was estimated month-wise. The significance of the deviation of sex-ratio from 1:1 on the null hypothesis was tested by the Chi-square test (Snedecor, 1956).

Statistical analysis:

Statistical analysis was done by using Microsoft$^{(R)}$, MS Excel add-ins-DDXL and Graph pad Prisom 5 softwere.

a. Male

b. Female

Plate 4.2(a-b): Male and Female of *C. carpio* var. *specularis*.

RESULTS AND OBSERVATIONS

Sexual dimorphism

Sex differentiation is an important factor for scientific study of an organism. The sexual dimorphic characters of *C. carpio* var. *specularis* are very indistinct and the male and female are not easily distinguishable. Generally the fish shows some common sexual dimorphic characters superficially after attaining a minimum total length of 10cm. Unlike many fishes only the secondary sexual characters are observed during the breeding period. The following sexual dimorphic characters of *C. carpio* var. *specularis* were recorded during the study period.

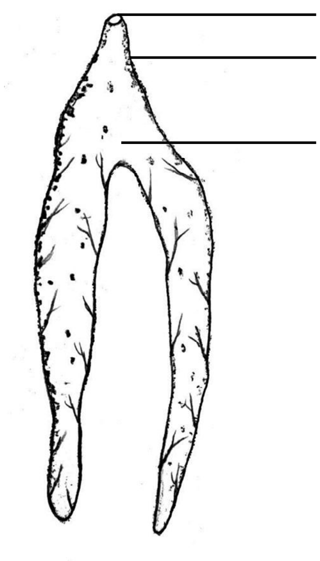
Characters of pectoral fin: Like other fishes, the pectoral fin characters are not distinct in *C. carpio* var. *specularis*. The characters were seemed superficial throughout the year. Although some characters appeared in acute observation. The pectoral fins of males were found little longer and seemed slightly blackish in colour with comparatively distinct fin rays. In females, the fins were shorter than the male, thin and light orange to reddish in colour with narrow fin rays.

Characters of abdomen and genital papilla: These characters are only prominent in the breeding period. In males, the abdomen was not bulgy and the genital papilla was thin, muscular, cone-shaped, more pointed and tipped with reddish colour. In female, the abdomen was bulgy, genital papilla was stouter, fleshy, broader, less pointed, round shaped and not tipped with red colour and the vent protrudes from the body.

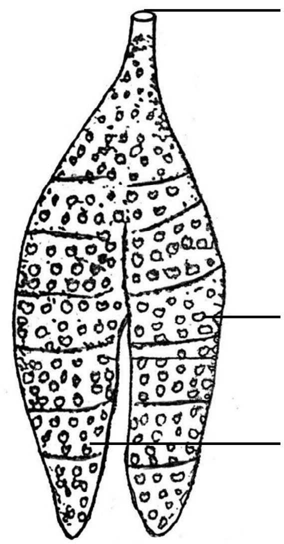
Colouration: The sexual dichromatism were observed between the male and female fishes. The pectoral fins of male were slightly blackish in colour but in female it seemed to be light orange to reddish. In males, the genital papilla was tipped with reddish colour and the diffused vent with red colouration where in female the genital papilla was not tipped with red colour.

Reproductive system

Generally the gonads and their associated ducts are the components of reproductive system of a fish. The reproductive system of *C. carpio* var. *specularis* was exceptional and basically different from most oviparous fishes as described below:



a. Male genital organ



b. Female genital organ

Fig. 4.1(a-b): The reproductive system of *C. carpio* var. *specularis* (Male & Female)

a. Male genital organ

b. Female genital organ

Plate 4.3(a-b): The reproductive system of *C. carpio* var. *specularis* (Male & Female)

Male reproductive organ

The male reproductive system of *C. carpio* var. *specularis* consists of a pair of elongated testes and its ducts. It originates in the abdominal cavity above the alimentary canal and below the kidney. The testes were not equal in size. The left one was larger than the right. The color and size vary according to stage of sexual maturity and ripenes.

Female reproductive organ

The female reproductive system of *C. carpio* var. *specularis* of a paired ovaries and short oviducts. The left ovary was always larger than the right. They were suspended from high on the sides of the body cavity by a pair of mesenteries (mesovaria) and thus situated above the alimentary canal. The anterior portion was slightly wider, blunt and rounded, projected into the body cavity and the posterior portion was tapered and open into a short common oviduct which leads out through the genital pore situated on an elevated urinogenital pore behind the anus just in front of the anal fin. The size and extent of occupancy of the ovary in the body cavity vary with the stage of sexual maturity of the female. The color varies from whitish in young through light yellow when maturing to yellow or deep yellow in ripe stage. Treasurer and Holliday (1981) was followed for the microscopic examination of ovary and microscopic structure of the oocytes.

Developmental stages of ovary

Development of ovary is a spontaneous process that required for the determination of the breeding season of a fish. The stages of maturation of the female can be traced by observing the colour of the ovary and taking the diameter of the ova (Hossain, 1976; Hossain *et al.,* 1989; Hoque and Hossain, 1993; Parween *et al.,* 1993; Hussain and Hossain, 2001). In the present study a total of 273 adult females were studied during (July, 2013 to June, 2015) to determine the different stages of ovaries and eggs.

However, the development of ovary could be divided into ten stages on the basis of colour, size and the occurrence of different stages of eggs. These are as follows:

Stage I: Immature

The immature ovary was only found in the immature female. In May to July it was thin and whitish in color and could not be visible easily and difficult to distinguish from the testes. Histologically the ovaries showed ovigerous lamellae, having nests of oogonia and immature oocytes.

The ovary occupied about one third of the body cavity. The tip of two lobes of the ovary appeared as flesh colored thread like structure (Fig.4.2a). Ova were not visible by naked eye. In this stages only immature ova were present. The ova diameter ranged from 0.22 to 0.32 mm.

Stage II: Maturing-I (Early developing)

Similar appearance of ovaries were found like stage I. The color of the ovary was still whitish/ opaque and become slightly thicker. The weight of ovary increased slightly than the immature stage, but the blood capillaries became inconspicuous. This stage occuiped about 40-55% of the body cavity (Fig. 4.2b). In this stage immature ova were frequently found. No granulosa layer and yolk vesicles were found. This stage was found in August to September. The ova diameter ranged from 0.26 to 0.51 mm.

Stage III: Maturing-II (Late developing)

In October the ovary grew in weight and size. The tunica became relatively thin. In this stage immature ova were frequently found. Ogonia and maturing ova were also found. The stage was found in late October to November. The color of the ovary became light yellow and occupied about 60% of the body cavity (Fig. 4.2c). The ova diameter ranged from 0.28 to 0.69 mm.

Stage IV: Mature

In December to February the ovary occuiped about 60-80% of the body cavity (Fig. 4.2d). The color of the ovary changed to yellow. The ovarian wall was slightly thin and the blood supply increased considerably, translucent and opaque ova were present. Immature and maturing ova were found with the mature ova. The percentage of occurrence of mature ova were maximum, immature and maturing ova were found in the central region and the posterior portion of the ovary and mature ova found in the periphery and the anterior and middle region of the ovary. The ova diameter ranged from 0.64 to 1.15 mm.

Stage V: Ripe ovary

Most of the gravid females caught during late December to April had a ripe ovary (Fig.4.2e). At this stage the ovary occupied about 90% of the body cavity and yellow / deep yellow in appearance. The ova were bright and easily visible through the thin and transparent ovarian wall by naked eye. The eggs were packed up in the ovary. They were partially free and oozed out with little pressure on the abdomen. In this stage, the ovary got rich blood supply. The ovaries fully occupied the abdominal cavity and extended just up to the base of the pectoral fin. The anterior region of the ovary became irregular in shape because not to get sufficient accommodation in the abdominal cavity.

The major part of the ovary was filled up with ripe ova, though small percentage of former stages of ova and sterile ova were also found. At this stage the oocytes became fully free and oozed out with little pressure. The ova diameter ranged from 0.99 to 1.98 mm.

Stage VI: Pre-spawning

This stage was very much similar to stage V from external observation. At this stage the oocytes became fully free and ooze out with little pressure on the abdomen. The ovary and oocytes were found yellow / deep yellow in color (Fig.4.2f)

Stage VII: Spawning

The thinner oviduct wall was found at this stage (Fig. 4.2g). The oocytes were sometimes found scattered around the genital pore of the female.

Stage VIII: Post-spawning

This stage exists for a very short period of time. The weight of the ovary reduced to about 80% but occupied about 50% of the body cavity very loosely (Fig. 4.2h).

Stage IX: Spent

At this stage the ovary lost all the oocytes (Fig. 4.2i). But still contained some ruptured and collapsed post ovulatory follicles and forming a irregular series of folds.

Stage X: Resting

At this stage the wall of the tunica contracted and reduced with a corresponding increase in diameter (Fig. 4.2j). This size and number of oocytes were also found in the appearing condition. The condition was observed in the matured females from late May to July. The ovaries occupied 1/2 or 2/3 of the abdominal cavity. Few ripe ova were discharged, atretic, oocytes and a few developing eggs were seen in the ovary. The color of the ovaries became pale.

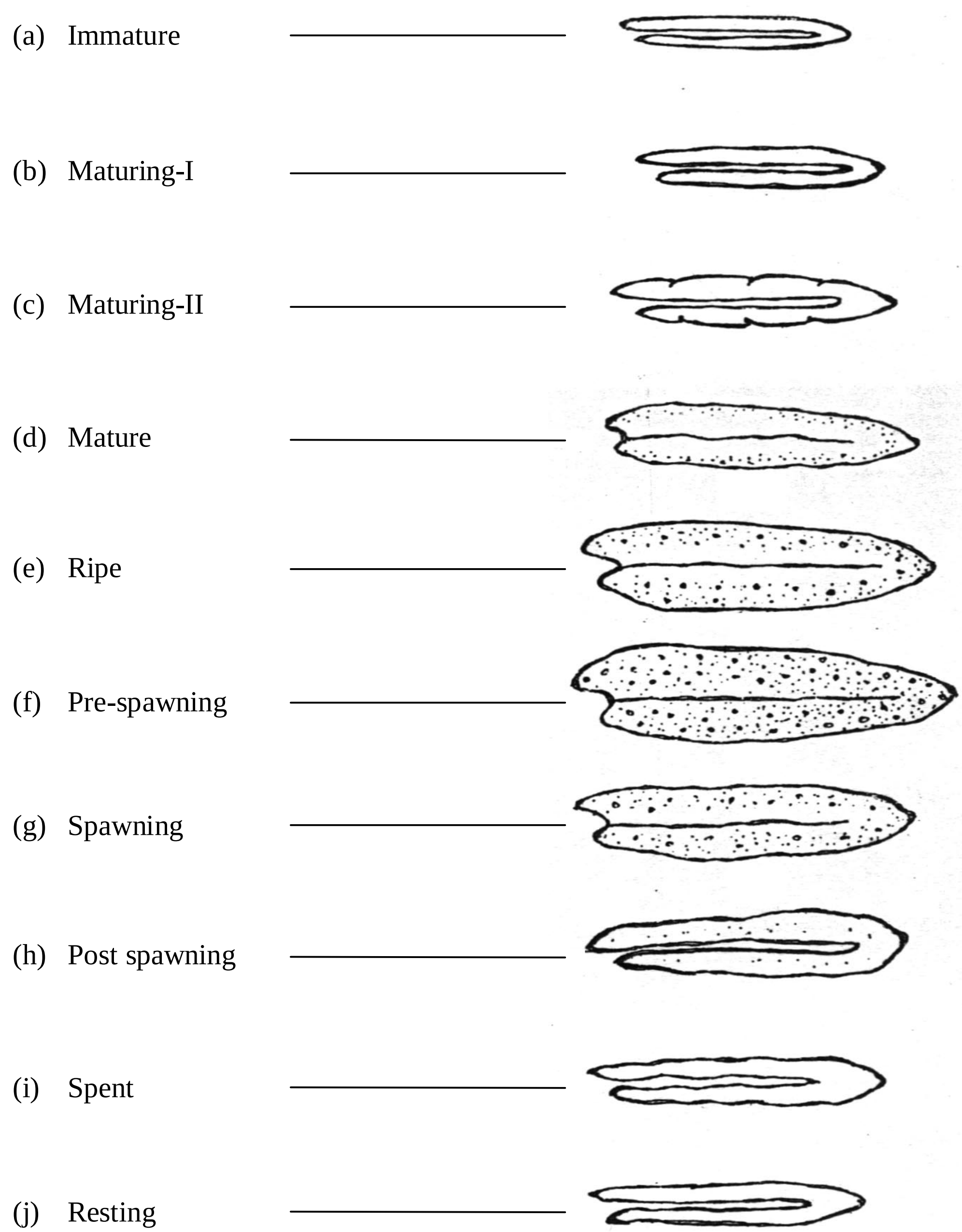


Fig. 4.2(a-j): Showing the stages of ovarian development of *C. carpio* var. *specularis.*

Maturation stages of ovary

The length and weight of immature ovaries were ranged from 2.00 to 2.70 cm (2.51±0.21) and 0.30 to 0.85 g (0.63±0.13) respectively. The maturing ovaries length and weight were ranged from 2.70 to 3.80 cm (3.33±0.35) and 0.85 to 2.18 g (1.52±0.43). The mature ovaries length were ranged from 3.80 to 6.40 cm (5.15±0.77) and with weight ranged from 2.18 to 5.00 g (3.56±0.79). The ripe ovaries length and weight were ranged from 6.50 to 18.80 cm (10.54±3.17) and 5.00 to 255.07 g (59.41±63.59) respectively (Table 4.1).

Table 4.1: Range and mean ± SD of lenth and weight of ovary in different stages of maturity of *C. carpio* var. *specularis.*

Stages of maturation	Length (cm)		Weight (g)	
	Range	Mean±SD	Range	Mean±SD
Immature	2.00-2.70	2.51±0.21	0.30-0.85	0.63±0.13
Maturing	2.70-3.80	3.33±0.35	0.85-2.18	1.52±0.43
Mature	3.80-6.40	5.15±0.77	2.18-5.00	3.56±0.79
Ripe	6.50-18.80	10.54±3.17	5.00-255.07	59.41±63.59

a. Ripe

b. Spent

Plate 4.4(a-b): Ripe and spent ovary of *C. carpio* var. *specularis.*

Maturation stages of ova

The developmental stages and maturation of the ovaries and oocytes are the important parameter for the determination of the breeding season of fish. In the present study the maturation of female was determined by observing the colour of the ovary and diameter of the ovum. The development of ovary could be divided into four major stages on the basis of colour, size and the occurrence of different stages of eggs as:

Immature: The ova were not clearly defined by the naked eye, but can be visible under microscope. The microscopic ova were nearly round shaped and semi-transparent with central nucleus. The ova were whitish in colour and yolk was not seen at all (Plate 4.5a). The ova diameter ranged from 0.22 to 0.32 mm with the mean of 0.26 ± 0.03 mm (Table 4.2).

Maturing: Increment of ova diameter was observed but was not clearly defined without microscope. The microscopic view of the ova revealed that the eggs were slightly opaque due to the deposition of the yolk at the central portion and the nucleus was seemed to visible. The outer boundary remained irregular. The ova became light yellow in colour (Plate 4.5b). The ova diameter ranged from 0.28 to 0.69 mm. The mean ova diameter was 0.54 ± 0.11 mm (Table 4.2).

Mature: In this stage eggs were more or less spherical in shape. It was observed from the microscopic view that the ova were opaque and the nucleus was invisible. The egg turned yellow in colour when the yolk deposition was completed (Plate 4.5c). The ova diameter ranged from 0.64 to 1.15 mm. The mean ova diameter was 0.90 ± 0.15 mm (Table 4.2).

Ripe: In this stage the eggs were spherical and yellow or deep yellow in colour (Plate 4.5d). The ova were clearly set through the wall of the ovary. A single oil globule can be seen in each ovum. In this stage the eggs attain the highest size and the ova diameter ranged from 0.99 to 1.98 mm with the mean of 1.26 ± 0.15 mm (Table 4.2).

A thin transparent layer of ootheca surrounds the eggs. The mature and ripe ova were found towards the periphery and the immature and maturing ova lay towards the center.

a. Immature

b. Maturing

c. Mature

d. Ripe

Plate 4.5(a-d): *C. carpio* var. *specularis* different development stage of ova and colour.

Table 4.2: *C. carpio* var. *specularis* Variation in ova diameter in different stages of maturity with distinguishing colour (N = 273).

Developmental stages of ova	Ova diameter (mm)			Colour
	Minimum	Maximum	Mean ± SD	
Immature (N = 25)	0.22	0.32	0.26±0.03	Whitish
Maturing (N = 54)	0.28	0.69	0.54±0.11	Light yellow
Mature (N = 86)	0.64	1.15	0.90±0.15	Yellow
Ripe (N = 108)	0.99	1.98	1.26±0.15	Yellow or Deep yellow

Determination of reproductive cycle

Reproductive behaviour in fishes are cyclic more or less regularly periodic (Lagler *et al.,* 1967). The reproductive act in some fishes occurs only once in a very short life time, while in other fishes, it occurs in moderately long life span. Several other species spawn more than once in a year more or less continually. After spawning new group of oocytes are formed, which gradually mature to become ready for the next season.

Reproductive cycle of fishes depend upon several factors. Both light and temperature are important factors in controlling the maturation of gonads in fishes. Variation in the duration of light and temperature may influence the rate of gonadal development. According to Kinne (1971), most of the stages of reproduction often depend on certain condition of water movement. In rainy season particularly in June and July months after a heavy shower, the physico-chemical condition of water bodies abruptly changes and which in turn probably stimulates spawning in most of the freshwater fishes (Jhingran, 1977). *C. carpio* to spawn especially between October to April when the average water temperature is above 17°C (Zanotta *et al.,* 2010). There are various methods for determining the reproductive cycle of a fish.

In the present study five methods were applied to determine the reproductive cycle of *C. carpio* var. *specularis* as follows:

Percentage of gravid female against time: The percentage distribution of gravid females were observed from July 2013 to June 2015 (24 Months). The result of two years data revealed that the gravid females of *C. carpio* var. *specularis* occurred during the period from November to April (Fig. 4.3). No gravid females were found in rest of the year. The highest percentage of gravid females were found in the month of February 2015 (92.00%), followed by February 2014 (91.30%), March 2015 (91.30%), March 2014 (87.50%), April 2015 (79.17%), April 2014 (75.00%), November 2013 (86.96%), November 2014 (83.33%), December 2013 (81.82%), December 2014 (86.96%), January 2014 (81.81%), January 2015 (86.36%) and the lowest percentage of gravid females were found in the month of August 2014 (3.85%) followed by August 2013 (8.00%), July 2013 (8.00%), July 2014 (8.70%), June 2014 (12.00%), June 2015 (12.50%), September 2013 (12.50%), September 2014 (8.33%), October 2013 (17.39%), October 2014 (12.50%), May 2014 (20.00%), May 2015 (20.00%) (App. Table 2). From the above observation, it may be concluded that *C. carpio* var. *specularis* is an annual breeder and the breeding season extending from November to April with the peak in February.

Limitation of the method

Accurate estimation of the proportion of the gravid females in the natural population is very difficult. The number of gravid females collected may not be the representative of the natural population. So far an accurate estimation of the breeding periodicity of *C. carpio* var. *specularis,* a number of methods has been applied. From the available data it would appear that the breeding season of this species range from November to April with the peak in February.

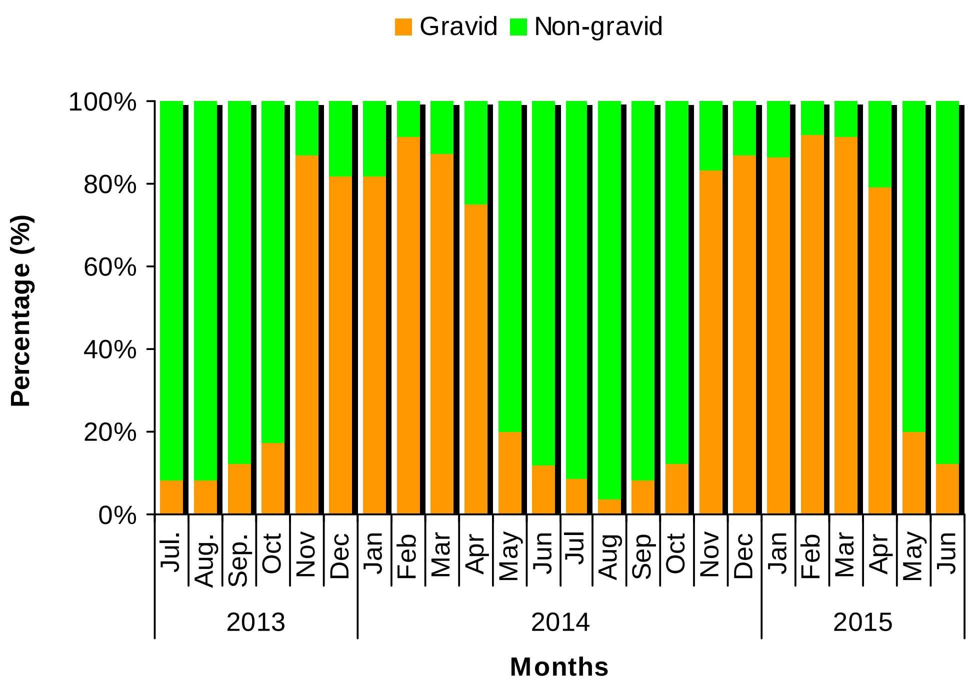


Fig. 4.3: *C. carpio* var. *specularis* ; Monthly percentage distribution of gravid and non-gravid females during July, 2013 to June, 2015.

Gonadal length index (GLI): The length of the ovary increases proportionately in relation to body length and season. GLI was calculated for each individual and monthly average values were shown in Fig. 4.4. The month wise range, mean and standard deviation of the GLI are given in App. Table 3. It can be seen from the Fig. 4.4 that the higher values of GLI were obtained during November to April with the peak in February which indicate the active breeding period of *C. carpio* var. *specularis*.

This method is very useful for the determination of the reproductive cycle, for its simplicity and accuracy, because there is no high sensitive measurement in this method *i.e.*, the lengths can easily be measured by using scale and fine point divider.

Gonado somatic index (GSI): Not only the length of ovary increase with the appearance of the ripe ova but also the weight of ovary is also increase accordingly. The monthly variation in GSI of *C. carpio* var. *specularis* was observed during the study period because in certain months of the year the mature females bear mature and ripe ovaries, which increases the value of GSI. Such higher values of GSI have been observed during the months from November to April with the peak in February where the values were found considerably low in rest of the months (Fig. 4.5). This variation in GSI only occurs in mature fishes which are reproductively active. After releasing the ripe ova, the length and weight of the ovary is suddenly reduced. So, fluctuations of gonado-somatic index in the same season are also commonly met with. The month wise range and mean of GSI are given in App. Table 3.

Ova diameter: The monthly variation in ova diameter is an important phenomenon to detect the reproductive cycle of a fish. It is well known that the diameter of ova are highest in the mature and ripe stages of an ovary. The ova with larger mean diameter were found from November to April that showed a distinct fluctuation throughout the year with a meaningful cycle (Fig. 4.6, App. Table 3). These variations were found only in mature female in relation to seasonal cycle.

The month wise ranges of the ova diameters are not so affected by the seasonal variation, but mean values are influenced by the seasonal factors. The reason behind this is the frequent occurrence of mature and ripe ova from November to April where the maturing and immature ova in the ovary are more frequent in rest of the months. The method revealed that the reproductive activities in *C. carpio* var. *specularis* continue from November to April with the peak in February.

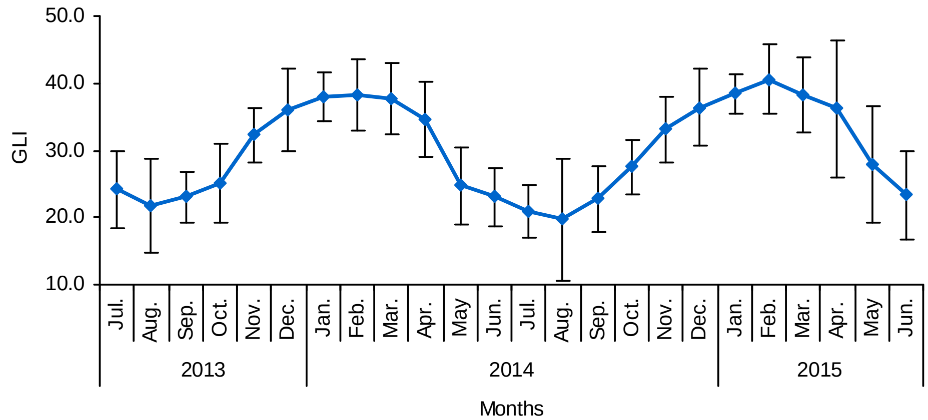


Fig. 4.4: Monthly variation in gonadal length index (GLI) in female of *C. carpio* var. *specularis*.

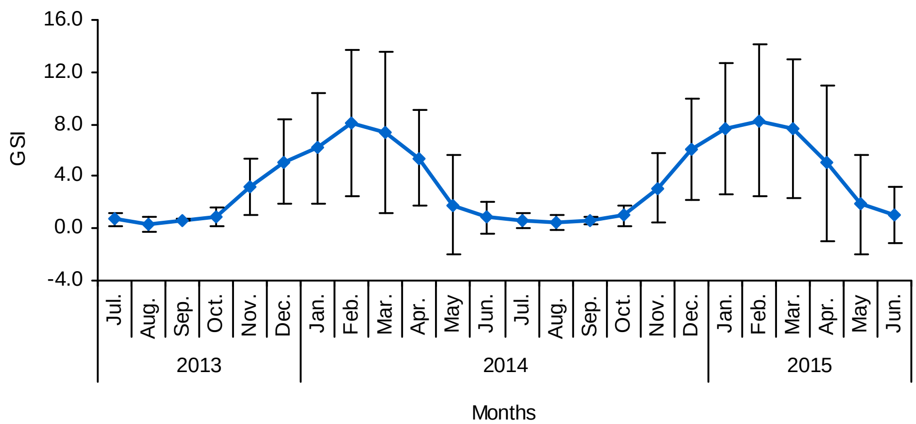


Fig. 4.5: Monthly variation in gonado somatic index (GSI) in female of *C. carpio* var. *specularis*.

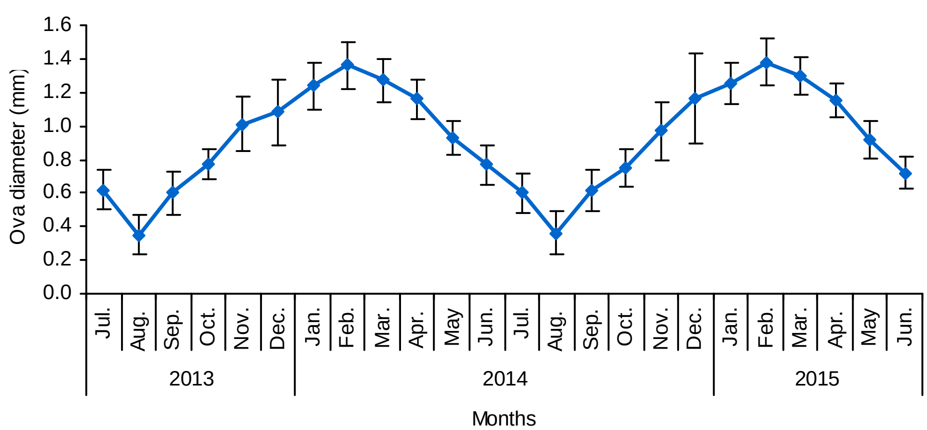


Fig. 4.6: Monthly variation in mean ova diameter of *C. carpio* var. *specularis*.

Colouration of the ovary: Colour of the ovary changes according to the degree of maturity of the ova. Colour of the ovaries varies from species to species such as green, red, white, orange, yellow, brown etc. (Lagler, 1956). Gradually the colour becomes more intense and bright with the attainment of maturity and so it is a helpful method for determining the reproductive cycle of a fish. It was observed that the mature and ripe ovary of *C. carpio* var. *specularis* was yellow to deep yellow in colour (Table 4.2). In the present investigation the occurrence of yellow or deep yellow coloured ovary was found from November to April which indicates that these months are the breeding period of *C. carpio* var. *specularis*.

Fecundity

Fecundity is the general term used to describe the number of eggs produced by an individual contained in the ovary. In fishery science, the fecundity is defined as the number of ripe eggs produced by a female individual up to the next spawning. The number of eggs contained in the ovary of a fish is termed the fecundity (Nikolsky, 1963).

Estimation of fecundity

For the estimation of fecundity of *C. carpio* var. *specularis* ripe ovaries were considered. During the spawning period a total of 273 gravid females were examined from the collected samples. It was observed that the number of eggs varied from minimum 5928.91 (for a fish with total length 11.66 cm size group 10-13 cm and total weight 296.67 g) to maximum 209120.18 (for a fish with total length 35.78 cm size group 34-37 cm and total weight 1294.58 g). However the smallest fish group 10-13 cm with a total length 11.66 cm and total weight 296.67g shows the fecundity of 5928.91 and the largest fish group 40-43 cm with a total length 41.38 cm and total weight 1776.54g. shows the fecundity 201187.50. The mean±SD fecundity was 87377.98±79544.17, total length 26.50±10.04 cm and total weight 880.34±468.29 g respectively (App. Table 4).

The variation of fecundity is very common in fish and the number of eggs produced by an individual female is dependent on various factors such as size, age, condition, types of species of samples (Lagler *et al.*, 1967).

Relationship of fecundity with different parameters

The relationship between fecundity and total body length, fecundity and total body weight, fecundity and standard length, fecundity and gonadal length, fecundity and gonadal weight were estimated from the observed data and the values of intercepts (a), regression coefficient (b) and co-efficient of correlation (r) were computed (Table 4.3) from those data by using the statistical formula $y = a + bx$ (regression equation).

Relationship between fecundity (F) and total length (TL)

The relationship between the fecundity and the total length of *C. carpio* var. *specularis* is shown by the scatter diagram in Fig. 4.7. The mean value of fecundity was 87377.98±79544.17 and the mean value of total length was 26.50±10.04 cm (App. Table 4). The regression equation was found to be linear and the co-efficient of correlation was highly significant ($r = 0.9129$) Table 4.3.

Relationship between fecundity (F) and standard length (SL)

The scatter diagram shows the relationship between fecundity and standard length of *C. carpio* var. *specularis* in Fig. 4.8. The mean fecundity was 87377.98±79544.17 and mean standard length 19.96±8.56 cm (App. Table 4) the regression equation was found to be linear and the co-efficient of correlation was highly significant ($r = 0.9319$) Table 4.3.

Relationship between fecundity (F) and total weight (TW)

This is a correlation between the fecundity and total weight of the females. The individual values of the body weight were plotted against the respective fecundity and showed by the scattered diagram. The relationship between these two variables of *C. carpio* var. *specularis* are shown in Fig. 4.9 which was of linear type. The mean total body weight was 880.34±468.29g (App. Table 4).

The regression equation was found to be linear and the co-efficient of correlation was highly significant ($r = 0.9519$) Table 4.3.

Relationship between fecundity (F) and gonadal length (GL)

The scatter diagram of fecundity and gonadal length suggested a linear relationship between the two variables in Fig. 4.10. The mean value of gonadal length was 9.72±4.00 cm (App. Table 4).

The value of co-efficient of correlation was highly significant (r = 0.9121) Table 4.3.

Relationship between fecundity (F) and gonadal weight (GW)

The scatter diagram of fecundity and gonadal weight showed a linear relationship between the two variables in Fig. 4.11. The mean value of gonadal weight was 61.90±55.02g (App. Table 4).

The value of co-efficient of correlation was highly significant (r = 0.9948) Table 4.3.

It was observed that all the relationships were highly significant with 'r' values being P>0.001 excluding the relationship between fecundity and gonadal length. In this case the 'r' value was 0.9121 which is near to the expected level.

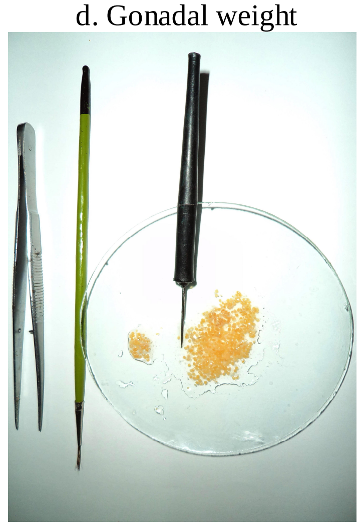
a. Total length

b. Total weight

c. Gonadal length

d. Gonadal weight

e. Fraction of gonad weight



f. Count of ova

Plate 4.6(a-f): Showing different parameters to estimate the fecundity of *C. carpio* var. *specularis*.

Table 4.3: *C. carpio* var. *specularis* Values of regression coefficient 'b' intercept 'a' and coefficient of correlation 'r' in F/TL, F/SL, F/TW, F/GL, F/GW (N = 273) Equation; y = a + bx.

Relation		Value of 'a'	Value of 'b'	Value of 'r'
Ordinate	**Abscissa**			
Fecundity (F)	Total length (TL)	-113424	7620.60	0.9129***
Fecundity (F)	Standard length (SL)	-91651	8971.00	0.9319***
Fecundity (F)	Total weight (TW)	-91523	17033.00	0.9519***
Fecundity (F)	Gonadal length (GL)	-102077	19615.00	0.9121***
Fecundity (F)	Gonadal weight (GW)	-1882.4	1442.00	0.9948***

***Correlation is highly significant at the 0.001 level.

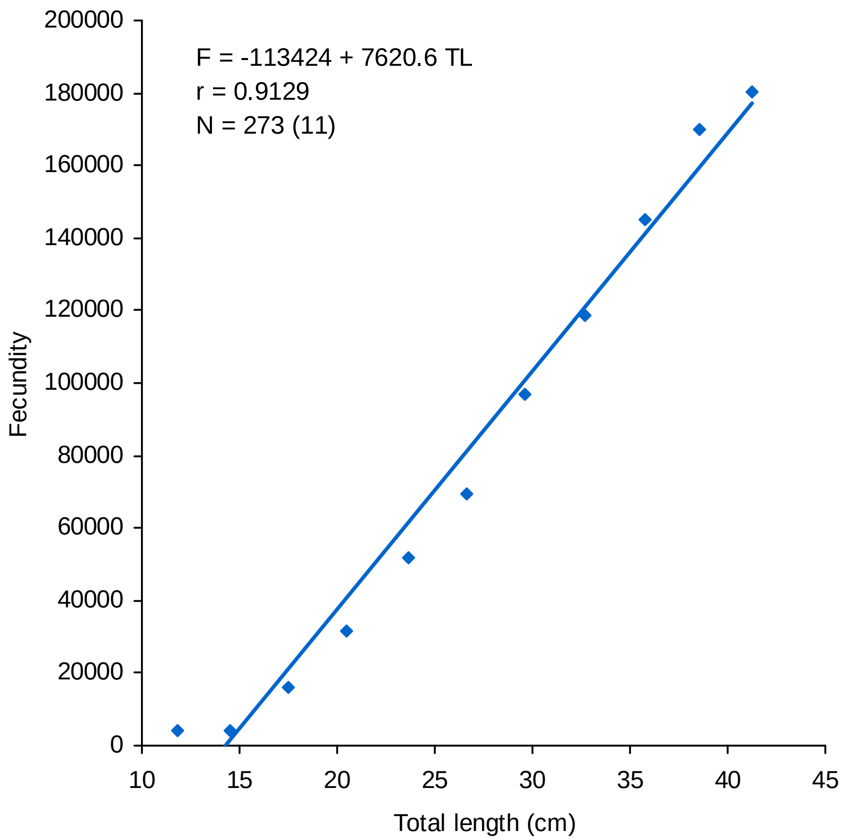


Fig. 4.7: Relationships between fecundity variable total length (TL) of *C. carpio* var. *specularis*.

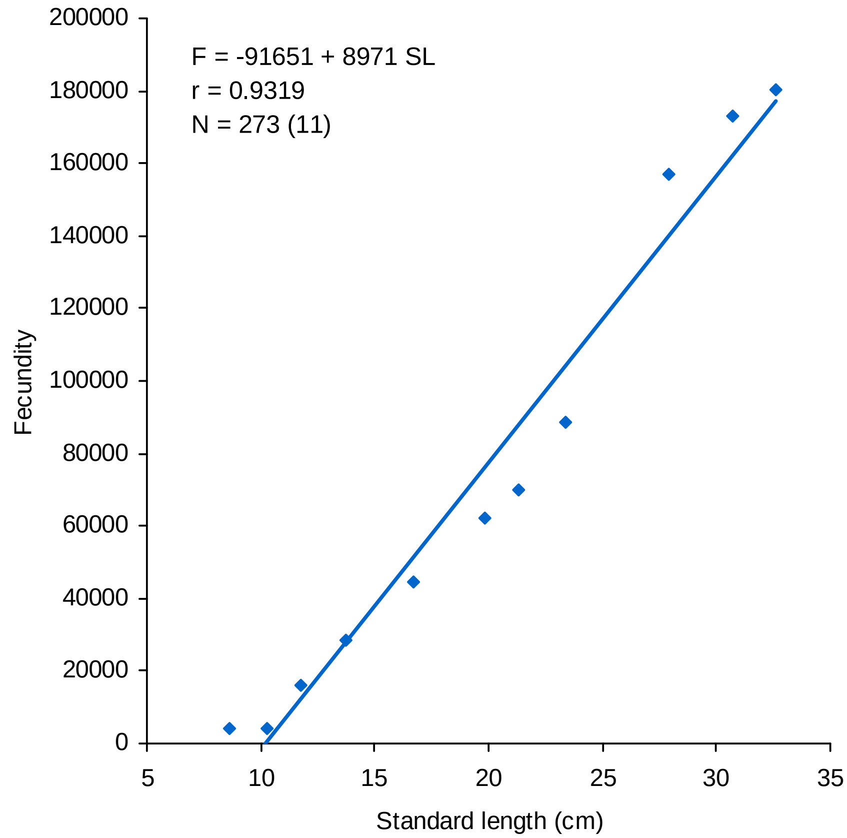


Fig. 4.8: Relationships between fecundity variable standard length (SL) of *C. carpio* var. *specularis*.

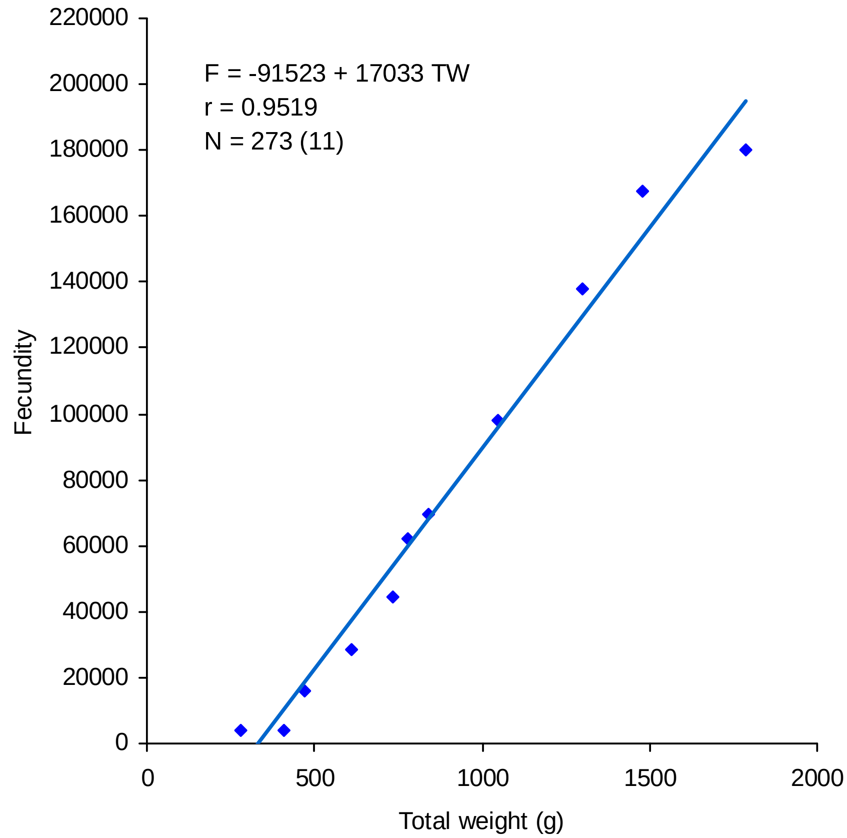


Fig. 4.9: Relationships between fecundity variable total weight (TW) of *C. carpio* var. *specularis*.

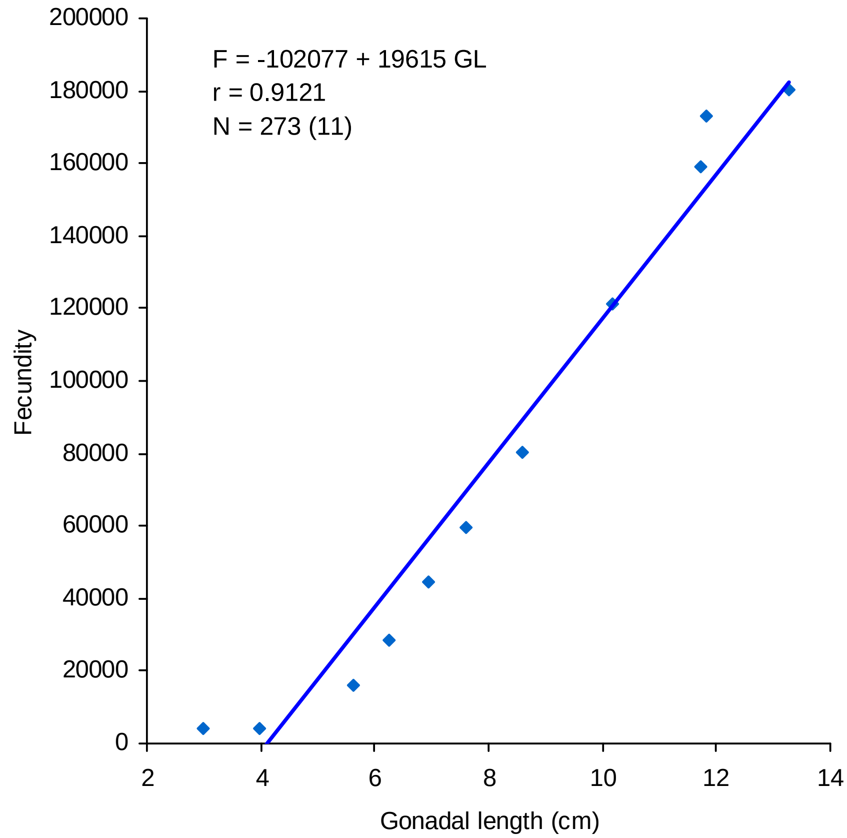


Fig. 4.10: Relationships between fecundity variable gonadal length (GL) of *C. carpio* var. *specularis.*

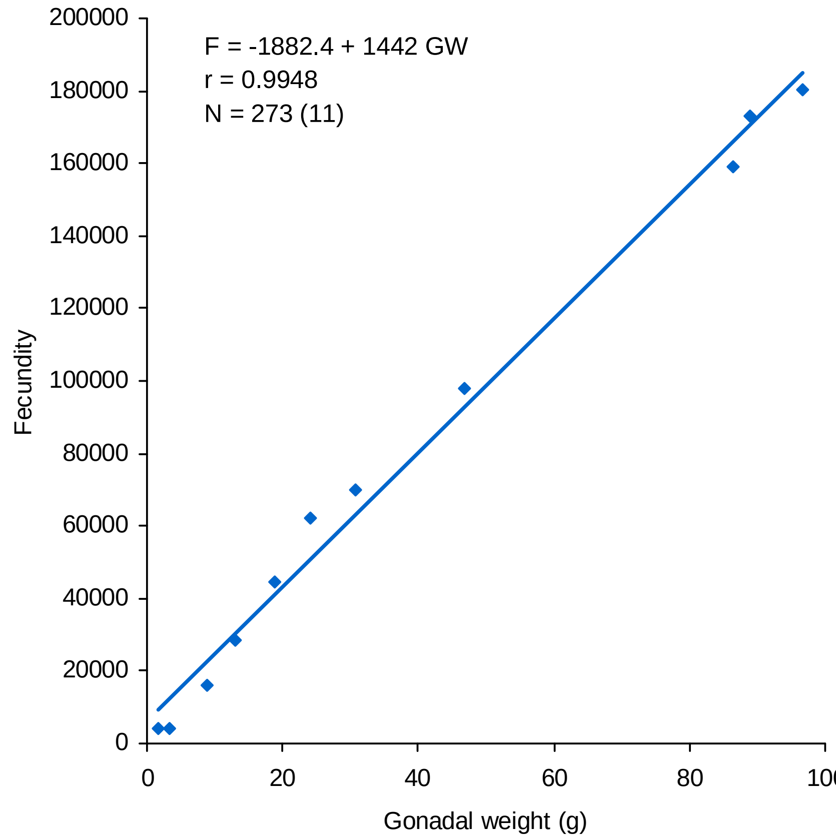


Fig. 4.11: Relationships between fecundity variable gonadal weight (GW) of *C. carpio* var. *specularis.*

Fecundity factor

Fecundity factor can be defined as the number of eggs in the female of a given length (App. Table 4). In other words fecundity factor is a tabular presentation weith respect to length of the animal. The highest value of fecundity was 209120.18 and the lowest value of fecundity was 5928.91. The mean fecundity was 87377.98±79544.17.

Sex ratio

Sex-ratio for adult *C. carpio* var. *specularis* was determined from 1084 specimens of which 512 males and 572 females. The overall sex ratio was found in favour of females *i.e.*, 1:1.12. The month wise male and female ratio was also calculated and found to vary during the study period that shown in App. Table 5. The females were found mostly predominating all over the year (Fig. 4.12 and 4.13).

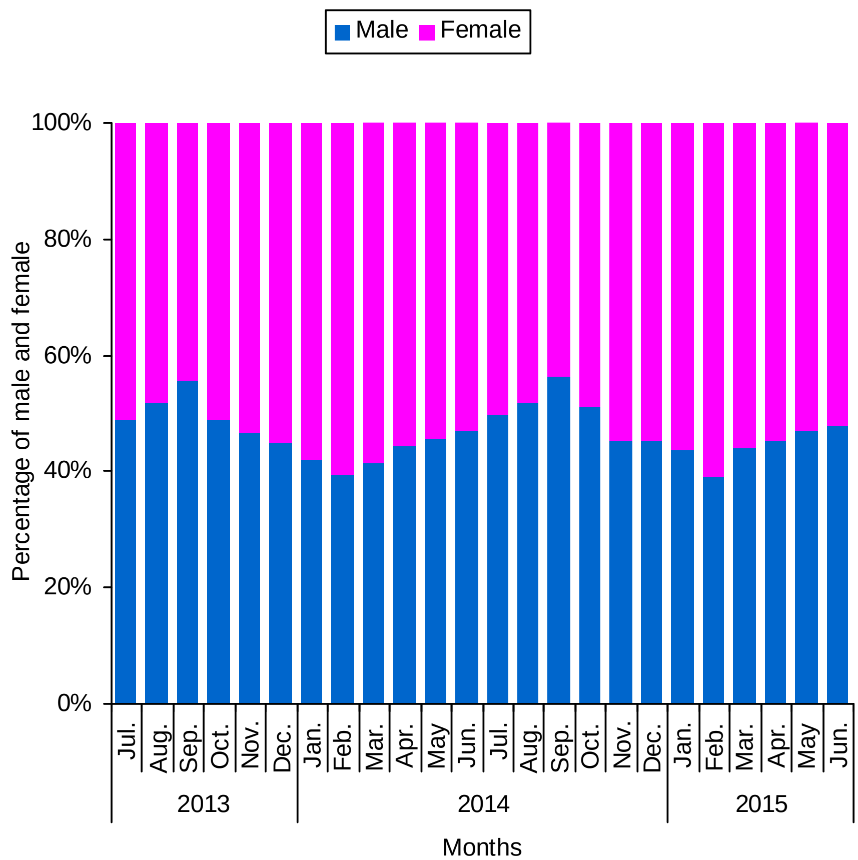


Fig. 4.12: Monthly percentage distribution of male and female of *C. carpio* var. *specularis* (July, 2013 to June, 2015).

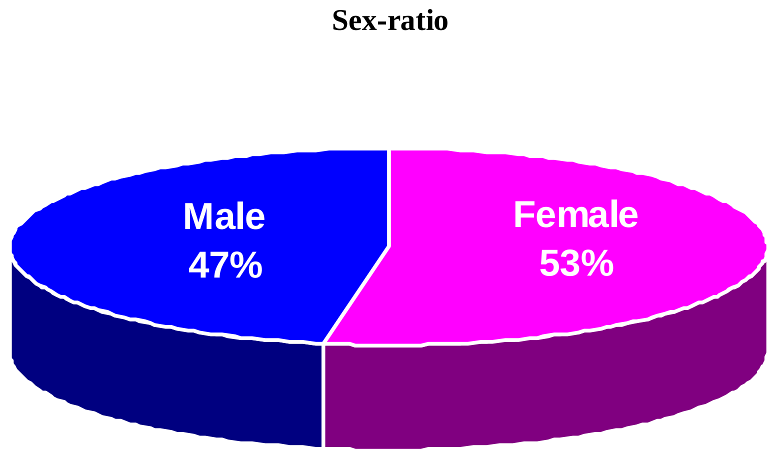


Fig. 4.13: Sex ratios of *C. carpio* var. *specularis.*

The chi-square test was made to see whether the sex difference was statistically significant or not. The test was made from the actual number of males and females. If the population was unbiased in terms of the distribution pattern of the male and female sexes, then the occurrence of the two sexes would be 50% in each case in the random sampling. The results of the test are given in App. Table 6. The calculated value of total χ^2 was 5.97, the overall χ^2 value was 3.32 and the χ^2 heterogeneity values was 2.65, which were significantly different.

DISCUSSION

Sexual dimorphism

The sexes are separate in fishes but the sexual dimorphism is not a very common phenomenon (Yadav, 1999). The characteristics of sexual differences or sexual dimorphism that enable identification of the sexes are classified as primary and secondary sexual characteristics. Primary sexual characters are those, which are concerned actually with the reproductive systems. Testes and their ducts in the male, and ovaries and their ducts in the females constitute primary sexual characters. Secondary sexual characters themselves are really of two kinds: those which have no primary relationship with the reproductive act at all, and those which are definitely accessory to spawning (Lagler *et al*., 1977). The secondary sexual characters and accessory structures are necessary for courtship, sexual display and pairing of male and female, simultaneously along with the development of primary sex organs (Yadav, 1999). Some species of fishes exhibit well-marked sexual dimorphisms between the two sexes during their spawning season. It is very difficult to distinguish the sex of many species of fish from their external appearance. Many fishes show virtually no sexual dimorphisms in body shape or colour differences even when spawning. The differentiation of sexes is rather difficult except the breeding season, when the gravid female become obvious by the bulged-out belly (Moyle and Cech, 2000). In the present investigation, same sexual dimorphisms have been observed in *C. carpio* var. *specularis* that used to determine the sex of the mature fish. The pectoral fins of male were found little longer than female and slightly blackish with comparatively distinct fin rays. Where in female, they look comparatively shorter than the male, thin, light orange to reddish in colour having narrow fin rays.

In the adult males, the genital papilla is thin, muscular and more pointed. In the female, genital papilla is stouter, fleshy, broader, less pointed and protrudes from the body. Sexual dimorphism at maturity stage of fishes was determined by Davis (1959) in channel catfish *Ictaluras punctatus*; Flickinger (1969) in flat head minnow; Breder and Rosen (1966) in bitterling; Wiley and Collette (1970) in 25 families of fish. Huq (1977) examined the six species e.g., *Rita rita, Mystus corsula, Mystus tengara, Ailia coila, Clarias batrachus* and *Heteropneustes fossilis* of

Bangladesh for finding a practical way of discerning sexes. The size of head and colouration of the body of both sexes of these fishes were identical, but the area of genital pore was quite helpful for identification of sexes. Smith and Bell (1975) used the genital papilla as a key to determine sexes of adult female salmon.

Besides the structure of the genital papilla and the colouration of the body parts showed significant differences between the male and female during breeding season. It was observed that the males and females showed sexual dichromatism during breeding season. Similar condition was also reported by Shafi *et al.,* (1978) in *H. ilisha,* Hossain and Islam (1983) in *Clupisoma atherinoides,* Berra (1984) in *P. maraena,* Nabi and Hossain (1996) in *M. aculeatus,* Mabragana *et al.,* (2002) in *Sympterygia bonapartii,* Islam (2002) in *G. giuris,* Martin *et al.,* (2005) in *Psammobatis bergi,* Knight *et al.,* (2007) in Oxleyan pygmy perch *Nannoperca oxleyana*.

Male reproductive organs

The reproductive strategies of fishes are often reflected in the anatomical differences between the sexes. Testes in fishes, generally, are paired structures situated in either side somewhat lateral to and below the kidneys (Kumar and Tembhre, 1998). A huge number of works are available in this regard. The testis of *C. carpio* var. *specularis* was also found paired structure with very short duct and suspended from the dorsal body wall by mesentery.

Colour and size of the testes vary according to the stage of sexual maturation (Berra, 1984). The testes appeared as a translucent, whitish and tube like structure. In the body cavity the gut wall was externally well supplied with fat. Abu-Hakima (1984), Berra (1984) and Nabi and Hossain (1996), described this condition as the immature stage of testicular development in *Acanthopagrus* sp, *P. maraena* (Gunther) and *M. aculeatus* respectively.

In the successive months, the testes attained maturing (September to October) stage and finally become fully mature or ripe in the month from November to April. The colour and size of the testes also changes accordingly. In these months, the peak of testicular development was found in most fishes. The testes become large, smooth and whitish-red in colour. The fat from the gut wall was completely disappeared.

By late January to March most of the fish are found with spent, flaccid testes and width decreased. Testes started to regain their original shape and size. Colour of the testes again changed to white and fat deposition resumed in the gut wall. This type of developmental changes was reported from a variety of fish species e.g., *C. macrocephalus* (Mollah and Tan, 1982), *M. aeglefinus* (Robb, 1982), *Acanthopagrus* sp. (Abu-Hakima, 1984), *P. maraena* (Berra, 1984), *Amblypharyngodon mola* (Afroze and Hossain, 1990), *Setipinna phasa* (Alam *et al*., 1996), *M. aculeatus* (Nabi and Hossain, 1996), *Clupisoma atherinoides* (Bhuiyan *et al*., 1999a), *Gobius roulei* (Kovacic, 2001), *Gonialosa manminna* (Hossain *et al*., 2002), *Esox lucius* (Lenhardt and Cakic, 2002), *A. latus* (Abou-Seedo *et al*., 2003), *Crystallogobius linearis* (Caputo *et al*., 2003), *N. notopterus* (Shankar and Kulkarni, 2005), *Siganus luridus* (Azzurro *et al*., 2007), *Scaphirhynchus platorynchus* (Colombo *et al*., 2007), *Pseudocorynopoma doriai* (Ferriz *et al*., 2007), *Apogon imberbis* (Klein, 2007), *Ameca splendens* (Ortiz-Ordonez *et al*., 2007) and *Parastromateus niger* (Dadzie and Abuo-Seedo, 2008).

Female reproductive system

The female reproductive system of *C. carpio* var. *specularis* comprised of paired ovary and its duct. The female gonad exists at the same location as in male. The ovary was spindle shaped filled with ova of different sizes. The shape, size and colour of the ovary depend on the stages of maturation of the developing ova. The wall of the ovary is fairly thickened during the non-breading season and becomes highly vascular during the spawning period.

Depending on the degree of maturation, many authors like (Kestaven, 1960; Famer, 1974; Treasurer and Holliday, 1981; Hossain, 1989; Hussain and Hossain, 2001; Azzurro *et al*., 2007; Dadzie and Abou-Seedo, 2008) categorized the maturation stages of ovaries into ten stages *viz*., immature, maturing (early developing), maturing (late developing), mature, ripe, pre-spawning, spawning, post spawning, spent and resting. In the present study the maturing stages of ovaries were categorized into ten stages.

Immature ovaries were thin, whithish thread like structure, occupying one third of the abdominal cavity. In this stage only immature ova were present. Ova diameter ranged from 0.22 to 0.32 mm. The maturing (early developing) ovaries occupy about 40-55% on the body cavity. The color of the ovary still remained whitish in this stage also. Only immature ova were present but the larger ova were more numerous. No. granulosa layer and yolk vesicles were found. Ova diameter ranged from 0.26 to 0.51 mm. The maturing (late developing) ovaries gained weight and size, color became light yellow and occupied about 60% of the body cavity. Ova diameter ranged from 0.28 to 0.69. The mature ovary occupied about 60-80% of the body cavity. The color of the ovaries changed to yellow. In this stage immature and maturing ova were found the mature ova. The percentage of occurrence of mature ova were maximum. The ova diameter ranged from 0.64 to 1.15 mm.

Ripe ovaries were yellow / deep yellow and occupied about 90% of the body cavity. The ova were clearly seen through the wall of the ovary. Oocytes were transparent. The ova diameter ranged from 0.99 to 1.98 mm.

After spawning, the ovaries became reduced in size and dull colored. After a short spent phase the ovaries regained their original whitish color and strap shape rapidly. This condition was observed in the matured females form December to April. Similar conditions were reported by several authors in their studies on ovarian development in other *Teleosts* (e.g. Foucher and Beamish, 1977, 1980; Taylor and DiMichelle, 1980; Treasurer and Holliday, 1981; Robb, 1982) which breed once in a year like *C. carpio* var. *specularis*.

Reproductive cycle

The time and season when a species normally breed is termed as the "breeding" or "spawning" season of that species. The breeding season repeats in cyclic order, in which the organism undergoes maturation changes and thereby gets ready to breed again. This repeated phenomenon is known as reproductive cycle (Milton and Arthington, 1983).

Reproductive behaviour in most animals is cyclic- more or less regularly periodic; this is so far nearly for all fishes. The reproductive act in some fishes occurs only once in a very short lifetime, while in other fishes, it occurs once in a moderately long life span. Several other species spawn more than once in a year more or less continually (Lagler *et al.,* 1977). After spawning, new group of oocyte is formed which gradually mature to become ready for the next season (Khanna, 1978).

The reproductive cycle in fishes and shellfishes is guided by several environmental factors together with the endocrine activities. Both light and temperature are important factors controlling the maturation of gonads in fishes. Variation in the duration of light and temperature may influence the rate of gonadal development. Most of the stages of reproduction often depend on certain conditions of water movement, e.g. for the liberation of spermatozoa and egg fertilization (Kinne, 1971).

Seasonal changes in the ovaries of teleosts have been studied histologically as well as measuring ova diameter and with the help of gonado-somatic index (Khanna, 1978).

Stephenson (1934) observed four types of reproductive cycle in the animals of tropical water which are:

i. Annual reproductive cycle
ii. More than one reproductive cycle in a year
iii. Continuous breeder and which breeds throughout the year
iv. Discontinuous breeder.

The reproductive activities of most aquatic animals in tropical and sub-tropical areas where environment is relatively uniform throughout the year are controlled at least in part by temperature and length of day light (Hoque and Ahmed, 1993). deVlaming (1972) concluded that temperature appeared to be extremely important in regulating reproductive cycle of cypriniformes. For the enhancement of gametogenesis leading to maturity and spawning, the role of temperature is important (Asahina and Hanyu, 1983; Summers, 1996). However, photoperiod appeared to be more critical than temperature in determine the timing of the reproductive cycle in *Gambusia affinis* (Milton and Arthington, 1983). After heavy shower during monsoon period (late May to early August) some physico-chemical changes in the water bodies probably stimulate the reproductive activities of Indian major carps (Jhingran, 1983). According to Weber and Brown (2009), *C. carpio* prefers calm and shallow waters, such as flooded grasslands, to spawn. The reproductive cycle and pattern of gonadal development of *C. carpio* in natural ecosystems greatly depends on the ambient temperature. Spawning occurs at a water temperature of around 18°C (Billard and Breton 1978).

In the present investigation *C. carpio* var. *specularies* was found to breed during November to April during winter which decrease the water temperature, increase the dissolve oxygen (DO), and also form a unique fresh environment.

The annual reproductive cycle of marine and freshwater invertebrates could be determined by different methods such as:

(i) Percentage of ovigerous female against time: (Pollock, 1985 in *A. australis*; Nabi, 1987 in *M. aculeatus*; Afroze and Hossain, 1990 in *A. mola*; Hossain *et al.*, 1991a in *N. nandus*; Hossain *et al.*, 1992b in *O. pabda*; Nargis and Hossain, 1992 in *A. testudineus*; Parween *et al.*, 1993 in *E. danricus*; Alam *et al.*, 1996 in *S. phasa*; Bhuiyan *et al.*, 1999a in *C. atherinoides;* Hussain and Hossain, 2001 in *M. pancalus;* Martin *et al.*, 2005 in *P. bergi;* Juchno *et al.*, 2007 in *C. taenia*; Klein, 2007 in *A. imberbis;* Fazli *et al.*, 2008 in *L. aurata*).

(ii) Occurrence of larvae: (Chhapgar and Deshmukh, 1964).

(iii) Appearance of ripe gametes in the gonad (diameter of ova): (Micale *et al.*, 1987; Nabi, 1987; Hossain *et al.*, 1989; Afroze and Hossain, 1990; Hossain *et al.*, 1991a; Nargis and Hossain, 1992; Parween *et al.*, 1993; Azadi *et al.*, 1995; Hussain and Hossain, 2001; Islam *et al.*, 2001; Alp *et al.*, 2003; Caputo *et al.*, 2003; Grabowska, 2005; Sindhe and Kulkarni, 2005; Dadzie and Abou-Seedo, 2008).

(iv) Gonado somatic index (GSI): (Hossain *et al.*, 1989; Afroze and Hossain, 1990; Hossain *et al.*, 1991a; Nargis and Hossain, 1992; Hossain *et al.*, 1992b; Parween *et al.*, 1993; Alam *et al.*, 1997; Barros and Regidor, 2002; Mabragana *et al.*, 2002; Abou-Seedo *et al.*, 2003; Sindhe and Kulkarni, 2004; Kennedy *et al.*, 2006; Santic *et al.*, 2006; Ferriz *et al.*, 2007; Glamuzina *et al.*, 2007).

(v) Chemical composition of the gonad and also other organs related to reproduction: (Chandran, 1968 in *Charybdis variegata*; Diwan and Nagabhushanam, 1974 in *Barytelphus cancicularis*).

(vi) Relative condition factor (K_n): (Mallikarjuna, 1966 in *Macrobrachium rosenbergii*; Hossain, 1976 in *Thenus orientalis*; Hossain and Parween, 1982 in *Macrobrachium dayanum*; Nash, 1982 in *Leuseurigolaius friessi*; Nabi, 1987 in *Macrognathus aculeatus;* Karmakar, 1998 in *N. chitala*).

(vii) Integrated method of analyzing the immature, maturing, mature and spent, ovigerous with respect to virgin and copulated adult female (Haley, 1972 in *Ocypode quadrata*).

(viii) Maximum indices of abundance of spermatozoa; (Heydorn, 1965 in *Jasus lalandii*; Hossain, 1976 in *T. orientalis*; Nabi, 1987 in *M. aculeatus*; Alam *et al.,* 1997 in *S. phasa*; Hussain and Hossain, 2001 in *M. pancalus*).

(ix) Maximum egg bearing capacity: (Shyamasundari, 1972 in *C. triaenonyx*; Hossain, 1976 in *T. orientalis*; Afroze and Hossain, 1990 in *A. mola*).

(x) Gonadal length index (GLI): (Nabi, 1987; Hossain *et al.,* 1989; Afroze and Hossain, 1990; Hossain *et al.,* 1991a; Hossain *et al.,* 1992b; Nargis and Hossain, 1992; Parween *et al.,* 1993; Alam *et al.,* 1997; Hussain and Hossain, 2001; Islam *et al.,* 2001; Barros and Regidor, 2002; Mabragana *et al.,* 2002; Abou-Seedo *et al.,* 2003; Sindhe and Kulkarni, 2004; Kennedy *et al.,* 2006; Santic *et al.,* 2006; Ferriz *et al.,* 2007; Glamuzina *et al.,* 2007).

(xi) Colouration of gonad (ovary): (Nabi, 1987; Hossain *et al.,* 1989; Afroze and Hossain, 1990; Hossain *et al.,* 1991a; Hossain *et al.,* 1992b; Nargis and Hossain, 1992; Parween *et al.,* 1993; Alam *et al.,* 1997; Hussain and Hossain, 2001; Islam *et al.,* 2001; Barros and Regidor, 2002; Mabragana *et al.,* 2002; Abou-Seedo *et al.,* 2003; Sindhe and Kulkarni, 2004; Kennedy *et al.,* 2006; Santic *et al.,* 2006; Ferriz *et al.,* 2007; Glamuzina *et al.,* 2007).

(xii) Histology and histochemical aids: (Hossain, 1976; Dalela *et al.,* 1977; Nabi, 1987).

(xiii) Neurosecretory activity of certain NS-cells. (Hossain, 1976 in *T. orientalis*).

(xiv) Isolation of adult female (condition favourable or ecologically good for larvae) from natural population or normal ecological condition (Boolootian, 1965 in *Pachygrapsus crassipes*).

(xv) Activity of the cement gland (Hossain, 1976 in *T. orientalis*).

(xvi) Structure and nature of oosetae (Hossain, 1976 in *T. orientalis*).

(xvii) Structural modification of secondary sexual characters in fin fishes (Hossain, 1989).

From the aforementioned methods, only five (i, iii, iv, x, xi) were applied in the present study to determine the breeding cycle of *C. carpio* var. *specularis*.

The methods are:

1. Percentage of ovigerous female against time.
2. Appearance of ripe gametes in the gonad (diameter of ova).
3. Gonado somatic index (GSI).
4. Gonadal length index (GLI).
5. Colouration of gonad (ovary).

All the five methods showed that *C. carpio* var. *specularis* naturally breed once in a year which from November to April in the habitat of the Atrai river in favourable condition. Similar results have been obtained in different fishes e.g. Alikunhi (1966) found in the wild condition of *C. carpio* breed once in a year. Hussain and Hossain (2001) in *M. pancalus*; Abou-Seedo *et al.,* (2003) in *A. latus*; (Piedras *et al.,* 2006 and Garcia *et al.,* 2008) in *C. carpio* var. *communis*; (Vrneij and Dudley, 2000, Smith and Walker, 2004, Tempero *et al.,* 2006) in common carp; Koc *et al.,* (2007) in *L. Cephalus*; Dadzie *et al.,* (2008) in *Parastromateus niger*.

Several similar results were also reported by Hossain *et al.,* 2002 in *Gonialosa manminna* (January to June); Alp *et al.,* 2003 in *Salmo trutta macrostigma* (November to January); Pajuelo *et al.,* 2006b in *Pagrus auriga* (September to February); Fazli *et al.,* 2008 in *Liza aurata* (October to December); Filer and Sedberry, 2008 in *Hyperoglyphe perciformis* (September to May); Oker *et al.,* 2008 in *Uranoscopus scaber* (September to March). Zanotta *et al.,* 2010 and Debora *et al.,* 2012 in *Cyprinus carpio* var. *communis* (October to April).

Similar result also reported in the same species, (Karim, 1975 and Mohsin, 2010) in *C. carpio* var. *specularis* (December to April).

Out of all these methods, gonadal length index (GLI) was the most suitable method for this species. The breeding period mainly from November to April with the peak in February.

Fecundity

A perfect knowledge on the fecundity of fishes is essential for evaluating the commercial potentialities of its stock, for study of life history, practical culture and actual management of its fishery (Lagler, 1966; Hossain, *et al.,* 1997).

Fecundity appears to bear some relationship to the case according to the eggs (Lagler *et al.*, 1977). For example in poorly fecund fish parental care is highly developed. So, to make the population constant, fecundity does not fluctuate widely due to low mortality rate and predator. Again the fishes with high fecundity show great fluctuation in their abundance. They do not take care of their eggs; the eggs are subjected to several injuries due to excessive temperature, parasitic and predatory attack etc. After spawning most of them do not survive up to hatching.

The study of fecundity of any species is important to have a full understanding of its biology and population dynamics. Study on fecundity is also undertaken to determine the index of density dependent factor affecting the population size (Simpson, 1951; Das, 1977). The fecundity of an individual female varies according to many factors including her egg size, species, food availability, water temperature and season (Lagler *et al.*, 1977). But the estimation of fecundity on the basis of number of ova are not purely accurate (Farmer, 1974), because of the following drawbacks:

i) Not all the ova contained in an ovary is released,

ii) Some of the ova are re-absorbed in the ovary, which are known as atretic oocytes (Chubb and Potter, 1984) and

iii) Constituents of the germinal stand which is present at all the time are retained egg laying

In the present investigation a marked increase in the fecundity was noticed with the increase in total length, standard length, total weight, ovary length and ovary weight, which indicate that the number of eggs increased linearly with the increase of these variables. All the relationships showing values of the regression coefficient were highly significant ($P<0.001$). Similar results were also reported from different fishes worked out by a number of scientists.

A comparison of correlation co-efficient of fecundity-total length ($r=0.9129$), fecundity-standard length ($r=0.9319$), fecundity-total weight ($r=0.9519$), fecundity-gonadal length ($r=0.9121$) and fecundity-gonadal weight ($r=0.9948$) indicated that variation in fecundity could be better in terms of total weight and gonadal weight than other terms. But this result is disagreed with Ahmed *et al.,* (1979) in *C. batrachus*. They found that relationship between fecundity and gonadal weight could not be obtained an exact linear relationship, which might be due to the fact that the ova were not uniform in size. However in case of *C. carpio* var. *specularis* the ova were more uniform in size than previous observation as evident from relationship between gonadal weight and fecundity.

In *C. carpio* var. *specularis*, the fecundity minimum 5928.91 and maximum 209120.18 with mean ± SD 87377.98 ± 79544.17 which revealed that the species was high fecund. The number of eggs produced by a female is dependent on various factors such as size, age and condition (food availability, water temperature, and season) (Lagler *et al.,* 1977). Considering the individual fecundity of the species, it has been found that the same sized fish had different number of eggs in their ovaries. It is also observed that in some cases, fecundity of a fish of larger size is much less than that of a fish of smaller size. Individual physiology of the fishes and their surroundings may be the controlling factors for such variation (Hossain *et al.,* 1992b). This type of variation was also common in other fishes e.g., *Xenentodon cancila* (Bhuiyan and Islam, 1990); *Puntius stigma* (Islam and Hossain, 1990); *A. coila* (Alam *et al.,* 1994b); *S. phasa* (Alam *et al.,* 1997); *P. sophore* (Bhuiyan and Parveen, 1998); *C. idella* (Mustafa *et al.,* 1998); *B. gonionotus* (Bhuiyan *et al.,* 2000); *Gobius roulei* (Kovacic, 2001); *C. striatus* (Latifa *et al.,* 2002); *Salmo trutta macrostigma* (Alp *et al.,* 2003); *Crystallogobius linearis* (Caputo *et al.,* 2003); *M. holotrachys* (Morley *et al.,* 2004); *Siganus luridus* (Azzurro *et al.,* 2007); *Pseudocorynopoma doriai* (Ferriz *et al.,* 2007). This is perhaps owing to the variations in the size and heterogeneous distribution of the ova.

Several similar result were also reported as Alikunhi (1966) *C. carpio;* (Bhuiyan and Akhter, 2002) *C. carpio* var *communies*; (Dadzie *et al.,* 2008) *Parastromateus niger;* (Fazli *et al.,* 2008) *Liza aurata;* (Oker *et al.,* 2008) *Uranoscopus scaber*; (Mathewos, 2013) *C. carpio* var. *communies*; (Jan *et al.,* 2014) *Schizothorax plageostomus;* (Bhattacharya *et al.,* 2015) *Ompok Pabo*. During the estimation of fecundity it was found the present study that that *C. carpio* var. *specularies* spawns once in a year mainly from November to April with the peck in February in the study area the Atrai river when stay in favourable condition of the species.

The different data were statistically analysed. Fecundity is an important aspect of fish biology. The knowledge of fecundity is essential for management of the fish population cryopreservation of eggs, fish breeding, larval rearing etc., which are necessary for undertaking conservational measures.

Sex ratio

Knowledge of the sex ratio of a population was considered essential in the management of a fishery as it will be necessary to devise means of ensuring a proportional fishing of the two sexes. The sex-ratio of *C. carpio* var. *specularis* for 512 males and 572 females are shown in monthwise distribution (App. Table 5).

The total male and female ratio was 1:1.12 the female number was more than male. The females were predominant during the months of November to July of each year. While the males were predominant in the rest of the months. The chi-square test showed that the males and females distribution in the natural population was significantly different from the expected ratio. Similar type of results was also reported by (Islam and Hossain, 1990; Bhuiyan *et al.*, 1993; Bhuiyan and Afrose, 1996; Alam *et al.*, 1997; Bhuiyan *et al.*, 2000; Bhuiyan and Aktar, 2002 and Mathewos, 2013).

However, it is not clear that which factor might be responsible in the fluctuation of the male and the female population distribution of *C. carpio* var. *specularis*.

CHAPTER-5

BIOCHEMICAL COMPOSITION

INTRODUCTION

Good health is achieved by eating the right kinds and amount of food and vegetables. Well-balanced human food and vegetables should contain adequate amounts of nutrients, the shortage of which leads to malnutrition (Morris *et al.,* 1993). A large number of populations in Bangladesh are suffering from malnutrition. There are many kinds of consumable food in Bangladesh but all of these do not contain equal amount and same kind of nutrients. For the ignorance of people, they do not know the nutritive value of different kinds of foods. Soft water fishes are the most important sources of fish in Bangladesh. These fishes are either wild or farmed.

The annual fish production in Bangladesh was 36,84,245 metric tons and fish contributed about 60% to the nation's animal protein intake (Islam *et al.,* 2012; Minar *et al.*, 2012 and DoF, 2016). Fish is an essential and irreplaceable food item in the rural Bangladeshi diet. Fish body composed of mainly water, lipid, ash and protein though small amounts carbohydrates and non-protein compounds are present in a small amount (Love, 1980; Cui and Wootton, 1988; Wootton, 1990; Siddique *et al.,* 2012 and Azim *et al.,* 2012). Most of fish usually consists of water (70-80%), protein (20-30%) and 2-12% of lipid (Love, 1980; Ali *et al.*, 2005). But it may change within and between spacies and also with size, sexual condition, feeding, time of the year, seasonal change and physical activity (Weatherley and Gill, 1987).

Being an non migratory fish who remains in one habitat throughout its life (Mathur and Robbins, 1971; Mathur, 1973). Earlier reports stated that the fish was commonly distributed throughout the rivers, haors, baors, beels, jheels, canals and ponds of Bangladesh (Rahman, 1989 and Bhuiyan *et al.*, 1992).

A variety of factors such as food, space, temperature, salinity, physical activity influence the growth of fish (Weatherley and Gill, 1987; Ahmed *et al.*, 2012) and the fish body elements may change due to these factors (Kamal *et al.*, 2007).

The importance of fish as a rich source of animal protein is well established and this is frequently used to justify fish as a valuable food, whereas very little attention has been given to the role of fish in supplying vitamin A and minerals in the diet (Darntorn-Hill *et al.*, 1988).

From the national survey in rural Bangladesh, the mean total protein intake was 48 g/person/d, of which fish contributed 3g (Ahmed and Hosson, 1983). The value of fish in the Bangladeshi diet should not focus on the contribution made to protein, because protein recommendations in the tropical diet are met provided that the energy recommendations are met (Roos, 2001). Rather, focus should be placed on the composition of the fish and the contribution of micronutrients, especially vitamin A and minerals, from the different types of fish species. The variation in vitamin A content in fish species is extreme (Roos *et al.*, 2002).

The mineral content of fish, unlike its vitamin A content, is apparently not species specific. Small fish are generally eaten with bones, although some bones may be discarded as plate waste, whereas in large fish most or all bones are discarded as plate waste. Therefore, small fish are an excellent source of calcium. In studies with both humans and rats it is shown that the bioavailability of calcium from whole small fish (mola) is as high as that from milk. In humans, the fractional calcium absorption is found to be 24 ± 6% from small fish and 22 ± 6% from milk (Larsen, *et al.*, 2000).

Lipid is an important constituent of all type of cell. The cell membrane consists of three classes of amphipathic lipids: phospholipids, glycolipids, and cholesterols. The amount of each depends upon the type of cell, but in the majority of cases phospholipids are the most abundant (Lodish *et al.*, 2004).

Fish oil is derived from the tissues of oily fish. Fish and fish oils contain long-chain polyunsaturated omega-3 fatty acids, more specifically, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kris-Etherton *et al.*, 2002).

Fish oil play vital role in remediation of various diseases. Fish oil is a protective mean of various types of abnormalities such as heart diseases, diabetes mellitus, atherosclerosis, various types of cancers, inflammation, hypertension, obesity rheumatoid arthritis, osteoporosis, schizophrenia, etc. In addition EPA and DHA in fish oil can also combat some diseases such as asthma, multiple sclerosis, systemic lupus erythematous etc. Studies have shown that omega-3 fatty acids can help improve asthma in children and increase survival rate in cancer patients (Iso H *et al.,* 2001 and Nagakura *et al.,* 2000). Fish oil can also fill nutritional gaps and increase weight in cancer patients. Low levels of DHA have been linked to depression; people who have supplemented omega-3 fish oil have noticed an increase in mood and relief from symptoms of depression. Omega-3 fish oil may even help with mental illnesses like bipolar disorders. One study suggests that fish oil can help with rheumatoid arthritis (Volker *et al.,* 2000).

Successful antiplatelet and anticoagulant therapies are often accompanied by an increased risk of bleeding. Fish and fish oil supplements, although generally well tolerated, have as their main adverse side effect a mild gastrointestinal discomfort, appearing as a fishy aftertaste, belching, nausea, flatulence or loose stools. Published fish oil intervention studies with healthy subjects do not provide indications for increased bleeding, even after a daily intake of 6g (n-3) PUFA. Various papers explicitly mention the absence of easy bruising or clinical signs of (postoperative) bleeding after fish oil intake by patients with cardiovascular disease (Wojenski *et al.,* 1991; Roulet *et al.,* 1997; Landmark and Aursnes, 2004) Positive interactions between (n-3) PUFA intake and oral anticoagulants have been noted, but these appear to occur in the absence of clinically relevant bleeding (De Caterina, *et al.,* 1990). There is only an incidental report of a patient with minor nasal bleeding after a fish oil diet with concomitant anticoagulant therapy (Eritsland, *et al.,* 1995). In another study, fatal coagulopathy occurred in a patient after abdominal aortic aneurysm resection, but here a causal relationship of fish oil supplementation and the bleeding diathesis was reported to be uncertain (Smith, *et al.,* 1989).

Only the Zutphen epidemiologic study, investigating the association between fish consumption and stroke incidence in the Netherlands, concluded that large amounts of seafood may increase hemorrhagic subtypes of stroke, whereas the consumption of only small amounts of fish reduce the incidence of ischemic stroke (Bowles, *et al.,* 1991). Vasodilation is another reported effect (usually advantageous) of (n-3) PUFA on the vascular system. However, a meta-analysis of clinical trials showed that the vasoligating effect was most pronounced in hypertensive patients and had little effect on healthy normotensive people (Keli *et al.,* 1994). Thus, from the current literature, there seems to be little reason to be concerned about disadvantageous effects, at least when fish oil is not combined with anticoagulant treatment.

Many studies have been on the biochemical and nutritional studies of some freshwater fish, prawn and shrimp species of Bangladesh (Kamaluddin *et al.,* 1977; Gheyasuddin *et al.,* 1979; Rubbi *et al.,* 1987; Chakrabarty *et al.,* 2003; Mansur *et al.*, 2004; Kamal *et al.*, 2007; Naser *et al.*, 2007; Mohajira, *et al.*, 2012; Mahfuj, *et al.,* 2012; Ali, 2014;). But no attempt has been found hence forth to determine the biochemical composition of exotic fish like *Cyprinus carpio* var. *specularis* (Mirror carp) in Bangladesh. More over this fish like other fish contribute to fulfill the nutrient demand of the people of Bangladesh.

Therefore the present study was under taken to know the biochemical composition of *C. carpio* var. *specularis* with the following objectives:

i) To study the fish species that contain health favorable nutrient compositions.

ii) To compare biochemical composition between different sexes and seasons.

MATERIALS AND METHODS

Sample collection

Samples of *C. carpio* var. *specularis* fishes were collected from the different landing center in Atrai river of north western (Naogaon District) Bangladesh and the study was conducted from a period of January, 2014 to December, 2014 (12 months). The specimens were collected during the early hours of the day and carried in fresh condition to the laboratory in ice box. Besides, the specimens were properly cleaned and preserved in the deep freezer before analysis. Fishes were selected randomly regardless of sex.

Experimental place

The whole experiment was conducted at the Protein and Enzyme Research Laboratory, Department of Biochemistry and Molecular Biology, University of Rajshahi, Bangladesh and The Fish Nutrition Laboratory, Department of Aquaculture, Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Parameter estimation

The following observations were made for assessment of biochemical composition of fish species:

Determination of moisture content of *C. carpio* var. *specularis*

Moisture content was determined by the following method of AOAC, (1990).

Materials:

a) Porcelain crucible

b) Electrical balance

c) Oven

d) Desiccator

Procedure:

5-6 grams of *C. carpio* var. *specularis* fishes were weighed in a porcelain crucible (which was previously cleaned, heated to about 100°C, cooled and weighted).

The crucible with the sample was heated in an electrical oven for about six hours at 100°C. It was then cooled in desiccators and weighted again.

Calculation:

Percent of moisture content (g per 100 g of *C. carpio* var. *specularis* fish)

$$= \frac{\text{Weight of moisture}}{\text{Weight of the sample}} \times 100$$

Determination of ash content of *C. carpio* var. *specularis*:

Ash content was determined by the following method of AOAC, (1990).

Materials:

a) Porcelain crucible

b) Muffle furnace

c) Electrical balance

d) Desiccator

Procedure:

5-6 g of *C. carpio* var. *specularis* fishes were weighed in a porcelain crucible (which was previously cleaned, heated to about 100C°, cooled and weighed). The crucible with its content was placed in a muffle furnace for four hours at about 600°C. It was then cooled in a desiccator and weighed again. To ensure the completion of ashing, the crucible was again heated in the muffle furnace for half an hour, cooled and weighed again. This was repeated till two consecutive weights became same and the ash was almost white in color.

Calculation:

Percent of ash content (g per 100 g of *C. carpio* var. *specularis* fish)

$$= \frac{\text{Weight of ash obtained}}{\text{Weight of the sample}} \times 100$$

Determination of total lipid content of *C. carpio* var. *specularis*.

Lipid content of *C. carpio* var. *specularis* fishes determined by the method of Bligh and Dyer, (1959).

Reagent

A mixture of chloroform and ethanol (2:1V/V).

Procedure

About 5 g of *C. carpio* var. *specularis* fishes were first grinded in a morter and pastle with about 10 ml of distill water. The grinded flesh was transferred to a separating funnel and 30 ml of chloroform-ethanol mixture was added. The mixture was mixed well. It was then kept overnight at room temperature in the dark. At the end of this period 20 ml of chloroform and 20 ml of water were further added and mixed. Generally three layers were seen. A clear lower layer of chloroform containing the entire lipid, a colored aqueous layer of ethanol with all water soluble materials and a thick pasty interphase were seen.

The chloroform layer was carefully collected in a pre-weighed beaker (50 ml) and then placed on a steam bath for evaporation. After evaporation of the chloroform, the weight of the beaker was determined again. The difference in weight gives the amount of the lipid.

Calculation

Percent of lipid content (g per 100 g of *C. carpio* var. *specularis* fish).

$$= \frac{\text{Weight of lipid obtained}}{\text{Weight of the sample}} \times 100$$

Determination of water-soluble protein content of *C. carpio* var. *specularis*

Water soluble protein content of *C. carpio* var. *specularis* fishes were determined following the method of Lowry *et at*., (1951). The extraction was carried out with distilled water.

Reagents

(a) 2%Na_2CO_3 solution in 0.1N NaOH

(b) 0.5 copper sulfate in 1% sodium-potassium tartarate.

(c) Folin-Ciocaltean reagent (FCR): (Diluted with equal volume of H_2O, just before use).

(d) Protein standard: 150mg/100 ml in water.

Procedure

1) Mix reagents (a) and (b) in the ratio 50:1 and dilute reagent (c) just before use.

2) Eight glass test tubes, 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.8 ml of the standard protein solution, respectively were taken and the volume was made up to 1 ml by distilled water. The sample was transferred to a 50 ml. volumetric flask and the volume was made up to the mark by distilled water. Water was carefully added avoiding formation of emulsion. 1 ml of the sample was taken in a test tube and a duplicate was made. To each of the tubes 5.0 ml of (a:b) mixture was added and after 10 minutes 0.5 ml (FRC) solution was added. Absorbances of the solutions were recorded after 30 mins at 650 nm. A graph was drawn with the data obtained from the standards and the amount of protein in the sample was calculated from the graph (Fig. 5.1).

Calculation:

Percent of protein content (g per 100 g of *C. carpio* var. *specularis* fish).

$$= \frac{\text{Weight of water soluble protein}}{\text{Weight of the sample}} \times 100$$

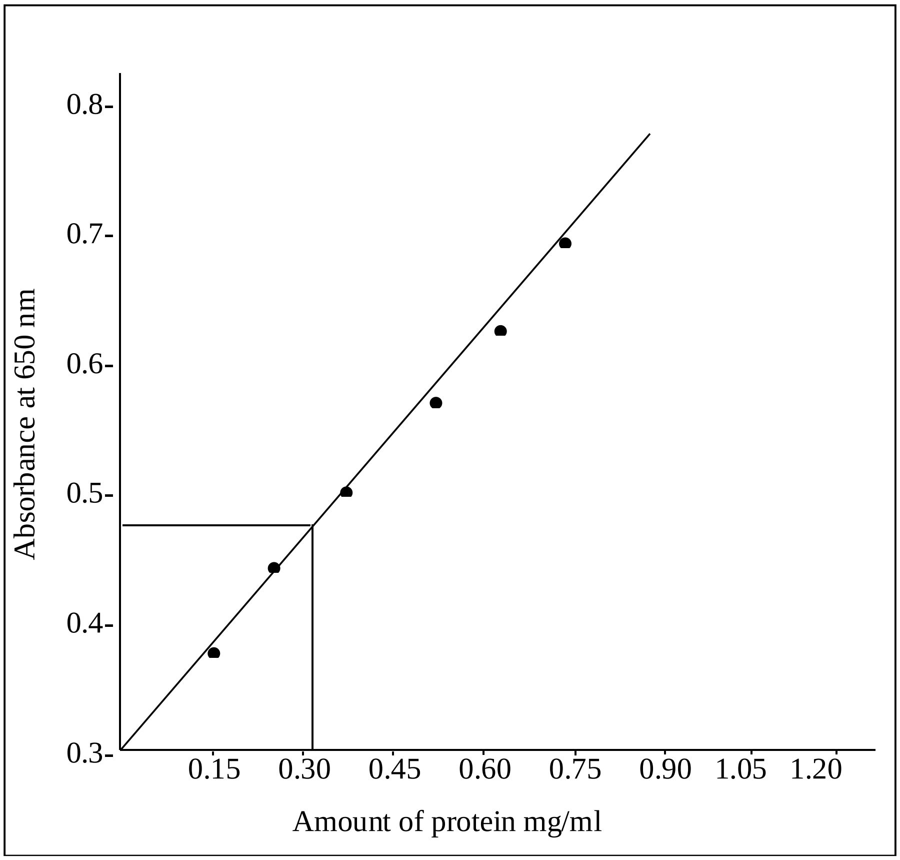


Fig. 5.1: Standard curve of protein for the estimation of water soluble protein.

Determination of free /total sugar content (Carbohydrate) of *C. carpio* var. *specularis*:

The sugar content of *C. carpio* var. *specularis* fishes were determined calorimetrically by the following anthrone method of Loomis and Shall, (1937).

Reagents:

a. Anthrone reagent: 0.2% in Conc. H_2SO_4.

b. Standard glucose solution: 10mg/ 100 ml distilled water.

Extraction of sugar from *C. carpio* var. *specularis*:

Extraction of sugar from *C. carpio* var. *specularis* fishes were done by the following method as described by Loomis and Shall, 1937.

4-6 g of *C. carpio* var. *specularis* fishes were plunged into boiling ethyl alcohol and allowed to boil for 5-10 minutes (5 to 10 ml of alcohol was used for each g of *C. carpio* var. *specularis*) fishes. The extract was cooled and crushed thoroughly in a mortar with a pestle. Then the extract was filtered through two layers of muslin cloth and re-extracted the ground fish for three minutes in hot 80 percent alcohol, using 2 to 3 ml of alcohol for each g of fish sample. This second extraction ensured complete removal of alcohol soluble substances. The extract was cooled and passed through muslin cloth. Both the extracts were filtered through Whatmann no-41 filter paper. The volume of the extract was evaporated to about $^1/_4$ of the volume over a steam bath and cooled. This reduced volume of the extract was then transferred to a 100 ml volumetric flask and made up to the mark with distilled water. Then 1 ml of diluted solution was taken into another 100 ml volumetric flask and made up to the mark with distilled water (working standard).

Procedure:

Aliqout of 1 ml of the fish extract from each part was pipetted into different test tubes and 4 ml of the anthrone reagent was added to each of this solution and mixed well. Glass marbles were placed on the top of each to prevent loss of water bath and then cooled. A reagent blank was prepared by taking 1 ml of water and 4 ml of anthrone reagent in a tube and treated similarly. The absorbance of the blue-green solution was measured at 680 nm in a colorimeter.

A standard curve of glucose was prepared by taking 0.0, 0.1, 0.2, 0.3, 0.4, 0.5 0.6, 0.8 and 1 ml of standard glucose solution in different test tubes containing 0.0, 0.01 mg, 0.02 mg, 0.03 mg, 0.04 mg, 0.05, 0.06, 0.08 and 0.1 mg of glucose respectively and made the volume up to 1 ml with distilled water. Then 4 ml of anthrone reagent was added to each test tube and mixed well. All these solutions were treated similarly as described above. The absorbance was measured at 680 nm using the blank containing 1 ml of water 4 ml of anthrone reagent.

The amount of free sugar was calculated from the standard curve of glucose (Fig. 5.2). Finally, the percentage of free sugar present in the *C. carpio* var. *specularis* fishes were determined using the formula given below:

Percentage of free sugar (g per 100 g of *C. carpio* var. *specularis* fish).

$$= \frac{\text{Weight of sugar}}{\text{Weight of the sample}} \times 100\%$$

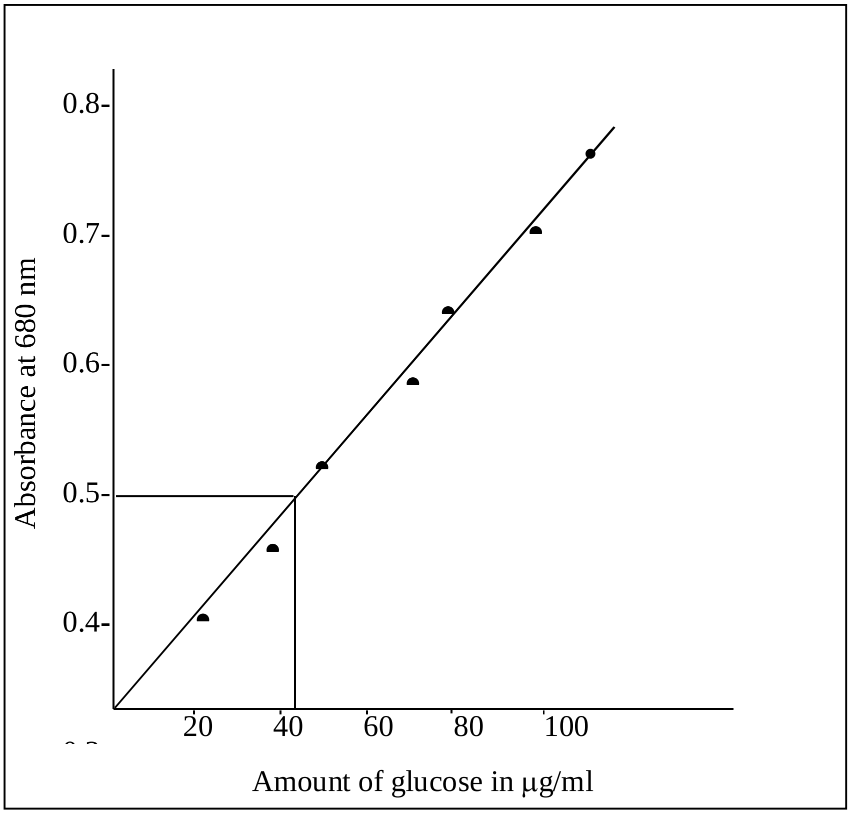


Fig. 5.2: Curve for the determination of free sugar

Statistical analysis

The data were collected from the experiment was tabulated and the final result was prepared by using both MS Excel and SPPS 20 version computer based software.

Plate 5.1(a-f): Showing the different activity to estimation the biochemical composition of *C. carpio* var. *specularis* in research laboratory.

Plate 5.2(g-m): Showing the different activity to estimation the biochemical composition of *C. carpio* var. *specularis* in research laboratory.

RESULTS AND OBSERVATIONS

The present study deals with the biochemical composition (moisture, ash, lipid, protein and carbohydrate) of freshwater fish *C. carpio* var. *specularis* with relationship to its seasons and sexes. Generally the body composition of fish seems to depend on age, sex, seasons and diet (FAO, 1995). In the present investigation the biochemical composition of *C. carpio* var. *specularis* was carried out to know the food value of the species. The research work indicates variation in biochemical composition on the basis of sex and season.

Sex and seasonal variation in biochemical composition of *C. carpio* var. *specularis* as follows:

Moisture

In the present investigation, the moisture content was found as one of the major component. The moisture content varied from season to season as well as in case of sexes. The male fishes carried moisture 72.54±0.551% in summer whereas at winter season it rises up to 74.53±1.078%. In case of female fishes, at summer, rainy and winter season the moisture was found 73.32±0.778, 74.13±1.146 and 75.42±0.538% respectively (Fig. 5.3, App. Table 7). The moisture content was respectively high in female at winter season and low in male at summer season. It was observed that the moisture content of both sexes were relatively low at summer season and higher moisture content recorded at winter season. Moisture content was significantly different (P <0.05) in summer and winter season whereas there was no significant difference was found in rainy season.

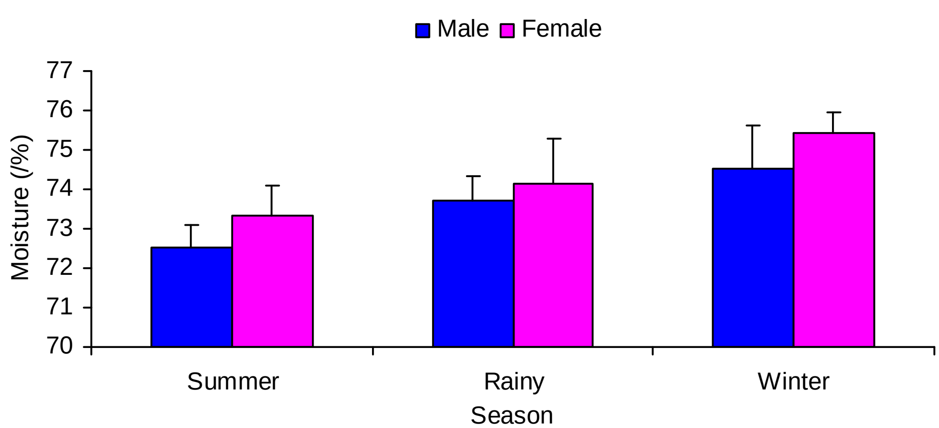


Fig. 5.3: Seasonal variation in biochemical composition of Moisture (%) content of *C. carpio* var. *specularis* in both male and female fishes.

Ash

Ash content at summer, rainy and winter season of male fishes were recorded 3.02±0.637, 3.13±0.423, 3.44±0.536% and female fishes the values were 2.64±0.393, 3.41±0.630 and 3.77±0.766% respectively (Fig. 5.4, App. Table 7). Highest and lowest ash content was found in winter and summer season at both male and female fishes. No significant difference ($P < 0.05$) was found in ash content of both male and female fishes.

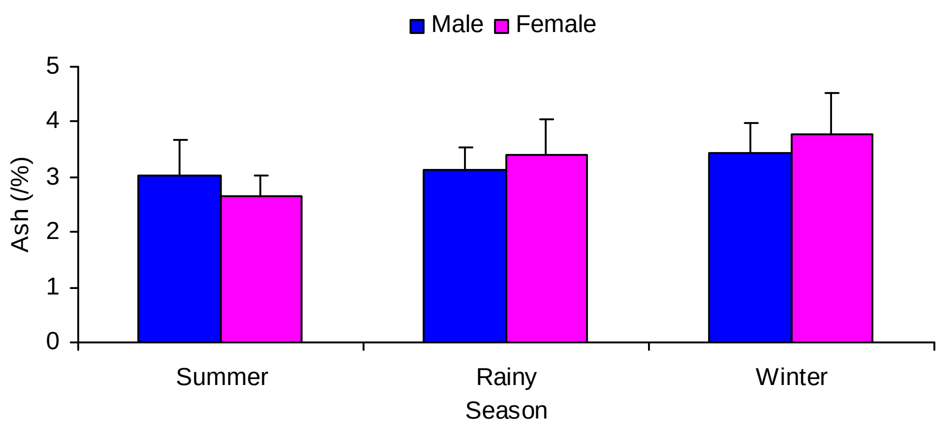


Fig. 5.4: Seasonal variation in biochemical composition of Ash (%) content of *C. carpio* var. *specularis* in both male and female fishes.

Lipid

The mean value of lipid was found 4.19±0.231, 3.80±0.362, 3.36±0.400% in male fishes and 4.08±0.400, 3.58±0.684 and 2.95±0.349% in female fishes at summer, rainy and winter season respectively (Fig. 5.5, App. Table 7). Maximum lipid content was (4.19±0.231%) found in male fishes at summer and minimum was (2.95±0.349%) recorded in female fishes at winter season. The lipid content showed decreasing trend from summer to winter season (Fig. 5.5). In both male and female fishes lipid content significantly different ($P < 0.05$) among summer and winter season (App. Table 7).

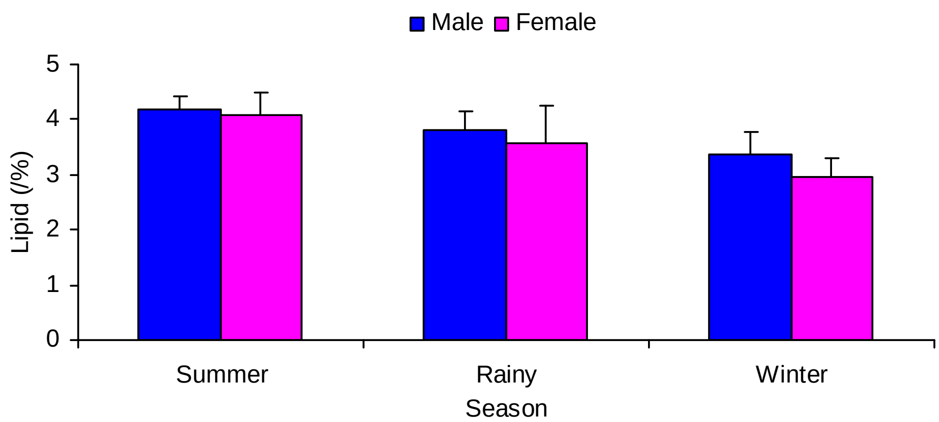


Fig. 5.5: Seasonal variation in biochemical composition of lipid (%) content of *C. carpio* var. *specularis* in both male and female fishes.

Protein

The average protein content for male fishes at summer, rainy and winter season was 20.07±0.190, 18.71±1.079, 18.25±1.189 % and for female fishes it was found 19.56±0.538, 17.99±1.226 and 17.03±0.925% respectively (Fig. 5.6, App. Table 7). The highest protein content was found in male (20.07±0.190%) at summer season and the lowest was found in female (17.03±0.925%) of winter season. In case of both sexes the protein content reached at its higher value in summer season and lower values was obtained at winter season. No significant differences ($P < 0.05$) were found in case of both sexes in summer and rainy season but significant differences were observed among both males and females protein content at winter season (App. Table 7).

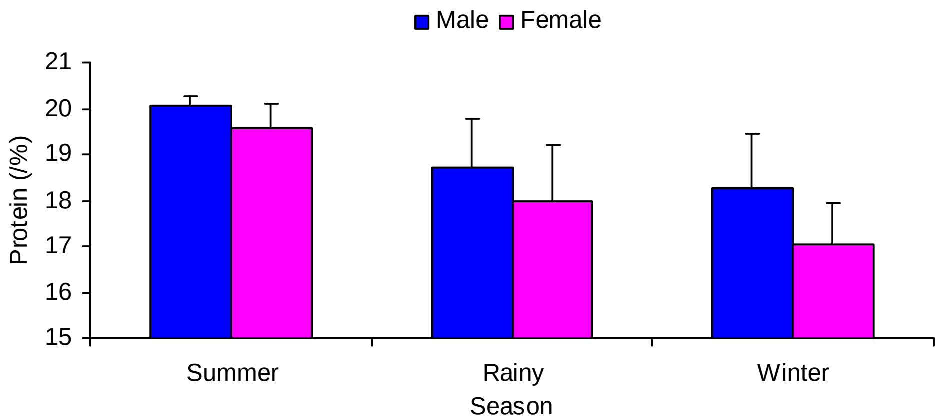


Fig. 5.6: Seasonal variation in biochemical composition of Proten (%) content of *C. carpio* var. *specularis* in both male and female fishes.

Carbohydrate

Carbohydrate content was lowest among all food nutrients. The amount of carbohydrate was 0.035±0.002, 0.042±0.005, 0.040±0.001% in male fishes and 0.037±0.004, 0.041±0.005 and 0.042±0.003% in female fishes in summer, rainy and winter season respectively (Fig. 5.7, App. Table 7). The highest and lowest carbohydrate content was found in male fishes, it was highest (0.042±0.005%) at rainy season and lowest (0.035±0.002%) at summer season (App. Table 7). No significant differences ($P < 0.05$) were observed in case of both sexes in three seasons.

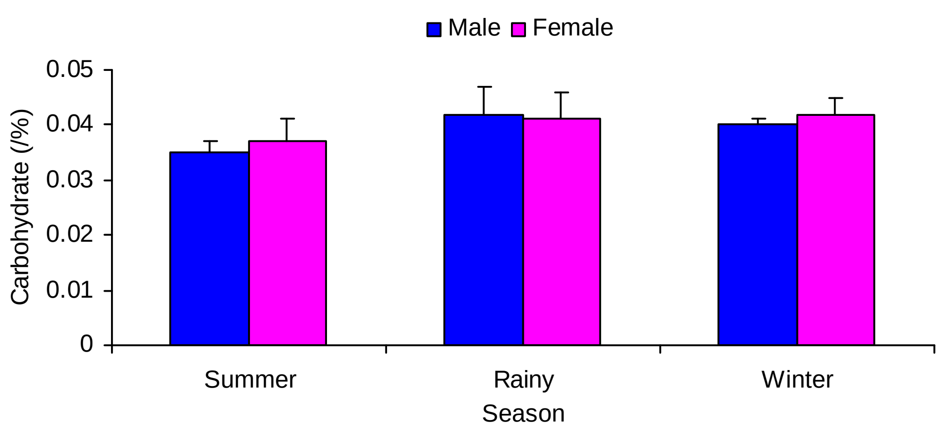


Fig. 5.7: Seasonal variation in biochemical composition of Carbohydrate (%) content of *C. carpio* var. *specularis* in both male and female fishes.

DISCUSSION

In both male and female fishes, the highest value of moisture were obtained in winter season *i.e.* at spawning period. The finding is more or less similar to other fishes as well as in other vertebrates due to maturation of gonads (Dembergs, 1964; Marais and Erasmus, 1977). The low value of moisture during certain seasons have been observed in several other fishes by various authors Nabi and Hossain (1989), Osako *et al.*, (2002) and Mahfuj *et al.*, (2012). The moisture content was lower in summer and higher in winter. The similar findings was also found in Gobi fish (Ahmed *et al*., 1977), *Anabas testudineus* (Bloch) (Nargis, 2006), *Glossogobius giuris* (Islam and Joadder, 2005). In the present study moisture content varied from 72.54 to 75.42%. Which is more or less coincide with the findings of Ali *et al.*, (2005) who found that the water content of some other fish species namely *Labeo rohita*, *Cirrhinus mrigala* and *Catla catla* was 72.10, 69.50 and 68.84%, respectively, similar findings was also found by Mansur *et al.*, (2004) who recorded the moisture content in *C. carpio* var. *communis* was 76.86%.

The fluctuation of ash content made difficult to show any relationship with the season and sex. During the present study, ash content varied from 2.64 to 3.77%. Which is more or less coincide with the findings of Chakwu and Shaba (2009) and Ali (2014) who found ash content of *C. gariepinus* and *T. alalunga* were 3.06% and 3.27% respectively.

Protein and lipid are important for growth and development of the body, maintenance and repairing of torn out tissues and for production of enzymes and hormones required for many body processes. In female fish the protein and lipid content were found lowest (17.03% and 2.95%) at winter season i.e. at spawning period of the species and highest values were found (19.56% and 4.08%) at summer season. In case of male, the protein and lipid content was higher than female in all seasons. The protein and lipid cycle appears to be having a strong correlation with feeding and spawning of female fishes reported in a number of fish species (Chakraborty *et al*., 1985). During the present study the maximum protein and lipid values recorded in male and female during summer season coincide with a period of intense feeding and pre-breeding season of this fish. This intense feeding perhaps is more in the months, i.e., immediately after spawning as the fish while spawning incurs energy expenditure along with the loss of gonadal elements and recoups to compensate the expenditure through vigorous feeding activity, Stansby (1954) observed the similar findings in certain freshwater fishes. In the present study protein and lipid content varied from (17.03 to 20.07%) and (2.95 to 4.19%) which is more or less similar to the findings of Mansur *et al.*, (2004) and Mahfuj *et al.*, (2012) who found protein and lipid contents of *C. carpio* var. *communis* (19.21% and 4.18%) and *L. bata* (18.51% and 3.79%) respectively.

In the present study carbohydrate percentage range from 0.035-0.042%. The similar findings was also found by Mahfuj *et al.,* (2012) who recorded carbohydrate content of *L. bata* was 0.045%.

The above information clearly showed that the freshwater fish Mirror carp is a high protein and lipid fish. The results indicate the seasonal variation of moisture, ash, lipid, protein and carbohydrate of *C. carpio* var. *specularis.* It was observed that the proportion of the components varied with the change of season. The seasonal variation found in females fishes greater then the males. The variation of biochemical composition in season due to breeding period and availability of food. The present findings agreed with findings of over mentioned researchers. It was also observed that the fish have a good potential to serve protein and lipid for all the people being a source of animal protein. It can play a vital role in the commercial aspects also as a source of nutrition and income generation.

CHAPTER-6

SUMMARY

Physico-chemical conditions

The physico-chemical conditions of the Atrai river exhibited more or less variations according to the change of months and seasons.

In the study area, four physical and three chemical parameters have been considered. The two years mean values of these parameters in the river Atrai stand as air temperature 28.24°C, water temperature 26.81°C, water transparency 0.45 m, rainfall 122.24 mm, pH 7.91, dissolved oxygen 6.21 mg/l and free carbon dioxide 7.00 mg/l. So form a unique fresh environment of the river area became calm.

Reproductive biology

The male and female reproductive systems of *C. carpio* var. *specularis* are described separately. At the breeding season the difference of the genitalia was used for sex differentiation of the fish, otherwise the fishes were sexed according to the primary sex characters, after dissection.

The male reproductive system of *C. carpio* var. *specularis* consists of a pair of elongated testes and its ducts. It originates in the abdominal cavity above the alimentary canal and below the kidney. The colour and size vary according to stage of sexual maturation. Gross anatomy of *C. carpio* var. *specularis* testes showed progressive enlargement from August to the spawning season in November to April. The testes change from a translucent, whitish, narrow, thread like structure to pear shaped mass of deep creamy colour. In this time milt oozes out by slight pressure on the abdomen. After spawning most of the fishes were found with flaccid testes.

The female reproductive system of *C. carpio* var. *specularis* of a paired ovaries and short oviducts. The left lobe of the ovary was always larger than the right lobe. Two lobes unite posteriorly before opening through the genital pore situated just in front of the anal fin. The shape, size and colour of the ovary vary according to the degree of maturation. The colour varies from whitish in young through whitish to brownish when maturing to brownish in ripe stage. After spawning the ovaries become reduced in size and dull in colour. On the basis of developmental stages of the ovaries are divided into ten stages: immature, maturing-I (early developing), maturing-II (late developing), mature, ripe, pre-spawning, spawning, post spawning, spent and resting stage. The ova are also divided into four stages: immature stage, maturing stage, mature stage and ripe stage.

Five methods have been employed to determine the breeding seasons of *C. carpio* var. *specularis*. These methods are, percentage of gravid females against time, diameter of ova, gonado-somatic index, gonadal length index and coloration of the ovary. All these methods showed that the breeding season of this species is meanly November to April with the peak in February.

The fecundity of *C. carpio* var. *specularis* ranged from 5928.91 to 209120.18 eggs and mean value was 87377.98±79544.17. A comparison of correlation co-efficient relation between fecundity with total length (r = 0.9129), standard length (r = 0.9319), total weight (r = 0.9519) gonadal length (r = 0.9121) and gonadal weight (r = 0.9948) of the female showed straight line linear regressions. All the value of co-efficient of correlation (r) was highly significant.

The fecundity factor of the species was also provided. The sex ratio, 1084 specimens collected during 24 months of study was determined. The total male (512) and female (572) ratio was 1:1.12 i.e. the female number was more than that of the male. The chi-square test shows that the males and females distribution in the natural population was significantly different.

Biochemical composition

The freshwater fish *C. carpio* ver. *specularis* is very important due to its high nutritional value in terms of lipid, protein content and other related substances which are not commonly available in other food.

The biochemical composition of *C. carpio* var. *specularis* fish was studied to obtain a detailed information on the variation of moisture, ash, lipid, protein and carbohydrate in different seasons of male and female. The percentage of moisture

(M 72.54±0.551 to 74.53±1.078 and F 73.32±0.778 to 75.42±0.538%), ash (M 3.02±0.637 to 3.44±0.536 and F 2.64±0.393 to 3.77±0.766%), lipid (M 3.36±0.400 to 4.19±0.231 and F 2.95±0.349 to 4.08±0.400%), protein (M 18.25±1.189 to 20.07±0.190 and F 17.03±0.925 to 19.56±0.538%) and carbohydrate (M 0.035±0.002 to 0.042±0.005 and F 0.037±0.004 to 0.042±0.003%) respectively. An increasing trend of moisture, ash and carbohydrate were found both males and females in winter season whereas lipid and protein content showed a decreasing trend in both sexes in winter season (winter season was spawning period of this fish). In case of male protein and lipid content was higher than female fishes in all seasons. The results indicates variation in biochemical composition on the basis of sex and season. As the fish contains high level of lipid and protein, it will be helpful to meet the nutrition demand of the country.

CHAPTER-7

CONCLUSION AND RECOMMENDATION

Changes of environmental factors due to global warming are directly affecting the life of aquatic animals. As a result the aquatic animals are increasingly in under pressure to change their normal behaviour. The research work is successfully carried out some targeted physico-chemical conditions, reproductive biological and biochemical composition aspects of *C. carpio* var. *specularis*. The result provided some new and updated information on the physico-chemical conditions, reproductive biological and biochemical composition status of the fish which will be helpful to the fisheries scientists all over the world. Though *C. carpio* var. *specularis* is a taste fish, it has very good consumer demand as a live fish with low price and availability in respect to other live fishes. The growth of the carp is very rapid, particularly in favourable environments. Its rapid growth, tasty flesh, good reproductive ability and modest requirements have led to the carp's becoming the stable fish of warm water fisheries.

The present study gives an information about the different physico-chemical parameters of the habitat of *C. carpio* var. *specularis*. The production and availability of fish were found to depend on the physico-chemical conditions of the water in which the fish lives. The different environmental factors which determined the characters of water were found to exert significant impacts on the growth, maturity, reproduction and development of fish. The abundance of *C. carpio* var. *specularis* in the Atrai river continuous throughout the year.

The physico-chemical parameters of the habitat were also found in the good condition. As a result the fish can easily lead its life to produce new generation, if the man made hazard do not hamper its habitat. The species has also the strength to survive in any type of water body due to its hardy nature having air breathing character. So, commercial production will be very effective in a sense.

C. carpio var. *specularis* was found in the study area the Atrai river in favorable condition to breed in nature from November to Aril with peak in February and the fecundity of *C. carpio* var. *specularis* ranged from 5928.91 to 209120.18 which revealed that the species was high fecund. All the relationships showing values of the regression co-efficient were highly significant. Chi-square test shows that the distribution of males and females in the natural population was significantly different.

The gonadal development was found to be affected by epidemic and endemic factors like periodic lowering of rainwater intrusion resulting in the change in different physico-chemical condition parameters. Among the developmental stages gravid females were very common in entire winter season.

The present study revealed the biochemical composition of *C. carpio* var. *specularis* was more or equal to other larger fish species in Bangladesh. The Mirror carp contains comparatively high amount of protein and lipid. The fish will be able to mitigate the nutrient demand of the poor people. These results also suggest that the biochemical composition of fish species greatly varies due to sex and seasonal changes in environmental conditions, i.e. spawning characteristics might greatly affect the biochemical composition. The present findings agreed with findings of over mentioned researcher. It was also observed that the fish has a good potential to serve protein and lipid for all the people being a source of animal protein. It can play a vital role in the commercial aspects also as a source of nutrition and income generation. This study provides valuable information of fish species studied. Necessary steps should be taken to increase the production of the species by aqua farming and proper management of natural habitat of the species.

The findings of the present work may play an important role in the production and conservation of the species is urgently needed through cultural practices of the species, as the fish is a highly consumer preferred fish. However, further scientific research is recommended for the proper management of the species.

CHAPTER-8

REFERENCES

Abou-Seedo, F. S., Dadzie, S. and Al-khanaan, K. A. 2003. Sexuality, sex change and maturation patterns is the yellow fin seabream, *Acanthopagrus latus* (Teleostei: Sparidate) (Houttuyn, 1782). *J. Appl. Ichthyol*, 19: 65-73.

Abu-Hakima, M. 1984. Some aspects of the reproductive biology of *Acanthapagrus* sp. (Family: Sparidae). *J. Fish. Biol.,* 25: 515-526.

Adiyodi, K. G. and Adiyodi, R. G. 1974. *Comparative physiology of reproduction in arthropods: Comparative physiology and biochemistry* (edited by O. Lowenstien). Academic press. New york. London. 5: 37-107.

Afroze, S. and Hossain, M. A. 1983. The fecundity and sex-ratio of *Amblypharyngodon mola* (Hamilton) (Cyprinifromes:Cyprinidae). *Univ. j. zool. Rajshahi Univ.,* 2: 29-32.

Afroze, S. and Hossain, M. A. 1990. The reproductive cycle of the freshwater fish *Amblypharyngodon mola* (Ham.) (Cypriniformes: Cyprinidae). *Univ. j. zool. Rajshahi Univ.,* 9: 17-21.

Afroze, S., Nabi, M. R. and Mustafa, G. 1999. Fecundity of 'Chapila', *Gudusia chapra* (Hamilton) from Jahangirnagar University ponds. *Bangladesh J. Life Sci.,* 11(1&2): 45-51.

Ahmad, K. & Hassan, N. 1983. Nutritional Survey of Rural Bangladesh 1981–82. Institute of Nutrition and Food Sciences, Dhaka University, Dhaka, Bangladesh.

Ahmed, A. T. A. Mustafa, G. and Hai, A. 1979. Fecundity of the catfish *Clarias batrachus* (Lin.). *J. Asiatic Soc. Bangladesh Sci.,* 5(2):7-12.

Ahmed, J.W. and sarker, A. 1997. Limnology and productivity of two reservoirs in the North Eastern India. *J. Inland Fish. Soc. India*. 29(2): 1-10.

Ahmed, K., Malek, M. A. and Sanaullah, 1977. Deshio Khadyer Pustiman. Inst. Nutrition and Food Sci., Dhaka University, Dhaka.

Ahmed, S., A.F.M. Arifur Rahman, M.D. Ghulam Mustafa, M.B. Hossain and N. Nahar, 2012. Nutrient composition of indigenous and exotic fishes of rainfed waterlogged paddy fields in Lakshmipur, Bangladesh. W. J. Zool., 7: 135-140.

Alam, M. F. and Thompson, K. J. 2001. Current constraints and future possibilities for Bangladesh fisheries. *Food Policy*. 26: 297-313.

Alam, M. J. Kamal, D. and Kader, M. A. 1997. Fecundity of the Gangetic Anchovy, *Setipinna phasa* (Hamilton-Buchanan) from the Bay of Bengal. *J. Asiat. Soc. Bangladesh,* (Sci.). 23(1):1-8.

Alam, M. J., Kamal, D. and Kader, M. A. 1996. A preliminary study of the maturation and spawning of *Setipinna phasa* (Ham.) from the Bay of Bengal. *Bangladesh J. Zool.*, 24 (1): 65-71.

Alam, M. R., Mortuza, M. G., Islam, M. S. and Hossain, M. A. 1994b. The fecundity of *Ailia coila* (Hamilton-Buchanan) (Siluriformes:Schibeidae) from the river Padma near Rajshahi. *J. bio-sci.*, 2:113-116.

Alam, M.R. Mortuza, M.G., Islam, M.S. and Hossain, M.A. 1997. The sex-ratio and reproductive cycle of *Ailia coila* (Hamilton-Buchannun) (Siluriformes : Schilbeidae). *Ibid*. 5: 213-217.

Ali Aberoumand, 2014, Nutritional Properties of Some Novel Selected Fish Species In Khuzestan Province, IRAN, *Journal of Food Technology Research,* 1(2): 52-59.

Ali, M., F. Igbal, A. Salam, S. Iram and M. Athar, 2005. Comparative study of body composition of different fish species from brackish water pond. Int. J. Environ. Sci. Technol., 2: 229-232.

Ali, M.M. and Islam, M.A. 1983. Studies on the plankton of a lake in Bangaldesh Agricultural University campus, Mymensingh, *Ibid*. 2-5(1): 51-61.

Alikunhi, K.H. 1966. Synopsis of biological data on common carp, *Cyprinus carpio* Linnaeus, 1758 (Asia and the Far East). *FAO Fish, Synops,* (31.1): pag. var, 83 p.

Alp, A., Kara, C. and Büyükçapar, H. M. 2003. Reproductive biology of brown trout, *Salmo trutta macrostigma* Dumeril 1858, in a tributary of the Ceyhan River which flows into the eastern Mediterranean Sea. *J. Appl. Ichthyol.,* 19: 346–351.

AOAC (Association of Official Agrichemicals). 1990. Official Methods of Analysis of the Association of Official Agricultural Chemist. Helritz, K. (Ed.). 15th Ed. Vol.2. Association of Official Analytical Chemists, Inc., Suite 400, 2200 Wilson Boulevard, Arlington, Virginia 22201 USA. p. 685-1298.

Asahina, K. and Hanyu, I. 1983. Role of temperature and photoperiod in the annual reproductive cycle of the rose bitterling, *Rhodeus ocellatus ocellatus* (Agassiz). *Can. J. Zool.,* 49: 61-67.

Azadi, M. A., Islam, M. A., Nasiruddin, M. and Quader, M. F 1995. Reproductive biology of *Notopterus notopterus* (pallas) in Kaptai Reservoir, Bangladesh. *Bangladesh J. Zool.,* 23 (2): 215-220.

Azim, M.A., M.R. Islam, M.B. Hossain and M.H. Minar, 2012. Seasonal variations in the proximate composition of gangetic sillago, *Sillaginopsis panijus* (Perciformes: Sillaginidae). Middle-East J. Sci. Res., 11: 559-562.

Azzurro, E., Carnevali, O., Bariche, M. and Andaloro, F. 2007. Reproductive features of the non-native *Siganus luridus* (Teleostei, Siganidae) during early colonization at Linosa Island (Sicily Strait, Mediterranean Sea). *J. Appl. Ichthyol.,* 23: 640-645.

Bagenal, T. B. 1978. Aspects of fish fecundity. In: Ecology of freshwater fish production (ed.S. D. Gerking). Willey Int., New York. pp. 75-101.

Bamforth, S. 1958. Ecological studies on the planktonic protozoa of a small artificial pond. *Limnol.* and *Oceanogr*. 3:398-412.

Banu, N. Ali, S., Shaha, T. R. and Vakta, N. C. 1985. The studies on the fecundity of *Anabas testudineus* (Bloch) in a confined pond of Dhaka district. *Bangladesh J. Aquaculture*, 6-7(1): 45-49.

Barros, S. E. and Regidor, H. 2002. Reproduction in *Odontesthes bonariensis* (Atherinidae: Pisces) from north-western Argentina. *J. Appl. Ichthyol.*, 18: 27-28.

Berra, T. M. 1984. Reproductive anatomy of the Australian greyling *Prototroctes maraena* Gunther. *J. Fish. Biol*., 25: 241-251.

Berreur-Bonnenfant, J. and Charniaux-Cotton, H. 1970. Sexualite de crustaces superieurs. *Ann. Biol*., 9:427-439.

Bhattacharya, P. and Banik, S., 2015. Study of Fecundity of *Ompok pabo* (Hamilton, 1822) an Endangered Fish Species of Tripua, India. J. Fisheries Livest Prov. 3: 4.

Bhuiyan, A. S. and Afrose, R. 1996. The fecundity and sex-ratio of *Oreochromis nilotica* (L.) (Perciformes: Cichlidae). *Univ. j. zool. Rajshahi Univ.,* 14 & 15: 29-32.

Bhuiyan, A. S. and Perveen, A. S. 1998. Fecundity of *Puntius sophore* (Hamilton) (Cyprinidae : Cypriniformes). *J. bio-sci*., 6: 153-154.

Bhuiyan, A. S., Begum, M. and Nessa, Q. 1997. Observation of the food and feeding habit of *Clupisoma atherinoides* (Bloch) (Siluriformes: Schilbidae). *Univ. j. zool. Rajshahi Univ.,* 16: 1-5.

Bhuiyan, A. S., Begum, M. and Nessa, Q. 1999a. The spawning periodicity of *Clupisoma atherinoides* (Bloch and Schneider) (Siluriformes: Schilbeidae). *Bangladesh J. Zool.,* 27(1): 83-89.

Bhuiyan, A. S., Islam, M. N. and Sultana, N. 1993. The fecundity and sex-ratio of *Aspidoparia morar* (Hamilton) (Cyprinidae:Cypriniformes) form the river Padma. *Univ. j. zool. Rajshahi Univ.,* 12: 59-63.

Bhuiyan, A. S., Nessa, Q. and Begum, M. 2000. Fecundity and sex-ratio of Thai silver barb *Barbodes gonionotus* (Bleeker). *Bangladesh J. Fish. Res.,* 4(1): 97-99.

Bhuiyan, A.S. and Akhter, S. 2002. Fecundity and sex-ratio of *Cyprinu carpio* var. *communies* (Lin.) (Cyprinidae:Cypriniformes). *J. Bio-Sci.* 10: 109-111.

Bhuiyan, A.S. and Islam, M.N. 1990. Fecundity of *Xenentodon cancila* (Hamilton). (Belonidae : Beloniformes). *Environment and Ecology*.8(3): 1004-1007.

Bhuiyan, A.S., M.N. Islam and T. Hossain, 1992. A check list of the fishes of Rajshahi. Rajshahi Univ. Stud., 20: 287-306.

Bhuiyan, A.S., Nessa, Q. and Begum, M. 2000. Fecundity and sex-ratio of Thai silver barb *Barbodes gonionotus* (Bleeker). *Bangladesh J. Fish. Res.,* 4(1): 97-99.

Billard R, Breton B. 1978. Rhythms of reproduction in teleost fish. In: Thorpe J (ed), Rhythmic activity of fishes. Academic Press, London, England, pp 31–53

Bligh E.G. and Dyer W. 1959. Total lipid Extraction and Purification. Can. *J. Bipchem. Physiol.* 37:99-110 p.

Boolootian, R. A. 1965. Aspects of reproductive biology in striped shore crab, *Pachygrapsus crassipes*. *Bull. Southern. Calif. Acad. Sci*., 64(1): 43-49.

Bowles, M. H., Klonis, D., Plavac, T. G., Gonzales, B., Francisco, D. A., Roberts, R. W., Boxberger, G. R., Poliner, L. R. & Galichia, J. P. 1991. EPA in the prevention of restenosis post PTCA. Angiology 42:187-194. 32.

Boyd, C.E. 1998. *Water Quality for Aquaculture*. Research and Development Series No. 43. Aubum University, Alabama, USA 37p.

Breder, C. M. and Rosen, D. E. 1966. *Modes of reproduction of fishes*. T.F.H. publication, Neptune City. 941p.

Caputo, V., Mesa, M. L., Candi, G. and Cerioni, P. N. 2003. The reproductive biology of the crystal goby with a comparison to that of the transparent goby. *Journal of Fish Biology,* 62: 375-385.

Casal, CMV, 2006. Global documentation of fish introductions: the growing crisis and recommendations for action. *Biological Invasions* 8: 3-11, http://dx.doi.org/10.1007/s10530-005-0231-3.

Chakrabarty, S.C., M.B. Uddinand M.N. Islam, 2003. A study on the composition of common freshwater fishes of Bangladesh. Bangla. J. Fish., 26: 23-26.

Chakraborty, R. D., Roy, P. and Singh, S. B. 1959. A quantitative study of the Plankton and physico-chemical conditions of the river Jamuna at Allahabad in 1954-55, *Indian J. Fish*. 6(1): 186-203.

Chakraborty, S. C., Rahmatullah, S. M. and Das, M. 1985. Nutritive value of Silver carp (*Hypophthalmichthys molitrix*) of Bangladesh Agricultural University fish pond. *Bangladesh J. Aquacul*., 6-7: 19-23.

Chakwu, O. and I.M. Shaba, 2009. Effect of drying method on proximate composition of cat fish *(Clarial gariepinus*). J. World Agric. Sci., 5: 114-116.

Chandran, M. R. 1968. Studied on the marine crab, *Charybdes variegata* I, reproductive and nutritional cycle in relation to breeding periodicities. *Proc. Ind. Acad. Sci*., 67: 203-207.

Chhapgar, B. F. and Deshmukh, S. K. 1964. Further records of lobsters from Bombay. *J. Bombay Nat. Hist. Soc*., 61(1): 203-207.

Chow, T. 1958. A study of water quality in the fish ponds of Hong Kong. *Hong Kong Univ. Fish. J.* 2:7-18.

Chowdhury, M. 1960. Experiments of induced spawning of Indian Carps with pituitary infections. *Indian J. Fish.* 7(11): 20-49.

Chowdhury, S. H. and Mazumder, A. 1981. Limnology of Lake Kaptai. I. Physico-chemical features. *Bangladesh. J. Zool*. 9(2): 59-72.

Chubb, C. F. and Potter, I. C. 1984. The reproductive biology and estuarine movements of the Gizzard Shad *Nematolosa vlaminghi* (Munro*). J. Fish. Biol*., 25: 527-543.

Colombo, R. E., Garvey, J. E. and Wills, P. S. 2007. Gonadal development and sex-specific demographics of the shovelnose sturgeon in the Middle Mississippi River. *J. Appl. Ichthyol.,* 23: 420-427.

Craig, J.F., Halls, A.S., Barr, J.J.F., Bean, C.W. 2004. The Bangladesh floodplain fisheries. *Fisheries Research*. 66: 271-286.

Cui, Y. and R.J. Wootton, 1988. Effectc of ration, temperature and body size on the body composition, energy content and condition of the minnow, *Phoxinua phoxinus* (L.). J. Fish Biol., 32: 749-764.

Dadzie, S. and Abou-Seedo, F. 2008. Reproductive guilds (maturation, spawning frequency and fecundity) in the black pomfret, *Parastromateus niger* (Carangidae) in the Kuwaiti waters of the Arabian Gulf. *J. Appl. Ichthyol.,* 24: 562–568.

Dadzie, S., Abou-Seedo, F. and Manyala, J. O. 2008. Length–length relationship, length–weight relationship, gonadosomatic index, condition factor, size at maturity and fecundity of *Parastromateus niger* (Carangidae) in Kuwaiti waters. *J. Appl. Ichthyol.*, 24: 334-336.

Dalela, R. C., Rani, M. and Verma, S. R. 1977. Seasonal histological changes in the gonads of two teleost fishes, *Notopterus notopterus* (Pallas) and *Colisa fasciatus* (Bloch. Schn.). *Gegenbaurs Morphol Jahrb*., 123(1):128-40.

Darnton-Hill, I., Hassan, N., Karim, R. & Duthie, M. R. (1988) Tables of nutrient composition of Bangladeshi foods. English version with particular emphasis on vitamin A content. Helen Keller International, Dhaka, Bangladesh.

Das, H. P. 1977. The fecundity of grey mullet, *Mugil cephalus* L. along Goa coast. *Mahasagar Bull. National Ins. of Oceanography,* 10(1+2): 79-82.

Davis, J. 1959. Management of channel cat fish in Kansas, University of Kansas. Natural History Museum, *Mise. Publ*. No. 21.56p.

De Caterina, R., Giannessi, D., Mazzone, A., Bernini, W., Lazzerini, G., Maffei, S., Cerri, M., Salvatore, L. & Weksler, B. 1990. Vascular prostacyclin is increased in patients ingesting omega-3 polyunsaturated fatty acids before coronary artery bypass graft surgery. Circulation 82:428-438.

Debora Fernanda Avila Troca and Valeria Marques Lemos, Antonio Sergio Varela Junior and Joao Paes Vieira 2012. Evidence of reproductive activity of the invasive common carp *Cyrinus carpio* (Linnaeus, 1785) (Teleostei: Cyprinidae) in a subtropical coastal system in sourthern Brazil, J. REABIC, BioInvasions Records, Volume 1, Issue 4: 289-293.

Dembergs, N. 1964. Extraction of fish muscles, 4. Seasonal variation of fat, water soluble protein and water in Cod (*Gadno morlia*) fillets. *J. Fish. Res. Canada*, 21: 705-709.

deVlaming, V. L. 1972. Environmental control of teleost reproductive cycle; a brief review. *J. Fish Biol*., 4: 131-140.

Diwan, V. P. and Nagabhushanam, R. 1974. Reproductive Cycle and biochemical changes in the gonads of the freshwater crab, *Barytelphusa canicularis* (West-wood, 1836). *Ind. J. Fish.,* 21(1): 164-176.

DoF, 2016. National Fish Week 2016. Department of Fisheries, Ministry of Fisheries and Livestock, Government of the Peoples Republic of Bangladesh, Dhaka. 148 p.

Ehshan, M. A., Hossain, M. S., Razzaque, A., Mollah, M. F. A. and Mazid, M. A. 1997. Limnology of a flood plain: Halti Beel. *Univ. J. Zool . Rajshahi Univ.* Vol. 16: pp. 95-98.

Ellis, J. R. and Shackley, S. E. 1997. The reproductive biology of *Scyliorhinus canicula* in the Bristol Channel, U.K. *Journal of Fish Biology,* 51: 361-372.

Eritsland, J., Arnesen, H., Seljeflot, I. & Kierulf, P. 1995. Long-term effects of n-3 polyunsaturated fatty acids on haemostatic variables and bleeding episodes in patients with coronary artery disease. Blood Coagul. Fibrinolysis 6:17-22.

FAO, 1995. Quality and changes in fish flesh. FAO Fisheries Technical Paper. p.195.

Farmer, A. S. 1974. Reproduction in *Nephrops norvegicus* (L). (Decapoda : Nephropidae). *J. Zool. London*. 174: 161-183.

Fatema, N., Mortuza, M. G., Parween, S. and Hossain, M. A. 1997. On the breeding periodicity of *Oxygaster bacaila* (Hamilton) (Cypriniformes: Cyprinidae). *Univ. j. zool. Rajshahi Univ.,* 16: 101-102.

Fazli, H., Janbaz, A. A., Taleshian, H. and Bagherzadeh, F. 2008. Maturity and fecundity of golden grey mullet (*Liza aurata* Risso, 1810) in Iranian waters of the Caspian Sea. *J. Appl. Ichthyol.,* 24: 610-613.

Fernandez-Delgado C. 1990. Life history patterns of the common carp, Cyprinus carpio, in the estuary of the Guadalquivir river in south-west Spain. Hydrobiologia 206: 19-28.

Ferriz, R. A., Fernandez, E. M., Bentos, C. A. and Lopez, G. R. 2007. Reproductive biology of *Pseudocorynopoma doriai* (Pisces: Characidae) in the High Basin of the Samborombón River, province of Buenos Aires, Argentina. *J. Appl. Ichthyol.,* 23: 226-230.

Filer, K. R. and Sedberry, G. R. 2008. Age, growth and reproduction of the barrelfish *Hyperoglyphe perciformis* (Mitchill) in the western North Atlantic. *Journal of Fish Biology,* 72: 861–882.

Flickinger, S. A. 1969. Determination of sexes in flat head minnow. *Middle Amer. Fish. Soc.*, 98: 526-527.

Foucher, R.P. and Beamish, R.J. 1977. A review of oocytes development in fishes with special reference to pacific Hake (*Merluccius productus*), *Fish. Mar. Serv. Tech. Rep.* No. 755: 16 pp.

Foucher, R.P. and Beamish, R.J. 1980. Production of nonviable oocytes by pacific Hake (*Merluccius productus*). *Can. J. Fish. Aquat. Sci.* 37: 41-48.

Garcia LO, Copatti CE, Wachholz F, Pereira Filho W, Baldisserotto B. 2008. Freshwater temperature in the state of Rio Grande do Sul, Southern Brazil, and its implication for fish culture. *Neotropical Ichthyology* 6(2), http://dx.doi.org/10.1590/S0101-81752004000100027

Geisler, R., Knoppel, H. A. and Sioli, H. 1975. The ecology of freshwater fishes in Amazonia - Present status and future tasks for research. Animal Research and Development, Institute for Scientific cooperation, Germany. 1:102-119.

Gheyasuddin, S., A. Rahmanand and M. Mumtazuddin, 1979. Biochemical composition of shellfishes of Bangladesh. Bangla. J. Sci. Res., 2: 15-23.

Glamuzina, B., Bartulovic, V., Dulcic, J., Conides, A. J., Tutman, P., Matic-Skoko, S., Gavrilovic, A., Jug-Dujakovic, J., Haskovic, E., Ivanc, A. and Zovko, N. 2007. Some biological characteristics of the endemic Neretvan nase, *Chondrostoma knerii* Heckel, 1843, in the Hutovo Blato wetlands (Bosnia and Herzegovina). *J. Appl. Ichthyol.,* 23: 221-225.

Grabowska, J. 2005. Reproductive biology of racer goby *Neogobius gymnotrachelus* in the Włocławski Reservoir (Vistula River, Poland). *J. Appl. Ichthyol.,* 21: 296–299.

Haley, S. R. 1972. Reproductive cycling in the Ghost crab, *Ocypode quadrata* (Fabr.) (Brachyura: Ocypodidac). *Crustaceana*, 23(1): 1-11.

Heydorn, A. E. F. 1965. The rock lobster of the South African West Coast. *Jasus lalandii* (H. Mile Edwards). I. Notes on the reproductive biology and determination of minimum size limits for commercial catches. *Invest: Rep. Div. Sea. fish St. Afr*., 53: 1-32.

Hickman, M. 1979. Seasonal succession, standing crop and determinants of the phytoplankton of Ministik lake Alberta, Canada, *Hydrobiologia*. 64: 105-121.

Hoggarth, D.D., Cowan, V.J., Halls, A.S., Aeron-Thomas, M.A., McGregor, J.A., Garaway, C.A., Payne, A.I. and Welcome, R.L. 1999. Management guidelines for Asian floodplain river fisheries, FAO Fisheries Technical Paper 384/2, Rome, 117 pp.

Hoque, M. A. and Hossain, M. A. 1993. Sexual maturity and fecundity of the freshwater cat fish *Mystus vittatus* (Bloch) (Cypriniformes: Bagridae). *Univ. j. zool. Rajshahi Univ.,* 12: 9-13.

Hoque, M. T. and Ahmed, A. T. A. 1993. Spawning periodicity of two Indian major carps, *Labeo rohita* Ham. and *Cirrhinus mrigala* Ham. *Bangladesh J. Zool*., 21(2): 9-26.

Hossain, M. A. 1976. Studies on some aspects of the biology of sand lobster, *Thenus orientalis (Lund.) (Decapoda: Scyllaridae)* from Andhra Coast. Ph. D. thesis, Andhra University, Waltair. 267p.

Hossain, M. A. 1989. On the methods of determining the reproductive cycle in fisheries species. A scientific paper presented in 76th Ind. Sc. Cong. Ass. Madurai.

Hossain, M. A. and Islam, M. S. 1983. Sexual dimorphism and sex ratio of *Clupisoma atherinoides* (Bloch). *Univ. j. zool. Rajshahi Univ.,* 2: 71-72.

Hossain, M. A. and Parween, S. 1982. Sexual periodicity in freshwater prawn *Macrobrachium dayanum* (Handerson). *Bang. J. Sci. Ind. Res*., 17(3-4): 236-245.

Hossain, M. A., Islam, M. N., Ahsan, M. K. and Sultana, N. 2002. Reproductive periodicity of *Gonialosa manminna* (Ham.) (Clupeiformes: Clupeidae). *Univ. j. zool. Rajshahi Univ.,* 21: 91-92.

Hossain, M. A., Parween, S and Rahman, M. A. 1997. Fecundity of *Nandus nandus* (Hamilton-Buchanan) (Nandidae: Perciformes). *J. bio-sci*., 5: 305-307.

Hossain, M. A., Rahman, M. A. and Parween, S. 1991a. A study on the sex-ratio and reproductive cycle of *Nandus nandus* (Hamilton-Buchanan) (Nandidae: Perciformes) *J. Asiat. Soc. Bangladesh* Sci., 17(2): 141-144.

Hossain, M. A., Taleb, A. and Rahman, M. H. 1989. Reproduction and reproductive periodicity of *Notopterus notopterus* (Pall). (Notopteridae: clupeiformes). *Bangladesh J. Aqua*., 11(2): 57-64.

Hossain, M. A., Taleb, A. and Rahman, M. H. 1992b. Reproduction and fecundity of *Ompok pabda* (Ham.). *Bangladesh J. Sci. Res.*, 10(1): 49-52.

Hossain, M.M. Islam, M.A. Ridgway, S., Matsuish, T. 2006. Management of inland open water fisheries resources of Bangladesh. Issues and options Fisheries research. 77: 275-284.

Huq, M. F. 1977. Determination of sexes in catfish of Bangladesh and Pakistan coasts. *Bangladesh J. Zool*., 5(1): 33-40.

Hussain, M. A. and Hossain, M. A. 2001. Notes on the reproductive cycle of *Mastacembelus pancalus* (Hamilton). *Univ. j. zool. Rajshahi Univ.*, 20: 87-88.

Islam, A.K.M.N. and Mendes, F. 1976. Limnological Studies of Jheel in Sher-E-Bangla Nagar. *Dhaka Univ. Stud. Pt.* B 24(2): 63-71.

Islam, A.K.M.N., Haroon, A.K.Y. and Zaman, K.M. 1974. Limnological studies of the river Buriganga. *Ibid*. P. B 22(2): 99-111.

Islam, M. A. and Azadi, M. A. 1989. Fecundity of *Mystus cavasius* (Bloch) from Kaptai Lake. *Proc. 14th Ann. bang. Sic. Conf.,* (Abst.); Sec. IX, Gazipur. p.19.

Islam, M. N. 2002. Cannibalism of freshwater gobi, *Glossogobius giuris* (Hamilton) in relation to its size, sex and season from the river Padma. *Univ. j. zool. Rajshahi Univ.*, 21: 63-64.

Islam, M. N. and Joadder, M. A. R. 2005. Seasonal variation of the proximate composition of freshwater gobi, *Glossogobius giuris* (Hamilton) from the river Padma. *Pakistan Journal of Biological Sciences*, 8(4): 532-536.

Islam, M. N., Flowra, F. A. Khatun, S. and Hossain, M. A. 2001. Reproduction and reproductive periodicity of *Setipinna phasa* (Hamilton) (Clueiformes: Engraulidae). *Univ. j. zool. Rajshahi Univ*., 20: 92-94.

Islam, M. S. and Hossain, M. A. 1990. The fecundity and sex-ratio of the common punti, *Puntius stigma* (Cuvier and Valenciennes) (Cypriniformes: Cyprinidae) from the river Padma near Rajshahi. *Univ. j. zool. Rajshahi Univ.*, 9: 69-74.

Islam, M.R., N. Sultana, M.B. Hossain and S. Mondal, 2012. Estimation of fecundity and gonadosomatic index (GSI) of gangetic whiting, *Sillaginopsis panijus* (Hamilton, 1822) from the Meghna River Estuary, Bangladesh. World Appl. Sci. J., 17: 1253-1260.

Islam, M.S. and Hossain, M.A. 1984. On the fecundity of chela, *Oxygaster bacaila* (Hamilton) (Cypriniformes: Cyprinidae) from the river Padma. *Univ. J. Zool. Raj. Univ.* 3: 45-48.

Iso H, Rexrode KM, Stampfer MJ, 2001. Intake of fish and omega-3 fatty acids and risk of stroke in women. JAMA. 285:304-312.

Jhingran, V. G. 1983. *Fish and Fisheries of India* (2nd edition). Hindustan Publishing Corporation, Delhi, India., 645p.

Jhingran, V.G. 1977. Fish and fisheries of India, 2nd Edit. Hind. Pub. Corp. India, 954 pp.

Jhingran, V.G. 1983. Fish and fisheries of India (Revised and enlarged 2nd ed). Hindustan Publishing Corporation (India) Delhi. 645 pp.

Juchno, D., Boron, A. and Goqaszewski, J. 2007. Comparative morphology and histology of the ovaries of the spined loach *Cobitis taenia* L. and natural allopolyploids of Cobitis (Cobitidae). *Journal of Fish Biology*, 70: 1392-1411.

Kahraman, A. E., Alicli, T. Z., Akayli, T. and Oray, I. K. 2008. Reproductive biology of little tunny, *Euthynnus alletteratus* (Rafinesque), from the north-eastern Mediterranean Sea. *J. Appl. Ichthyol.*, 24: 551-554.

Kamal, D., Khan, A. N., Rahman, M. A. and Ahamed, F. 2007. Biochemical composition of some small indigenous freshwater fishes from the river Mouri, Khulna, Bangladesh. *Pakistan Journal of Biological Sciences*, 10(9): 1559-1561.

Kamaluddin, A., M.A. Malek and M. Sanaullah, 1977. Deshio khaddeer pustiman. Ins, Nutr. Fd. SCI. Dhaka University.

Karim M.A. 1975. An Introduction to Fish Culture in Bangladesh. BAU, Mymensingh, Bangladesh 164 pp.

Karim, M. A. 1975. *An Introduction to Fish Culture in Bangladesh.* BAU, Mymensingh, Bangladesh. 164pp.

Karmakar, B. R. 1998. Some aspects of the biology of the freshwater fish *Notopterus chitala* (Hamilton) (Clupeiformes: Notopteridae). M. Sc. thesis, Department of Zoology, University of Rajshahi, Bangladesh. 121p.

Keli, S. O., Feskens, E. J. & Kromhout, D. 1994. Fish consumption and risk of stroke. The Zutphen study. Stroke 25:328-332.

Kendall, B. W. and Gray, C. A. 2008. Reproductive biology of two co-occurring mugilids, *Liza argentea* and *Myxus elongatus*, in south-eastern Australia. *Journal of Fish Biology*, 73: 963-979.

Kennedy, A. J., Sutton, T. M. and Fisher, B. E. 2006. Reproductive biology of female shovelnose sturgeon in the upper Wabash River, Indiana. *J. Appl. Ichthyol.*, 22: 177-182.

Kestaven, G.L. (ed). 1960. Manual of field methods in fisheries biology. FAO Man. Fish. Sci. No. 1 p. 152.

Khanna, S. S. 1978. *An Introduction to Fishes* (2nd edition). Central Book Dept. Allahabad, India. 486p.

Kibria, G. and Ahmed, K.K.U. 2005. Diversity of selective and nonselective fishing gear and their impact on inland fisheries in Bangladesh. NAGA, 28: 43-48.

Kinne, O. 1971. *Marine Ecology*. Wiley Int. Science Division, Johan Wiley Sons Ltd., New York. 1244p.

Klein, N. R. 2007. Age, growth and reproductive parameters of the Mediterranean cardinal fish, *Apogon imberbis. J. Appl. Ichthyol.*, 23: 675-678.

Knight, J. T., Butler, G. L., Smith, P. S. and Wager, R. N. E. 2007. Reproductive biology of the endangered Oxleyan pygmy perch *Nannoperca oxleyana* Whitley. *Journal of Fish Biology*, 71: 1494-1511.

Koc, H. T., Erdogan, Z., Tinkci, M. and Treer, T. 2007. Age, growth and reproductive characteristics of chub, *Leuciscus cephalus* (L., 1758) in the Ikizcetepeler dam lake (Balikesir), Turkey. *J. Appl. Ichthyol.,* 23: 19-24.

Kottelat, M. 1997 European freshwater fishes. *Biologia*, 52, Suppl. 5:1-271.

Kottelat, M. 2001. Fishes of Laos. WHT Publications Ltd., Colombo 5, Sri Lanka. 198p.

Kottelat, M. and J. Freyhof 2007 Handbook of European freshwater fishes. Publications Kottelat, Cornol, Switzerland. 646 p.

Kovacic, M. 2001. The biology of Roule's goby in the Kvarner area, northern Adriatic Sea. *Journal of Fish Biology,* 59: 795-809.

Kris-Etherton, PM, 2002. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation, Vol. 106, November 19: pp. 2747-57.

Kumar, S. and Tembhre, M. 1998. *Anatomy and Physiology of Fishes*. Vikas Publishing House Pvt. Ltd., Jangpura, New Delhi, India. 275p.

Lagler, K. F. 1966. *Freshwater Fishery Biology* (second edn). WM. C. Brown Company. Dubuque. Iowa. 421p.

Lagler, K. F. Bardach, J. E. and Miller, R. R. and Passino, D. R. M. 1977. *Ichthyology* (second edn.). John Willey and Sons. Inc. New York. London. Sydney. 506p.

Lagler, K.F. 1956. Enumeration of fish eggs. In freshwater fishery biology. 2^{nd} ed. W.M.C. Brown Co. Pub. Dabuque, pp. 106-110.

Lagler, K.F. Bardach, J.E. and Miller, A.R. 1967. Ichthyology. *Ibid.* Sydney. P. 545.

Lakshminarayana, J.S.S. 1965. Studies on the phytoplankton of the river Ganges, Varanashi, India, Part-1 and 2 *Hydrobiologia*. 25(1-2): 91-100.

Landmark K and Aursnes I Mercury, fish, fish oil and the risk of cardiovascular disease Tidsskr Nor Laegeforen. 2004 Jan 22;124(2):198-200.

Larsen, T., Thilsted, S. H., Kongsbak, K. & Hansen, M. 2000. Whole small fish as a rich calcium source. Br. J. Nutr. 83: 191–196.

Latifa, G. A., Rahman, M. M. and Parvin, S. 2002. Fecundity and maturation of gonad of snakehead fish *Channa striatus* (Bloch, 1793) (Channiformes: Channidae). *Univ. j. zool. Rajshahi Univ.*, 21: 65-67.

Lenhardt, M. and Cakic, P. 2002. Seasonal reproductive cycle of pike, *Esox lucius* L., from the River Danube. *J. Appl. Ichthyol.*, 18: 7-13

Lodish H, Berk A, Zipursky LS. 2004. *Molecular Cell Biology* (4th ed.). New York: Scientific American Books. ISBN 0716731363.

Loomis, W.E. and I. Shall, W.E. 1937. Methods in Plant Physiology, McGrow-Hill Company, London.

Lorenzo, M., Pajuelo, J. G., Me´ndez-Villamil, M., Coca, J. and Ramos, A. G. 2002. Age, growth, reproduction and mortality of the striped seabream, *Lithognathus mormyrus* (Pisces, Sparidae), off the Canary Islands (Central-east Atlantic). *J. Appl. Ichthyol.*, 18: 204–209.

Love, R.M., 1980. The Chemical Biology of Fishes. Academic Press, London.

Lowry, O.H.; N.J. Rosebrough; N.J. Farr and Randall, R.J. 1951. Protein measuremrnt with the folin-Phenol reagent. *J. Biol.chem.* 183: 265-275.

Mabragana, E., Lucifora, L. O. and Massa, A. M. 2002. The reproductive ecology and abundance of *Sympterygia bonapartii* endemic to the south-west Atlantic. *Journal of Fish Biology,* 60: 951-967.

Mahfuj, M.S., Hossain, M., Belal Hossain, M. and Minar, M.H. 2012. Biochemical Composition of and Endangered Fish, *Labeo bata* (Hamilton, 1822) from Bangladesh Waters, *American Journal of Food Technology* 7(10): 633-641.

Mahmood, N. 1986. Hydrobiology of the Kaptai Reservor. Final report on the Kaptai Reservoir Studies Program. FAQ/UNDP Aquaculture Development and Coordination Programs, Rome, Italy. Contract No. DP/BGD/615-4.

Mallikarjuna, R. R. 1966. Studies of the biology of *Macrobrachium rosenbergii* (Deman) of the Hoogly estuary with notes on its fishery. *Proc. Nat. Ins. Sci. India*, 33(56): 252-278.

Mansur, M.A., Chakraborty, S.C., Narejo, N.T., Haque, M.M., Hossain, M.I., Shikha F.H., Kabir, M.S., 2004. A comparative study on freshness of fish at landing centre and in market of a peri-urban area of central north region of Bangladesh, Sindh Univ. Res. Jour. (Sci. Ser.), Vol: 36(2) 11-20.

Marais, J. F. K. and Erasmus, T. 1977. Body composition of *Mugil cephalus*, *Liza domerle*, *L. richardsoni* and *L. tricaspidus* (Teleostei : Mugilidae) caught in the Swartkops estuary. *Aqua*, 10: 75-86.

Marshal, L. A. 1965. *The Life of Fishes*. Weidenfeld and Nichason, London.

Martín, M. J. S., Perez, J. E. and Chiaramonte, G. E. 2005. Reproductive biology of the South West Atlantic marbled sand skate *Psammobatis bergi* Marini, 1932 (Elasmobranchii, Rajidae). *J. Appl. Ichthyol.,* 21:504–510.

Mathewos Hailu 2013. Reproductive aspects of common carp (*Cyprinus carpio* L, 1758) in a tropical reservoir (Amerti: Ethiopia). Journal of Ecology and the Natural Environment, Vol. 5(9), pp. 260-264.

Mathur, D. and T.W. Robbins, 1.971. Food habits and feeding chronology of young white crappie, *Pomoxis annularis* (Rafinesque) in Conowingo reservoir. Trans. Am. Fish. Soc., 100: 307-311.

Mathur, D., 1973. Food habits and feeding chronology of the blackbanded darter, *Percina nigrofasciata* (Agassiz) in Halawakee Creek, Alabama. Trans. Am. Fish. Soc., 102: 48-55.

Mazlan, A. G. and Rohaya, M. 2008. Size, growth and reproductive biology of the giant mudskipper, *Periophthalmodon schlosseri* (Pallas, 1770), in Malaysian waters. *J. Appl. Ichthyol.*, 24: 290-296.

Micale, V., Perdichizzi, F and Santangelo, G. 1987. The gonadal cycle of captive white bream, *Diplodus sargus* (L.). *J. Fish. Biol.*, 31:435-440.

Michael, G.R. 1969. Seasonal trends in physico-chemical factors and plnkton of a freshwater fish pond and their role in fish culture. *Hydrobiol.* 33: 144-159.

Milton, D. A. and Arthington, A. H. 1983. Reproductive biology of *Gambusia affinis halbrooki* (Baird and Girard), *Xiphophorus helleri* (Gunther) and *X. maculatus* (Heckel) (Pisces: Poeciliidae) in Queensland. *Australian J. Fish. Biol*., 23: 230-41.

Minar, M.H., R.K. Adhikary, M. Begum, M.R. Islam and T. Akter, 2012. Proximate composition of Hilsa *(Tenualosa ilisha)* in laboratory condition. Bangla. J. Progres. Sci. Technol., Vol. 10.

Mohajira Begum and Maruf Hossain Minar, 2012. Comparative Study About Body Composition of Different Sis Shell Fish and Ilish; Commonly Available in Bangladesh. DAMA International Vol. 1, No. 1. 38-42.

Mohsin A.B.M. 2010. Mirror carp : *Cyprinus carpio* var. *specularis*. Bangladesh Fisheries Information Share Home. www.en.bdfish.org/2010/01/mirror-carp-*Cyprinus carpio* var. *specularis*. Accessed on 12 May 2016.

Mollah, M. F. A. and Tan, E. S. P. 1982. Some aspects of reproductive biology of Malaysian freshwater catfish (*Clarias macrocephalus* Gunther). *Malays. Appl. Biol.*, 11(2): 125-134.

Morley, S. A., Mulvey, T., Dickson, J. and Belchier, M. 2004. The biology of the big eye grenadier at South Georgia. *Journal of Fish Biology*, 64: 1514-1529.

Morris, M. C., Sacks, F. & Rosner, B. 1993. Does fish oil lower blood pressure? A meta-analysis of controlled trials. Circulation 88:523-533.

Moyle, P. B. and Cech, J. J. 2000. *Reproduction in Fishes: An introduction to ichthyology*. (4th edition), Prentice Hall Inc. 123-144p.

Jan Muneera, Jan Ulfat and Mustafa S.G., 2014. Studies on Fecundity and Gonadosomatic Index of *Schizothorax plagiostomus* (Cypriniformes: Cyprinidae), Journal of Threatende Taxa [www.threatendedtaxa.org] 6(1): 5375-5379.

Mustafa, G., Das, M. and Huda, M. N. 1998. Fecundity and some relationships of grass carp (*Ctenopharyngodon idella* Cuv. And Val.). *J. Asiat. Soc. Bangladesh Sci.*, 24(2): 207-213.

Nabi, M. R. 1987. *Studies on the biology of freshwater spiny eel, Macroganthus aculeatus (Bloch) with notes on its fishery in Chalan beel area*. Ph. D. thesis (unpublished), Department of Zoology, University of Rajshahi. 264p.

Nabi, M. R. and Hossain, M. A. 1989. Seasonal variation in the chemical composition and caloric content of *Macrognathus aculeatus* (Bloch) from the Chalon Beel waters. *J. Asiatic Soc. Bangladesh (Sci.)*, 25: 103-110.

Nabi, M. R. and Hossain, M. A. 1996. Reproductive biology of the freshwater spiny eel *Macrognathus acculeatus* (Bloch). *Bangladesh J. Zool*., 24(2): 115-120.

Nagakura T, Matsuda S, Shichijyo H, Hata K. Dietary supplementation with fish oil rich in omega-3 polyunsaturated fatty acids in children with bronchial asthma. Eur Respir J. 2000;16:861-865.

Nargis, A. 2006. Seasonal variation in the chemical composition of body flesh of Koi fish *Anabas testudineus* (Bloch) (Anabantidae:Perciformes). *Bangladesh J. Sci. Ind. Res*., 41(3-4): 219-226.

Nargis, A. and Hossain, M. A. 1988. The fecundity and sex-ratio of the climbing perch, *Anabas testudineus* (Bloch) (Anabantidae: Perciformes). *J. Asiat. Soc. Bangladesh Sci*., 14(1): 21-27.

Nargis, A. and Hossain, M. A. 1992. Reproductive periodicity of climbing perch *Anabas testudineus* (Bloch) (Anabantidae: Perciformes). *Bangladesh. J. Sci. Ind. Res*., 27(1 & 2): 87-93.

Naser, M.N., G.W. Chowdhury, M.M. Befjum and W, Haque, 2007. Proximate composition of prawn, *Macrobrachium rosenbergii* and shrimp, *Penaeus monodon*. Dhaka Univ. J. Biol. Sci., 16: 61-66.

Nash, R. D. M. 1982. The biology of fires Goby, *Lesueurigolaius friessi*, in the Firth on Clyde, Scotland and a comparison with other stocks. *J. Fish. Biol*., 21: 69-85.

Nikolsky, G. V. 1963. *The Ecology of Fishes*. Academic Press, London. 352p.

Norbis W, Paesch L, Galli O. 2006. Los recursos pesqueros de la costa de Uruguay; ambiente, biologia y gestion. In: Menafra R, Rodriguez-Gallego L, Scarabino F, Conde D (eds), Bases para la Conservacion y el Manejo de la Costa Uruguaya. Vida Silvestre, Montevideo, Uruguay, pp 197-210.

Oker, T. C., Akyol, O., Özaydýn, O., Leblebici, S. and Tosunog¢lu, Z. 2008. Determination of batch fecundity in *Uranoscopus scaber* Linnaeus, 1758 from the Aegean Sea, Turkey. *J. Appl. Ichthyol*., 24: 85-87.

Oliva-Paterna, F. J., Torralva, M. M., Fernandez-Delgado, C. 2002. Age, growth and reproduction of *Cobitis paludica* in a seasonal stream. *Journal of Fish Biology*, 60: 389-404

Ortiz-Ordonez, E., Urıa-Galicia, E., Lopez-Lopez, E., Maya, J. P. and Carvajal Hernandez, A. L. 2007. Reproductive cycle by histological characterization of the ovary in the butterfly goodeid *Ameca splendens* from the upper Río Ameca Basin, Mexico. *J. Appl. Ichthyol.*, 23: 40-45.

Osako, K., A. Yamaguchi, T. Kurokawa, K. Kuwahara, H. Saito and Y. Nozaki, 2002. Chemical components and body color of horse mackerel caught in different areas. Fish. Sci., 68: 587-594.

Pajuelo, J. G., Lorenzo, J. M., Bilbao, A., Ayza, O. and Ramos, A. G. 2006a. Reproductive characteristics of the benthic coastal fish *Diplodus vulgaris* (Teleostei: Sparidae) in the Canarian archipelago, northwest Africa. *J. Appl. Ichthyol.*, 22: 414-418.

Pajuelo, J. G., Socorro, J., Gonzalez, J. A., Lorenzo, J. M., Perez-Penalvo, J. A., Martinez, I. and Hernandez-Cruz, C. M. 2006b. Life history of the red-banded seabream *Pagrus auriga* (Sparidae) from the coasts of the Canarian archipelago. *J. Appl. Ichthyol*., 22: 430-436.

Parween, S., Begum, N., Rahman, M. H. and Hossain, M. A. 1993. On the breeding periodicity of *Esomus danricus* (Hamilton). *Univ. j. zool. Rajshahi Univ.*, 12: 31-34.

Patra, R. W. R. and Azadi, M. A. 1987. Ecological studies on the planktonic organisms of the Halda River. *Bangladesh J. Zool*. 15(2): 109-123.

Piedras S.R., Pouey J.L., Moraes P.R. 2006. Comportamento alimentar e reprodutivo de peixes exoticos e nativos cultivados na zona sul do Rio Grande do Sul. *Revista Brasileira de Agrociencia* 12(3): 341-344.

Pinheiro, P., Broadhurst, M. K., Hazin, F. H. V., Bezerra, T. and Hamilton, S. 2006. Reproduction in *Bagre marinus* (Ariidae) off Pernambuco, northeastern Brazil. *J. Appl. Ichthyol*., 22: 189-192.

Pollock, B. R. 1985. The reproductive cycle of yellowfish bream, *Acanthopagrus australis* (Gunther) with particular reference to protandrous sex inversion. *J. Fish. Biol*., 26: 301-311.

Quaatey, S. N. K. and Maravelias, C. D. 1999. Maturity and spawning pattern of *Sardinella aurita* in relation to weter temperature and zooplankton abundance off Ghana, West Africa. *J. Appl. Ichthyol*., 15: 63-69.

Rahman, A. K. A. 2005. *Freshwater Fishes of Bangladesh* (2nd ed.). Zoological Society of Bangladesh, Dhaka, Bangladesh. xviii+394 pp.

Rahman, A.K.A., 1989. Freshwater Fishes of Bangladesh. Zoological Society of Bangladesh, Dhaka, Bangladesh, Pages: 364.

Rahman, M. H., Hossain, M. A. and Parween, S. 1992. Notes on the sex ratio of *Lepidocephalus guntea* (Hamilton) (Cypriniformes: Cyprinidae). *Univ. j. zool. Rajshahi Univ.,* 10 & 11: 111-112.

Rahman, M. H., Parween, S., Mortuza, M. G. and Ahsan, M. K. 2002. Reproductive periodicity and spawning potentiality of *Rhinomugil corsula* (Ham.) (Mugiliformes: Mugilidae). *Univ. j. zool. Rajshahi Univ.,* 21: 99-100.

Rahman, M. M., Hossain, M. A., Miah, M. A. M. and Rahman, M. M. 1989. An investigation into fisheries knowledge of fish farms toward fish culture management. *Bangladesh Journal of Training and Development*. 1(1):9-16.

Rahman, M. S. 1992. *Water Quality Management in Aquaculture.* BRAC Prokashana, Dhaka, Bangladesh. 84pp.

Rao, C.B. 1955. On the distribution of algae in a group of six ponds II. Algal Productivity. *J. Ecol.* 43: 291-308.

Robb, A. P. 1982. Histological observations on the reproductive biology of the haddock, *Melanogrammus aeglefinus* (L.). *J. Fish. Biol.,* 20: 397-408.

Roos, N. 2001. Fish consumption and aquaculture in rural Bangladesh: nutritional contribution and production potential of culturing small indigenous fish species (SIS) in pond polyculture with commonly cultured carps. Doctoral thesis. Research Department of Human Nutrition, The Royal Veterinary and Agricultural University, Frederiksberg, Denmark.

Roos, N., Leth, T., Jakobsen, J. & Thilsted, S. H. 2002. High vitamin A content in some small indigenous fish species in Bangladesh: perspectives for food-based strategies to reduce vitamin A deficiency. Int. J. Food Sci. Nutr. 53: 425–437.

Roulet, M., Frascarolo, P., Pilet, M. & Chapuis, G. (1997) Effects of intravenously infused fish oil on platelet fatty acid phospholipid composition and on platelet function in postoperative trauma. J. Parenter. Enteral Nutr. 21:296-301.

Royce, W. F. 1972. *Introduction to the Fishery Science*. Academic Press, New York, San Francisco, London. 351p.

Rubbi, S.F., M.M. Rahman, A.R. Khan, S.S. Jahan and M. Begum, 1987, Proximate composition and quality of some commercial species of freshwater fish. Bangladesh J. Sci., 5: 1-20.

Saha, K.C., Sen, D.P., Chowdhury, A.K.R. and Chakrabarty, 1957. Studies on the factors influencing spawning of Indian major carps in “bundh” fisheries. *Indian J. Fish*. 4: 284-294.

Sahai, R. and Sinha, A.B. 1969. Investigation on bio-ecological of inland waters of Gorakhpur (U.P.), India. *Hydrobiol*. 34: 433-447.

Santhanam, R, Sukumaran, N and Natarajan, P, 1999. *A Manual of Freshwater Aquaculture*. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India. 193pp.

Santic, M., Pallaoro, A. and Jardas, I. 2006. Co-variation of gonadosomatic index and parameters of length–weight relationships of Mediterranean horse mackerel, *Trachurus mediterraneus* (Steindachner, 1868), in the eastern Adriatic Sea. *J. Appl. Ichthyol*., 22: 214-217.

Shafi, M. A. and Quddus, M. M. A. 1974. The fecundity of the common punti, *Puntius stigma* (Cuvier and Vallenciennes) Cyrpinidae : Cypriniformes. *Bangladesh J. Zool*., 2(2): 133-145.

Shafi, M. and Mustafa, G. 1976. Observations on some aspects of the biology of climbing perch, *Anabas testudineus* (Bloch) (Anabantidae: Perciformes). *Bangladesh J. Zool.*, 4(1): 21-28.

Shafi, M., Quddus, M. M. A. and Islam, N. 1978. Maturation and Spawning of *Hilsa ilisha* (Hamilton-Buchanan) of the River Meghna. *Dhaka university studies* Part-B, XXVI(2): 63-71.

Shankar, D. S. and Kulkarni, R. S. 2005. Somatic condition of the fish, *Notopterus notopterus* (Pallas) during different phases of the reproductive cycle. *J. Environ Biol.*, 26(1):49-53.

Shyamasundari, K. 1972. A method for the simultaneous distribution of neurosecretory and mucoid substances in invertebrates. *Surr. Sci.*, 42(2): 78.

Siddique, K. and Choudhury, S. N. 1996. Matsya- A Manual on Pond Pisiculture by the National Institute of Local Goverment. Agargaon, Sher-e-Bangla Nagar, Dhaka. 92-93 pp.

Siddique, M.A.M., P. Mojumder and H. Zamal, 2012. jProximate composition of three commercially available marine dry fishes *(Harpodon nehereus, Johnius dussumieri* and *Lepturacanthus savala).* Am. J. Food TechnoL, 7: 429-436.

Simpson, A. C. 1951. The fecundity of the Plaice. *Fish Invest. Series*, 11: 17p.

Sindhe, V. R. and Kulkarni R. S. 2004. Gonadosomatic and hepatosomatic indices of the freshwater fish *Notopterus notopterus* (Pallas) in response to some heavy metal exposure. *J. Environ. Biol.*, 25(3): 365-368.

Sindhe, V. R. and Kulkarni, R. S. 2005. Fecundity of the freshwater fish, *Notopterus notopterus* (Pallas) in natural and heavy metal contaminated water. *J. Environ. Biol.*, 26(2):287-290.

Smith B.B, Walker K.F. 2004. Spawning dynamics of common carp in the River Murray, South Australia, shown by macroscopic and histological staging of gonads. J. Fish Biol. 64:336-354.

Smith, L. S. and Bell, G. R. 1975. A Practical Guide to the Anatomy and physiology of pacific Salmon. *Fish. Mar. Serv. Misc. Spec. Publ.*, 27:14.

Smith, P., Arnesen, H., Opstad, T., Dahl, K. H. & Eritsland, J. 1989. Influence of highly concentrated n-3 fatty acids on serum lipids and hemostatic variables in survivors of myocardial infarction receiving either oral anticoagulants or matching placebo. Thromb. Res. 53:467-474.

Snedecor, G. W. 1956. *Statistical methods.* The Lowa State College Press, Lowa. 534p.

Spillman, C. J. 1961 Faune de France: Poissons d'eau douce. Fédération Française des Sociétés Naturelles,Tome 65. Paris. 303 p.

Stansby, M. E. 1954. Composition of certain species of freshwater fish. *Food Res*., 16: 231-234.

Stephenson, A. 1934. The breeding of reef animals Part II. Invertebrates other than corals. Great Barrier Reef Expedition, 1928-29. *Sci. Rept*., 3: 247-272.

Sulikowski, J. A., Driggers III, W. B., Ford, T. S., Boonstra, R. K. and Carlson, J. K. 2007. Reproductive cycle of the black-nose shark *Carcharhinus acronotus* in the Gulf of Mexico. *Journal of Fish Biology*, 70: 428-440.

Sultana, A., Begum, M., Parween, S., Rahman, M. S. and Hossain M. A. 2001. Breeding periodicity and fecundity of *Rohtee cotio* (Hamilton) (Cypriniformes: Cyprinidae). *J. bio-sci*., 9: 75-78.

Summers, D. W. 1996. Environmental influences on the timing of Atlantic salmon, *Salmo salar* L., in the river North Esk. *Fish. Mgmt. Ecol*., 3: 281-283.

Swingle, H.S. 1967. Standardization of chemical analysis for waters and ponds muds. *FAO Fish Rep*. 4(44): 397-421.

Talwar, P. K. and Jhingran, A. G. 1991. *Inland Fishes of India and Adjacent Countries* (Vol. 1 and 2). Oxford & IBH Publishing Co. Pvt. Ltd., India. 1158pp.

Taylor, M.H. and DiMichelle, L. 1980. Ovarian, changes during the lunar spawning cycle of *Pundulus heteroclitus. Capera*. pp. 118-125.

Tempero G.W., Ling N., Hicks B.J., Osborne M.W. 2006. Age composition, growth, and reproduction of koi carp (*Cyprinus carpio*) in the lower Waikato region. N. Z. J. Mar. Freshw. Res. 40:571-583.

Treasurer, J.W. and Holliday, F.G.T. 1981. Some aspects of the reproductive biology of perch *Perca fluviatilis* L. A histological description of the reproductive cycle. *J. Fish. Bio*. 18: 359-376.

Vermeij G.J., Dudley R. 2000. Why are there so few evolutionary transitions between aquatic and terrestrial eco-systems? Biol. J. Linn. Soc. 70:541–554.

Volker D, Fitzgerald P, Major G, Garg M. Efficacy of fish oil concentrate in the treatment of rheumatoid arthritis. J Rheumatol. 2000; 27:2343-2346.

Vyas, L.N. and Kumar, H.D. 1968. Studies on the phytoplankton and other algae of indrasagar tank Udaipur, India, *Hydrobiologia*, 27L 529-547.

Waterman, T. H. 1961. *The physiology of crustacea*. Academic Press, New York. Vol. 1 & 2: 356p.

Weatherley, A.H. and H.S. Gill, 1987. The Biology of Fish Growth. Academic Press, London, Pages: 443.

Weber M., Brown M. 2009. Effects of Common Carp on Aquatic Ecosystems 80 Years after "Carp as a Dominant": Ecological Insights for Fisheries Management. *Reviews in Fisheries Science* 17(4): 524–537, http://dx.doi. org/10.1080/106412609031 89243

Welcomme, R. L. 1985. River Fisheries. FAO Fish. *Tech. Pap*. 262. 330 pp.

Wiley, B. and Collette, B. 1970. Breeding tubercles and contact organism fishes: Their occurrence, structure and significance. *Bull. Amer. Mus. Nat. Hist*., 143(3): 145-216.

Wojenski, C. M., Silver, M. J. & Walker, J. 1991. Eicosapentaenoic acid ethyl ester as an antithrombotic agent: comparison to an extract of fish oil. Biochim. Biophys. Acta 1081:33-38.

Wootton, R.J., 1990. Ecology of Teleost Fishes. 1st Edn., Chapman and Hall, London, ISBN-13: 9780412317200, Pages: 404.

Yadav, B. N. 1999. *Fish and Fisheries*. Daya Publishing House, Rri Nagar, Delhi, India. 366p.

Yoneda, M., Tokimura, M., Fujita, H., Takeshita, N., Takeshita, K., Matsuyama, M. and Matsuura, S. 1998. Reproductive cycle and sexual maturity of the anglerfish *Lophiomus setigerus* in the East China Sea with a note on specialized spermatogenesis. *Journal of Fish Biology*, 53: 164–178

Zanotta D.C., Ducati J.R., Goncalves G.A. 2010. Surface temperature patterns of Lagoa dos Patos, Brazil, Using NOAA-AVHRR data; an anual cycle analysis. *Pesquisas em Geociencias* 37(3): 219-226.

Zorica, B., Sinovcic, G., Pallaoro, A. and Cikes-Kec, V. 2006. Reproductive biology and length–weight relationship of painted comber, *Serranus scriba* (Linnaeus, 1758), in the Trogir Bay area (middle-eastern Adriatic). *J. Appl. Ichthyol.*, 22: 260-263.

CHAPTER-9

APPENDICES

Appendix Table 1: Monthly mean values (mean±sd) of different physical and chemical parameters of the Atrai river of northwestern (Naogaon District) Bangladesh during July, 2013 to June, 2015.

Months	Years	**Physical parameters**				**Chemical parameters**		
		Air temperature (°C)	Water temperature (°C)	Water transparency (m)	Rainfall (mm)	pH	DO mg/l	CO_2 mg/l
July	2013	37.11±0,48	36.22±0.62	0.61±0.28	280.82	7.93±0.39	5.47±0.82	9.08±0.85
	2014	35.10±0,47	35.00±0.68	0.41±0.83	235.72	8.00±0.93	6.40±0.88	9.18±0.82
August	2013	33.20±0.37	32.42±0.86	0.27±0.24	270.02	7.75±0.29	5.21±0.85	8.88±0.92
	2014	33.00±0.97	31.41±0.86	0.17±0.14	275.97	7.88±0.88	6.24±0.92	8.81±0.84
September	2013	30.10±0.19	29.10±0.32	0.32±0.31	256.82	7.99±0.62	5.41±0.78	9.12±0.98
	2014	31.17±0.49	30.25±0.36	0.22±0.24	256.89	7.89±0.63	5.11±0.78	9.11±0.88
October	2013	28.11±0.47	27.41±0.91	0.49±0.34	247.16	7.98±0.22	5.92±0.49	5.22±1.47
	2014	28.00±0.28	27.60±0.80	0.39±0.44	235.46	8.01±0.24	6.12±0.55	5.29±1.42
November	2013	25.30±0.49	24.14±0.74	0.49±0.29	20.33	7.87±0.47	6.10±0.82	5.10±0.72
	2014	25.20±0.62	25.04±0.74	0.50±0.30	12.34	8.72±0.40	6.75±0.89	6.11±0.74
December	2013	19.98±0.43	17.11±0.78	0.56±0.47	11.95	8.01±0.37	6.03±0.79	5.42±0.34
	2014	17.50±0.14	16.38±0.78	0.56±0.25	Nil	8.04±0.38	6.93±0.70	6.42±0.99
January	2014	16.13±0.47	13.17±0.62	0.69±0.19	Nil	8.49±0.59	7.10±0.86	4.98±0.55
	2015	18.45±0.47	17.50±0.74	0.75±0.30	6.09	8.40±0.59	7.40±0.80	4.90±0.55
February	2014	20.40±0.24	18.54±0.69	0.60±0.24	8.42	7.98±0.47	7.01±1.04	5.44±0.94
	2015	21.40±0.88	20.42±0.69	0.65±0.15	9.00	6.98±0.82	7.14±1.04	4.40±0.92
March	2014	27.60±0.32	23.36±0.88	0.52±0.28	24.53	7.92±0.61	6.11±0.99	6.06±0.44
	2015	26.60±0.32	25.36±0.88	0.51±0.84	23.42	7.03±0.63	6.40±0.98	6.76±0.49
April	2014	31.28±0.57	30.99±0.47	0.40±0.49	85.35	7.75±0.22	6.05±0.77	7.04±0.82
	2015	30.20±0.67	29.08±0.42	0.41±0.25	75.35	7.85±0.25	6.09±0.97	8.04±0.99
May	2014	32.41±0.66	30.41±1.73	0.31±0.16	90.29	7.92±0.29	6.03±0.74	7.15±1.09
	2015	36.40±0.62	32.15±1.00	0.32±0.30	85.24	6.94±0.59	6.13±0.72	8.00±1.90
June	2014	35.13±0.37	33.25±0.32	0.26±0.23	216.82	8.00±0.43	5.98±0.59	8.74±0.95
	2015	38.10±0.34	37.15±0.32	0.32±0.55	205.82	8.44±0.42	6.00±0.50	8.72±0.95
Mean± SD		**28.24± 6.53**	**26.81± 6.75**	**0.45± 0.16**	**122.24± 112.89**	**7.91± 0.43**	**6.21± 0.60**	**7.00± 1.68**

First year observation : July, 2013 to June, 2014.
Second year observation : July, 2014 to June, 2015.

Appendix Table 2: *C. carpio* var. *specularis;* Monthly percentage of gravid female for two years (July, 2013 to June, 2015).

Months	Years	Total no. of specimens	No. of males	No. of females			Gravid percentage
				Total	Non-gravid	Gravid	
July	2013	49	24	25	23	2	8.00
	2014	46	23	23	21	2	8.70
August	2013	52	27	25	23	2	8.00
	2014	54	28	26	25	1	3.85
September	2013	54	30	24	21	3	12.50
	2014	55	31	24	22	2	8.33
October	2013	45	22	23	19	4	17.39
	2014	49	25	24	21	3	12.50
November	2013	43	20	23	3	20	86.96
	2014	44	20	24	4	20	83.33
December	2013	40	18	22	4	18	81.82
	2014	42	19	23	3	20	86.96
January	2014	38	16	22	4	18	81.81
	2015	39	17	22	3	19	86.36
February	2014	38	15	23	2	21	91.30
	2015	41	16	25	2	23	92.00
March	2014	41	17	24	3	21	87.50
	2015	41	18	23	2	21	91.30
April	2014	43	19	24	6	18	75.00
	2015	44	20	24	5	19	79.17
May	2014	46	21	25	20	5	20.00
	2015	47	22	25	20	5	20.00
June	2014	47	22	25	22	3	12.00
	2015	46	22	24	21	3	12.50
Total		**1084**	**512**	**572**	**299**	**273**	**47.73**

Appendix Table 3: Monthly variation in gonadal length index (GLI), gonado somatic index (GSI) and ova diameter of *C. carpio* var. *specularis* (N = 273).

Years	Months	No. of fish (N)	Gonadal Length Index (GLI)			Gonado Somatic Index (GSI)			Ova Diameter (mm)		
			Min	Max	Mean±SD	Min	Max	Mean±SD	Min	Max	Mean±SD
2013	Jul.	8	15.58	42.00	24.13±5.77	0.16	4.46	0.68±0.85	0.46	0.74	0.62±0.12
	Aug.	9	12.16	44.78	21.64±7.12	0.21	0.63	0.36±0.14	0.22	0.55	0.35±0.12
	Sep.	8	16.10	30.70	23.01±3.90	0.18	2.46	0.67±0.60	0.45	0.75	0.60±0.13
	Oct.	10	17.91	43.97	25.02±5.82	0.24	3.00	0.89±0.14	0.65	0.89	0.77±0.09
	Nov.	**13**	**23.58**	**39.74**	**32.30±4.08**	**0.36**	**11.43**	**3.20±3.14**	**0.78**	**1.22**	**1.01±0.16**
	Dec.	**12**	**21.43**	**49.06**	**35.99±6.94**	**0.34**	**12.60**	**5.12±3.25**	**0.85**	**1.35**	**1.08±0.20**
2014	**Jan.**	**14**	**28.43**	**40.00**	**35.26±3.66**	**0.48**	**15.56**	**6.18±4.24**	**1.02**	**1.42**	**1.24±0.14**
	Feb.	**16**	**27.12**	**51.37**	**38.24±5.38**	**0.49**	**17.88**	**8.34±6.18**	**1.15**	**1.55**	**1.36±0.14**
	Mar.	**15**	**22.13**	**46.03**	**37.64±5.30**	**0.35**	**19.56**	**8.09±5.56**	**1.06**	**1.44**	**1.27±0.13**
	Apr.	**14**	**24.56**	**45.61**	**34.66±5.59**	**0.40**	**24.82**	**5.40±5.65**	**0.96**	**1.31**	**1.16±0.12**
	May	12	18.52	39.34	24.70±5.62	0.21	17.82	1.38±3.80	0.78	1.02	0.93±0.10
	Jun.	10	17.45	33.23	23.01±4.20	0.16	5.86	0.83±1.22	0.62	0.91	0.77±0.12
	Jul.	8	14.34	57.92	25.73±9.16	0.22	2.32	0.64±0.59	0.44	0.75	0.60±0.12
	Aug.	9	11.99	26.88	20.89±3.83	0.21	1.88	0.58±0.35	0.24	0.58	0.36±0.13
	Sep.	8	13.71	30.00	22.74±4.93	0.10	2.82	0.66±0.56	0.46	0.78	0.62±0.12
	Oct.	9	19.39	35.16	27.55±4.04	0.21	3.85	1.03±0.79	0.60	0.90	0.75±0.11
	Nov.	**10**	**22.60**	**43.64**	**33.10±4.78**	**0.39**	**9.22**	**3.10±2.68**	**0.75**	**1.19**	**0.97±0.17**
	Dec.	**12**	**24.17**	**50.39**	**36.35±5.76**	**0.34**	**13.94**	**6.08±3.92**	**0.88**	**1.98**	**1.16±0.27**
2015	**Jan.**	**13**	**29.09**	**40.00**	**35.93±2.81**	**0.50**	**17.89**	**7.63±5.05**	**1.09**	**1.45**	**1.25±0.12**
	Feb.	**16**	**28.80**	**52.00**	**38.32±5.62**	**0.54**	**18.49**	**7.63±5.30**	**1.18**	**1.58**	**1.38±0.14**
	Mar.	**14**	**22.58**	**45.46**	**37.88±10.18**	**0.92**	**16.66**	**7.60±5.79**	**1.13**	**1.46**	**1.30±0.11**
	Apr.	**13**	**21.74**	**67.37**	**35.98±5.21**	**0.25**	**23.65**	**5.03±5.95**	**0.98**	**1.28**	**1.15±0.10**
	May	11	16.15	54.55	26.27±8.70	0.24	15.12	1.86±3.78	0.75	1.07	0.92±0.11
	Jun.	9	15.07	43.75	23.30±6.61	0.22	8.68	1.09±2.17	0.59	0.88	0.72±0.10

Note: The bold months indicate breeding season.

Appendix Table 4: Showing the estimated number of mature ova, total length, standard length, total weight, gonadal length and gonadal weight in different size groups in females of *C. carpio* var. *specularis* (N = 273).

Size groups (cm)	Mean					
	Total length (TL) in cm	Standard length (SL) in cm	Total weight (TW) in g	Gonadal length (GL) in cm	Gonadal weight (GW) in g	Fecundity
10-13 (N = 21)	11.66	8.58	296.67	3.58	4.91	5928.91
13-16 (N = 26)	13.72	9.88	404.15	4.23	5.17	6528.24
16-19 (N = 35)	17.50	11.81	695.03	6.52	12.86	17202.96
19-22 (N = 30)	20.59	13.84	751.12	7.52	19.60	28081.01
22-25 (N = 26)	23.53	16.55	807.00	8.90	34.28	48610.25
25-28 (N = 24)	26.47	19.83	464.25	9.71	47.83	68096.99
28-31 (N = 32)	29.74	22.36	678.21	10.79	56.40	77480.22
31-34 (N = 25)	32.61	24.74	1040.28	12.38	80.31	103599.63
34-37 (N = 22)	35.78	27.92	1294.58	14.05	143.07	209120.18
37-40 (N = 18)	38.48	30.61	1475.93	13.74	133.89	195321.84
40-43 (N = 14)	41.38	33.40	1776.54	15.48	142.57	201187.50
Mean±SD	**26.50±10.04**	**19.96±8.56**	**880.34±468.29**	**9.72±4.00**	**61.90±55.02**	**87377.98±79544.17**

Appendix Table 5: Monthly male and female distribution and sex ratio of *C. carpio* var. *specularis* (July, 2013 to June, 2015).

Months	Years	Total no. of specimens	No. of Males	No. of Females	Percentage of males	Percentage of females	Sex ratio male : female
July	2013	49	24	25	48.98	51.02	1:1.04
	2014	46	23	23	50.00	50.00	1:1.00
August	2013	52	27	25	51.92	48.08	1:0.93
	2014	54	28	26	51.85	48.15	1:0.93
September	2013	54	30	24	55.56	44.44	1:0.80
	2014	55	31	24	56.36	43.64	1:0.77
October	2013	45	22	23	48.89	51.11	1:1.05
	2014	49	25	24	51.02	48.98	1:0.96
November	2013	43	20	23	46.51	53.49	1:1.15
	2014	44	20	24	45.46	54.55	1:1.20
December	2013	40	18	22	45.00	55.00	1:1.22
	2014	42	19	23	45.24	54.76	1:1.21
January	2014	38	16	22	42.11	57.90	1:1.38
	2015	39	17	22	43.59	56.41	1:1.29
February	2014	38	15	23	39.47	60.53	1:1.53
	2015	41	16	25	39.02	60.98	1:1.56
March	2014	41	17	24	41.46	58.54	1:1.41
	2015	41	18	23	43.90	56.10	1:1.28
April	2014	43	19	24	44.19	55.81	1:1.26
	2015	44	20	24	45.46	54.55	1:1.20
May	2014	46	21	25	45.65	54.35	1:1.19
	2015	47	22	25	46.81	53.19	1:1.14
June	2014	47	22	25	46.81	53.19	1:1.14
	2015	46	22	24	47.83	52.17	1:1.09
Total		**1084**	**512**	**572**	**47.23**	**52.77**	**1:1.12**

Appendix Table 6: Calculated value total χ^2, overall χ^2 including χ^2 heterogeneity.

Years	Months	Sex	O	E	(O-E)	$(O-E)^2$	$\chi^2 = \frac{(O-E)^2}{E}$	Total χ^2	χ^2 Hetero-geneity
2013	July	Male	24	24.50	-0.50	0.25	0.01	5.97	2.65
		Female	25	24.50					
	Aug.	Male	27	26	1.00	1.00	0.04		
		Female	25	26					
	Sep.	Male	30	27	3.00	9.00	0.33		
		Female	24	27					
	Oct.	Male	22	22.5	-0.50	0.25	0.01		
		Female	23	22.5					
	Nov.	Male	20	21.5	-1.50	2.25	0.11		
		Female	23	21.5					
	Dec.	Male	18	20	-2.00	4.00	0.20		
		Female	22	20					
2014	Jan.	Male	16	19	-3.00	9.00	0.47		
		Female	22	19					
	Feb.	Male	15	19	-4.00	16.00	0.84		
		Female	23	19					
	Mar.	Male	17	20.5	-3.50	12.25	0.60		
		Female	24	20.5					
	Apr.	Male	19	21.5	-2.50	6.25	0.29		
		Female	24	21.5					
	May	Male	21	23	-2.00	4.00	0.17		
		Female	25	23					
	Jun.	Male	22	23.5	-1.50	2.25	0.10		
		Female	25	23.5					
	July	Male	23	23	0.00	0.00	0.00		
		Female	23	23					
	Aug.	Male	28	27	1.00	1.00	0.04		
		Female	26	27					
	Sep.	Male	31	27.5	3.50	12.25	0.45		
		Female	24	27.5					
	Oct.	Male	25	24.5	0.50	0.25	0.01		
		Female	24	24.5					
	Nov.	Male	20	22	-2.00	4.00	0.18		
		Female	24	22					
	Dec.	Male	19	21	-2.00	4.00	0.19		
		Female	23	21					
2015	Jan.	Male	17	19.5	-2.50	6.25	0.32		
		Female	22	19.5					
	Feb.	Male	16	20.5	-4.50	20.25	1.00		
		Female	25	20.5					
	Mar.	Male	18	20.5	-2.50	6.25	0.31		
		Female	23	20.5					
	Apr.	Male	20	22	-2.00	4.00	0.18		
		Female	24	22					
	May	Male	22	23.5	-1.50	2.25	0.10		
		Female	25	23.5					
	Jun.	Male	22	23	-1.00	1.00	0.04		
		Female	24	23					
Overall χ^2		Male	512	542	-30.00	900.00	1.66	3.32	
		Female	572	542	30.00	900.00	1.66		

Appendix Table 7: Seasonal variation in biochemical composition of male and female *C. carpio* var. *specularis* in three seasons.

Seasons	Sex	Biochemical composition (%)				
		Moisture (g)	Ash (g)	Lipid (g)	Protein (g)	Carbohydrate (g)
Summer	Male	72.54 ± 0.551^{b}	3.02 ± 0.637^{a}	4.19 ± 0.231^{a}	20.07 ± 0.190^{a}	0.035 ± 0.002^{a}
	Female	73.32 ± 0.778^{b}	2.64 ± 0.393^{a}	4.08 ± 0.400^{a}	19.56 ± 0.538^{a}	0.037 ± 0.004^{a}
Rainy	Male	73.70 ± 0.630^{ab}	3.13 ± 0.423^{a}	3.80 ± 0.362^{ab}	18.71 ± 1.079^{a}	0.042 ± 0.005^{a}
	Female	74.13 ± 1.146^{ab}	3.41 ± 0.630^{a}	3.58 ± 0.684^{ab}	17.99 ± 1.226^{ab}	0.041 ± 0.005^{a}
*Winter	Male	74.53 ± 1.078^{a}	3.44 ± 0.536^{a}	3.36 ± 0.400^{b}	18.25 ± 1.189^{a}	0.040 ± 0.001^{a}
	Female	75.42 ± 0.538^{a}	3.77 ± 0.766^{a}	2.95 ± 0.349^{b}	17.03 ± 0.925^{b}	0.042 ± 0.003^{a}

Figures bearing common letter(s) in a column as superscript do not differ significantly ($P < 0.05$).

*Winter season is the spawning period of the species.