STUDY ON REPRODUCTIVE BIOLOGY, CAPTIVE BREEDING, LARVAL REARING AND GROW-OUT OF GIANT SNAKEHEAD, CHANNA *MARULIUS* (HAMILTON, 1822)

THESIS

SUBMITTED IN FULFILMENT FOR THE AWARD OF THE DEGREE OF

Doctor of Philosophy

IN

APPLIED ANIMAL SCIENCES

Submitted By

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Dedicated to

My Beloved Parents

&

Teachers

DECLARATION

I hereby declare that this thesis entitled "**Study on Reproductive Biology, Captive Breeding, Larval Rearing and Grow-out of Giant Snakehead, *Channa marulius* (Hamiltom, 1822)''** submitted by me under the supervision and guidance of **Dr. Abha Mishra,** *Internal Supervisor* and **Dr. Sudhir Raizada**, *external supervisor* in accomplishment of the degree of Ph.D. in Applied Animal Sciences in the Department of Applied Animal Science, School of Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University, (A Central University) Lucknow (U.P.), India. It is up shot of my own endeavour of original research work.

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The thesis submitted to **Babasaheb Bhimrao Ambedkar University, Lucknow** satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations-1999 as amended in 2010* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

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(Kailash Chandra Yadav)

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List of abbreviation and symbols

±	Add or subtract
×	Multiply
µ	Micron
≤	Less than or equal to
≥	More than or equal to
≠	Not equal to
∑	Summation
%	Percentage
G	Gram
L	Liter
M	Meter
Mg	Milligram
mL	Milliliter
Cm	Centimeter
SD	Standard Deviation
∑	Total
Temp	Temperature
BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
EC	Electrical Conductivity
TDS	Total dissolved solids
LWR	Length Weight Relationship
GSI	Gonado Sonatic Index
FAO	Food and Agriculture Organization
Kg	Kilogram
W	Fish Weight
L	Standard Length
a	Constant (intercept)
b	Length exponent
K	Ponderal Index
R^2	Coefficient of determination
R	Correlation Coefficients

Chapter 1

INTRODUCTION

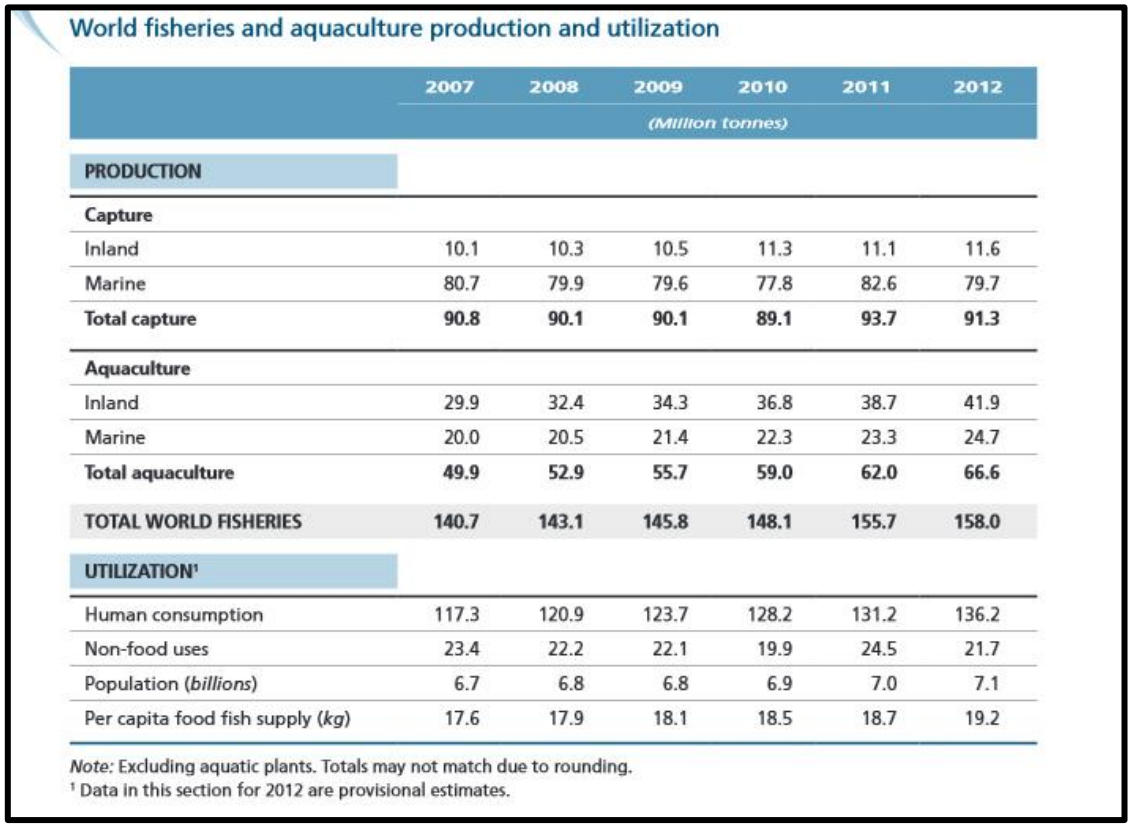
Chapter 1
INTRODUCTION

1.1. BACKGROUND

The global population is likely to increase from some 1.6 billion in 1900 to around 8.0 billion in 2025, suggesting an almost five times increase in the human mouths to be fed over one hundred and twenty-five years from the available food energy resources of the globe. The increasing human population is liable to result in increasing food insecurity at individual, national and global levels; leading to a multitude of socio-economic and political problems, ultimately resulting in political and economic instabilities. Food availability is thought to be the key-factor in achieving the food security, yet it may not be able to fight the problem of malnutrition particularly those living below the poverty line in the developing and under developed countries. Recent advances in agricultural sciences and agricultural technologies have averted the problem of starvation, to certain degree, yet have not been able to provide balanced nutrition to satisfy health needs to the majority of the human population. Diversifying the food base along with technologies to increase productivity are collectively required for fighting starvation and malnutrition together with cheaper availability of balanced food for the poverty stricken masses. The problem of malnutrition can thus be addressed by improving access to the quantity as well the quality of food which may require diversification of the present food base.

Global fish production growth continues to increase at an average annual rate of 3.2 percent, outpacing world population growth at 1.6 percent. In 2012, while global marine capture fishery production was stable at about 80 million tonnes, global aquaculture production set another all-time high at more than 66.6 million tonnes (excluding almost 24 million tonnes of aquatic plants) (Table 1). Aquaculture remains one of the fastest-growing food-producing sectors and is set to play a key role in meeting the rising demand for fishery products. The share of fisheries production used

for direct human consumption increased from about 70 percent in the 1980s to more than 85 percent (136 million tones, Table 1) in 2012 (FAO, 2014).



World fisheries and aquaculture production and utilization

	2007	2008	2009	2010	2011	2012
	(Million tonnes)					
PRODUCTION						
Capture						
Inland	10.1	10.3	10.5	11.3	11.1	11.6
Marine	80.7	79.9	79.6	77.8	82.6	79.7
Total capture	**90.8**	**90.1**	**90.1**	**89.1**	**93.7**	**91.3**
Aquaculture						
Inland	29.9	32.4	34.3	36.8	38.7	41.9
Marine	20.0	20.5	21.4	22.3	23.3	24.7
Total aquaculture	**49.9**	**52.9**	**55.7**	**59.0**	**62.0**	**66.6**
TOTAL WORLD FISHERIES	**140.7**	**143.1**	**145.8**	**148.1**	**155.7**	**158.0**
UTILIZATION[1]						
Human consumption	117.3	120.9	123.7	128.2	131.2	136.2
Non-food uses	23.4	22.2	22.1	19.9	24.5	21.7
Population (*billions*)	6.7	6.8	6.8	6.9	7.0	7.1
Per capita food fish supply (*kg*)	17.6	17.9	18.1	18.5	18.7	19.2

Note: Excluding aquatic plants. Totals may not match due to rounding.
[1] Data in this section for 2012 are provisional estimates.

Table 1: World fish production and utilization (FAO, 2014)

World per capita apparent fish consumption increased from an average of 9.9 kg in the 1960s to 19.2 kg in 2012 (Table 1 and Figure 1). A portion of 150 g of fish can provide about 50–60 percent of an adult's daily protein requirements. In 2010, fish accounted for 16.7 percent of the global population's intake of animal protein and 6.5 percent of all protein consumed. Moreover, fish provided more than 2.9 billion people with almost 20 percent of their intake of animal protein, and 4.3 billion people with about 15 percent of such protein. Protein from fish is a crucial nutritional component in some densely populated countries, where total protein intake levels may be low. Consuming fish is particularly important during

pregnancy and the first two years of life and can help lower the risk of coronary heart disease mortality (FAO, 2014).

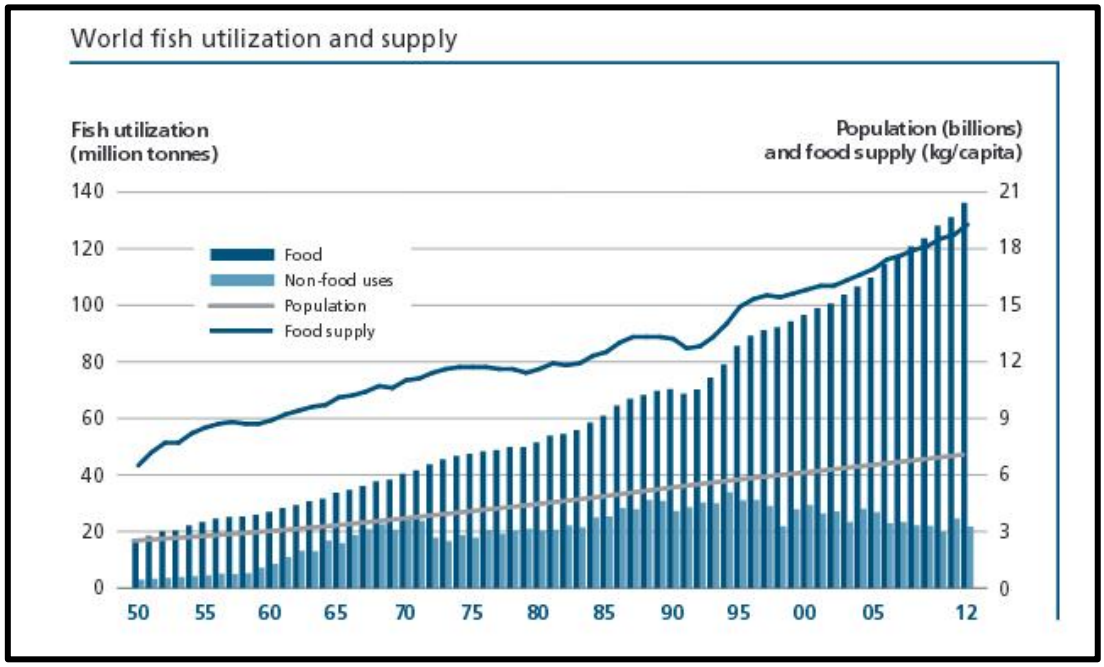


Figure 1: World fish utilization and supply (FAO, 2014)

The fisheries and aquaculture sector is also a source of employment and income, supporting the livelihoods of 10–12 percent of the world's population. In 2012, employment in the sector grew faster than the world's population, with almost 58.3 million people engaged in the primary sector of capture fisheries and aquaculture in 2012, 90 percent small-scale fishers and 15 percent of them women. In post-harvest activities such as processing, women can account for up to 90 percent of workers. The sector represented 4.4 percent of the 1.3 billion people economically active in the broad agriculture sector worldwide (FAO, 2014)

Protein is the basic food ingredient and hence is very important for human health and normal growth. Under the present trends, the major part of the protein requirements of the human populations is met from livestock and poultry resources. Aquaculture also provides the quality protein (Alikunhi, 1953), yet is not presently

exploited to its capacity. Whereas the production of farmed poultry and livestock is reaching its saturation point, aquaculture has expanded only during recent years and still has sufficient potential to grow to help save the human population from malnutrition occurred through protein deficiency. Fish is the most important single source of high quality white protein. It is generally believed that a consistent source of fish is essential for the nutritional health of a large segment of the world's population. Fish has the potential to ensure a quality food, providing some 17 % of the animal protein consumed by man and available estimates suggest that some one billion people, worldwide, rely on fish as their primary source of animal protein (FAO, 2014). Fish also has substantial social and economic importance.

The share of total fishery production exported in different product forms for human consumption or non-edible purposes grew from 25 percent in 1976 to 37 percent (58 million tonnes, live-weight equivalent) in 2012. Fishery exports reached a peak of US$129.8 billion in 2011, up 17 percent on 2010, but declined slightly to US$129.2 billion in 2012 following downward pressure on international prices of selected fish and fishery products.

The major source of the fish has primarily been the capture fisheries, and currently, on a global scale almost 58 percent of the total food fish supply comes from harvesting natural waters, mainly the marine waters (Figure 2). Inland capture fisheries till date has a relatively little contribution of around 12.7% in meeting the global demand of fish protein. With the increasing demand for fish in the market under increasing human population and health awareness, the stress on natural fisheries resources is increasing, particularly that of high valued species. Under such circumstances the capture fisheries has not been able to keep pace with the gradually increasing market demands. Evident signs of over-harvesting of the marine fisheries resources have started appearing in many areas, as is indicated by a decreased catch despite the increasing number and sophistication of the catching gadgets. On the contrary, the proportion of fisheries production used for direct

human consumption has been increasing. In the 1980s, about 71 percent of the fish produced was destined for human consumption, this share grew to 73 percent in the 1990s, and to 81 percent in the 2000s. In 2012, more than 86 percent (136 million tonnes) of world fish production was utilized for direct human consumption (FAO, 2014).

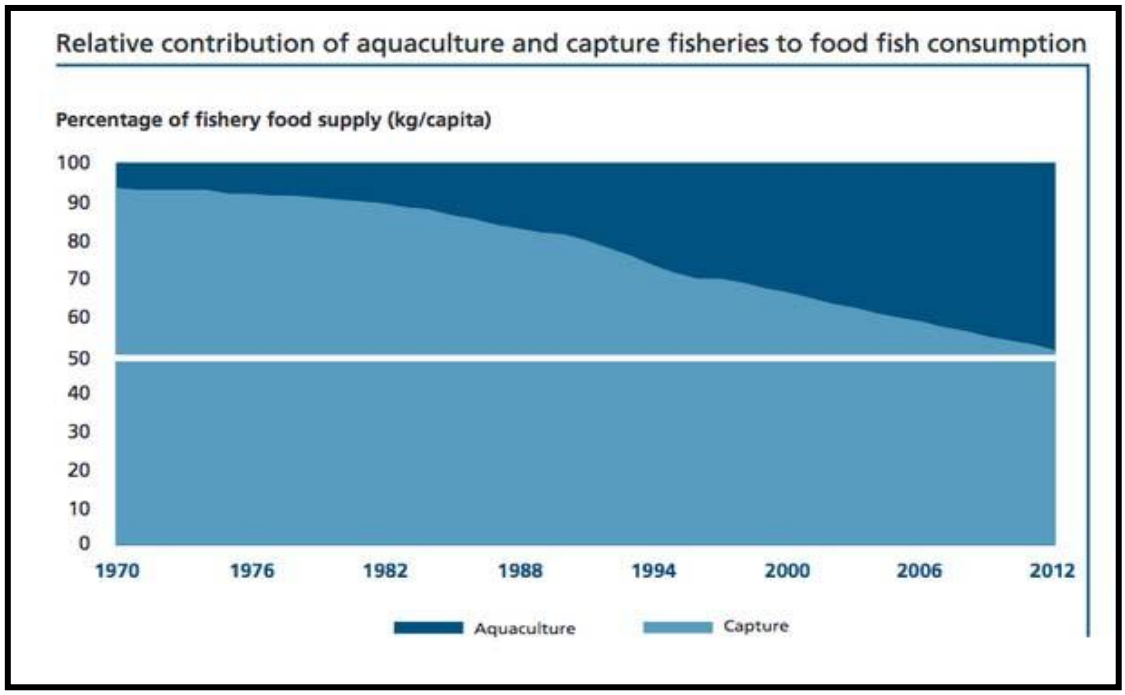


Figure 2: Relative contribution of aquaculture and capture fisheries to food fish consumption (FAO, 2014)

This has serious consequences on the natural fisheries resources, as the pressure of a higher demand forces the harvesters to put in more efforts. This has resulted in fishing much above the renewable potentials of the fish resource and hence the gradual depletion of the stock of natural commercial fisheries. It is believed that around half of the known ocean fisheries resources are completely exploited (FAO, 1999) and around 70% require immediate management measures to save such stocks for the future generations (MacLennan, 1995). Under such a pattern of harvesting the natural fish resources, the capture fisheries have become stagnant, and in many places are showing declining trends (FAO, 2008).

To meet the ever increasing demand for the fish, aquaculture in the recent years has expanded rapidly and is now becoming the fastest growing food-producing industry in the world. In contrast to the stagnation or even the decline of capture fisheries, world aquaculture production attained another all-time high of 90.4 million tonnes (live weight equivalent) in 2012 (US$ 144.4 billion), including 66.6 million tonnes of food fish (US$ 137.7 billion) and 23.8 million tonnes of aquatic algae (mostly seaweeds, US$ 6.4 billion). However, with the exception of China, the world aquaculture production has exhibited a somewhat lower annual growth rate (6.2%) during 2000-2012, more slowly than in the periods 1980–1990 (10.8 percent) and 1990–2000 (9.5 percent) (FAO, 2014).

The major present day concern is to increase per unit area production by applying different innovations / technologies rather than increasing the total area under the aquaculture. Apart from testing different advanced technologies for obtaining higher growth potential, the addition of new fish species into the aquaculture system is also being exercised to widen the aquaculture base and to fetch a higher market price. It is believed that aquaculture potential still exists for many fish species. However, the successful introduction of a new species into the aquaculture system needs careful study of its biology, though not much for the native species, but at least more for the exotic species for safe guarding indigenous species diversity and in turn ecosystem management. Many fish species introduced for aquaculture in the past in Indian waters like tilapia and Thai magur have caused severe loss to indigenous fish species. Therefore, any addition of new species in aquaculture system for diversification needs careful examination of its biology and likely impacts that may result on its being cultured. The ‘Giant snakehead’ is one of such native species which has been in great demand not only in India but all over the south-east Asia and USA due to several of its qualities given under to express its importance for the study.

1.2 DESCRIPTION OF GIANT SNAKEHEAD

The 'Giant snakehead' also known by various names as 'Great snakehead' or ‘Giant murrel' or ‘Bulls-eye snakehead or ‘Cobra snakehead or ‘Indian snakehead’ is the largest and fastest growing channidae fish among 35 fish species found globally (Figure 3). Taxonomically it is known as *Channa marulius* (Hamilton, 1822) and belongs to the family Channidae (superclass Osteichthyes, class Actinopterygii, subclass Neopterygii, infraclass Teleost, supraorder Acanthopterigii, order Perciformes, suborder Channoidei). The species is also known by several taxonomic names by different workers as *Ophicephalus marulius* (Hamilton, 1822); *Channa marulia* (Hamilton, 1822); *Ophiocephalus marulius* (Hamilton, 1822); *Ophiocephalus grandinosus* (Cuvier, 1831); *Ophicephalus sowara* (Cuvier, 1831); *Ophicephalus leucopunctatus* (Sykes, 1839); *Ophiocephalus theophrasti* (Sykes, 1839); *Ophiocephalus pseudomarulius* (Gunther, 1861); *Ophiocephalus aurolineatus* (Day, 1870); *Ophiocephalus marulius ara* (Deraniyagala, 1945); *Channa marulius ara* (Deraniyagala, 1945); and *Ophicephalus marulius ara* (Deraniyagala, 1945). However, the name *Channa marulius* (Hamilton, 1822) is the current accepted name and others are considered synonyms (Fishbase, 2014).

In India, the species is known by several vernacular names as *Hall* in Assam; *Sal, Gajal* in West Bengal; *Pumurl, Bhor* in Bihar; *Kubrah, Sawal, Dowlah* in Punjab; *Saal* in Odisha; *Poomeenu, Phoola-chapa, Phool-mural* in Andhra Pradesh; *Aviri, Puveral* in Tamil Nadu; *Chaeru-veraal, Curuva, Bral* in Kerala; *Hoovina-murl, Madhinhi, Aviu* in Karnataka (Talwar and Jhingran, 1992).

Figure 3: An adult specimen of *Channa marulius*

Geographically the species is distributed in Pakistan, India, SriLanka, Bangladesh, Nepal, Burma, Thailand and China (Talwar and Jhingran, 1992) (Figure 4) though this species has been established in inland water of North America due to introductions (U.S. Fish and Wildlife Service, 1922). It inhabits large lakes and rivers; prefers deep, clear stretches of water with sandy or rocky bottom, swamps, marshes and rice fields (Talwar and Jhingran, 1992; Kilambi, 1986) and reported to attain maximum length of 180 cm and weight up to 30 kg. The species is very tasty at plater and considered a favourite sport fish as can be caught with ladle or spoon.

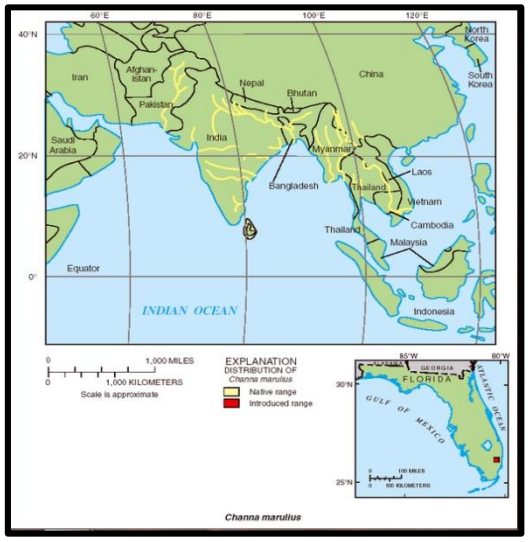


Figure 4:
Map showing natural distribution of *Channa marulius* from Pakistan to south-east Asia.
(Source: USGS, 2-12)

1.3 IMPORTANCE OF THE SPECIES

The snakeheads as a group constitute an important element of freshwater fishery in many parts of India (Parameswaran and Kamal, 1988; Talwar and Jhingran, 1992). They are in great demand as food fish due to its appealing flavor (Hossain *et al.*, 2008), few muscular spines, medicinal importance (Baie and Sheikh, 2000; Mat Jais *et al.*, 1994 and 1997; Somchit *et al.*, 2004; Wee, 1982; Zakaria *et al.*, 2004; Zuraini *et al.*, 2006) and air-breathing nature that facilitate easy transport in live condition to the markets (Wee, 1982). They are well known for their therapeutic and antinociceptive activities and hence consumed by many to induce healing and pain control after a

clinical operations. This has been concluded under various studies that demonstrated to contain high arachidonic acid (AA), which is a precursor of prostaglandin that may initiate blood clotting and be responsible for growth. Similarly fillet extract and mucus of the fish has been found to reduce pain in abdominal constriction and tail flick tests in mice. Snakehead also contains all the essential amino acids for wound healing, particularly glycine which is most important component of human skin collagen (Baie and Sheikh, 2000; Mat Jais *et al.*, 1994 and 1997; Zuraini *et al.*, 2006).

However, due to environment degradation and absence of aquaculture technology, the population of this species is declining with a rapid pace in last 3-4 decades in natural waters and there is a need to sustain their population through conservation and aquaculture techniques or better through principals of conservation aquaculture.

The snakehead (striped snakehead: *C. striatus*) culture is widely popular in Thailand and on limited scale in India, Philippines and Taiwan (Chacko, 1947; Chacko and Kurian, 1947; Chen, 1990; Wee, 1981 and 1982). By and large very little work has been carried out on captive breeding and culture of *C. marulius* due to poor understanding of breeding, larval rearing and grow-out requirements. Though species breeds in almost all type of aquatic systems including rivers, reservoirs, rice-fields, ponds and small cement tanks and their seed at least in limited quantities can be procured in the form of eggs (floating eggs), yolk-sac larvae, fry and fingerlings from such water bodies, no farmer oriented technique is available for mass breeding, larval rearing and grow-out in the captive conditions. The major hurdles in captive breeding of this species is the poor understanding of morphological determination of sexes, final maturity conditions, suitable environmental conditions, the hormone type, optimum hormone dosages for induced breeding. The second part is rearing i.e. larval rearing, fry and fingerling rearing and grow-out for table production which are much more difficult as the species is not only predatory but cannibalistic too and that habit start from the larval stage (Ng and Lim, 1990) and continue till adult stage consuming prey

fish more than half to two-third of its length (Diana *et al.*, 1985; Ng and Lim, 1990; Qin and Fast, 1996a; Wee 1982).

Several studies have demonstrated that although cannibalism aggression in fishes is difficult to stop, even after feeding the fish to satiation level but it can be reduced to a great extent by increasing natural food availability (Fox, 1975; Hecht and Pienaar, 1993; Polis, 1981; Qin *et al.*, 1997) or by weaning the fish to accept formulated feed (Diana *et al.*, 1985; Qin and Fast, 1996 a and b; Qin and Fast, 1997). Successful larval rearing of snakehead, therefore, depends on a more understanding of dietary requirements at the first-feeding and as fish grows.

1.4 OBJECTIVES OF THE PRESENT STUDY

The growth and reproduction of an organism, including fish, is controlled by environmental factors, like conditions of temperature, light and the availability of food, apart from range of tolerance to different biotic/ abiotic factors, the internal potentials of the species is controlled by its genomic set up, evolved during its evolutionary history (Morgan, 2008). The populations of *C. marulius* surviving under the conditions of northern India represent ecotype / ecotypes having specific genomes, adapted to a specific set of environmental conditions, which may vary in terms of growth and reproduction. The available literature from parts of the country and neighouring countries indicated that the breeding season of *C. marulius* is variable in different agro-climatic conditions. It is reported from June - August in southern Nepal (Shrestha, 1990), April - June in Pakistan (Mirza and Bhatti, 1993), June to October with peak from July to August in northern India (Qasim and Qayyum, 1961) but entirely different in southern India with two seasons, first being from May – June and second from November – January (Alikunhi, 1953; Srivastava, 1980). Thus, the present study has been conducted on the hypothesis that *C. marulius* under the conditions of the Uttar Pradesh has specific spawning season and growth rates.

The reproductive conditions of different species of fish stock has been variously judged through morphological and anatomical analysis of the gonads (Vitale *et al.*, 2005), yet the supportive histological studies have been frequently used for placing the macroscopic observations on sounder basis (Saborido-Rey and Junquera, 1998; Kjesbu *et al.*, 2003). Such studies are almost absent for *C. marulius* in the country, especially for the ecotype surviving in the waters of Uttar Pradesh in India.

Though *C. marulius* is a pond breeder but only one or two pairs breed at a time in a single water body which is entirely different from majority of fishes including Indian major carps and catfishes in which almost all the brooders spawn more or less at the same time. This needs to be explored through behavioral and gonadal maturity study. The species has inherent difference in breeding behavior from other commercialized fishes and due to which a standardized captive breeding technology could not be developed even after trials by several workers, which may be due to factors like (1) Mating pair observe courstship behavior prior to spawning and a spawning takes place between courting one male and one female only, (2) Behaviour of parental care by both sexes from laying of eggs to larval growth, (3) Low fecundity and a batch spawner. Therefore unlike breeding of Indian major carps, this species may require different strategy for successful spawning in the captive conditions.

Some work on the larval rearing of *C. striatus* and *C. punctatus* has been carried out in India and abroad but there is dearth of such literature on *C. marulius*. As larval rearing is undertaken by snakeheads under strict care almost up to 45 days in nature (Personal commumination) and larvae being carnivorous and also cannibalistic in nature, there is need to explore possibility of its rearing in captive conditions for commercial output so that the demand for grow-outs and conservation could be met out. Therefore, there is need to study the suitable diets and strategies at different stages of larval rearing for higher survivals, etc

With the above hypothesis, the specific objectives for the present study were:

1. Age related changes in length and weight and merismatic characters.
2. Morphological determination of sex and season related anatomical changes in gonads (gonadosomatic index, GSI).
3. Histological changes in the gonads, in relation to seasons.
4. Captive breeding in open and closed water systems using hormonal preparation.
5. Embroyonic development under controlled conditions.
6. Larval rearing and grow-out in captive conditions with a view to generate parameters / considerations for commercial out puts and conservation.

The study will help in understanding the maturity conditions and breeding requirements of this species in the captive and climatic conditions of northern India. It will also help in visualizing the strategie requirements for larval rearing and grow-out practices particularly in context to nutritional requirements, feeding strategies and formulation of artificial diets and size-grading intervals for growing larvae for commercial outputs. The information, thus generated, will be of immense practical importance in the commercial seed production and farming of this species. Once this is ascertained, it will provide platform for conservation aquaculture practices in which the species may be used both for conservation through ranching and aquaculture for diversification of species. The growth related studies on length-weight relationship (LWR) and merismatic variables will provide information of the available stock of the region for comparing the stock with other parts of its distribution range. As this species has been considered as one of the prioritized future aquaculture species, the information will be useful in the process of diversitifcation aquaculture.

Chapter 2

REVIEW OF LITERATURE

Chapter 2
REVIEW OF LITERATURE

2.1. GROWTH

2.1.1. Age Related Changes

Comparative studies on growth of different species of the genus *Channa* suggested that two species, i.e., *Channa marulius* and *C. striatus*, have better growth potential as compared with the other species of this genus (Devaraj, 1973a; Wee, 1982). *C. marulius* can attain the length of 122 cm (Bardach *et al*, 1972) and some individuals have attained a length of 180 cm and a weight of 30 kg under the condition of Maharashtra State, India (Talwar and Jhingran, 1992) though the specific conditions and age of such a fish was not mentioned. Some reports have appeared on growth rate of *C. marulius* maintained in different water bodies. The stock of this species maintained in a tank in the Karnataka State, India, showed that the individuals of this species with an average length of 3.7 cm attained the average length of 52.8 cm in 8 months. The study showed that the fish had a growth rate of 2.5 – 4.0 mm per day during the first 3 months of age, which decreased to 0.8 – 1.3 mm per day for the rest of the 5 months of the study. The average growth rate for the first 8 months of age was placed at 7.0 cm per month (Murugesun, 1978). *Channa marulius* attained a length of 30 cm during the first year of its life under the condition of the Maharashtra State, India (Talwar and Jhingran, 1992). Ahmad *et al.* (1990) also reported a decrease in the growth rate of *C. marulius* stock, surviving in the river Kali (northern India), with an increase in age. However, they have not specified the age of their fish stock. A decrease in growth with increasing age has also been reported for *C. marulius* specimen obtained from the river Ghaggar, Rajastan (India) but highest growth rate was reported for the second year of age (Johal *et al*, 1983). However, no report is available on changes in length and weight of *C. marulius* with age in natural and culture system.

2.1.2 Length-Weight Relationship

The fish biologist have always remained attracted towards the length-weight relationship as length being treated as a predictor variable, which may act as an indicator of weight and age, with certain limitations.

Many previously available studies (Wootton, 1990; Pauly, 1993; Petrakis *et al*, 1993; Ozaydin *et al.*, 2007; Moutopoulos and Stergiou, 2002; Froese, 2006) suggested that the analysis of length-weight relationship is important for fish biologists and managers as it allows: (a) conversion of growth in length to growth in weight or vice versa, (b) estimation of biomass from length observations, (c) estimate of the condition of the fish, and (d) between region comparisons of life histories of certain fish species. Such studies have also been considered important for the analysis of comparative growth (Froese and Pauly, 1998). The length-weight relationship is population specific, though this relationship changes with developmental stages of life, like, metamorphosis, growth and onset of the maturity (Thomas *et al.*, 2003).

The length-weight relationship is based upon the cube law, i.e. $W = a.L^{3}$, where 'a' is a species specific constant; 'W', the weight of fish and 'L', length of fish. Various studies suggested that the cube law is not applicable to all the fish species, and there are inter-specific or even inter-population variations, and hence the relation is now expressed as $W = a\,L^{b}$, where 'W', weight of fish (g); 'L', standard length, (Pauly, 1983) or total length of fish (cm) (Abowei, 2009) and 'a', constant (intercept), and 'b', length exponent (slope). Under the cube law the value of 3 for 'b' suggests isometric growth of weight in relation to length, while values lower than 3 indicate a negative allometric growth and conversely values higher than 3 indicate a positive allometric growth in the fish (Gayanilo and Pauly, 1997).The value of 'b' primarily depends upon the shape and fatness of the individuals, controlled by a number of different factors, including environmental factors, like, temperature, food availability, etc. (Pauly, 1984), which may vary seasonally (Bagenal and Tesch, 1978; Ozaydin *et al.*, 2007), gonadal

maturity, sex, stomach fullness, health, preservation techniques, season and habitat (Cherif *et al.*, 2008).

The value of 'b' calculated on 36 species captured from Aegean Sea (Greece) ranged between 2.27 and 3.70, though in one of the species (*Citharus linguatula*) it was as low as 1.29 (Froese and Pauly, 1998). No significant seasonal variation in value of 'b' was reported for the species for which seasonal data was available (Moutopoulos and Stergiou, 2002). The comparison of these result with those available previously (Petrakis and Stergiou, 1995) suggested a difference, which was attributed to difference in sample size, sampling area / season and length range (Mountopoulos and Stergiou, 2002). In another study on 11 marine fish species from the Gulf of Tunisia (Mediterranean Sea) the values of b ranged between 2.67 and 3.36 (Cherif *et al.*, 2008). Some low values of b have been suggested for elephant fish (*Mormyrus rume*; male =1.69, female = 2.13, combined = 1.99), in the river Ose (southwestern Nigeria), showing a strong negative allometric growth, though the species was still able to survive in the freshwater environment (Odedeyi *et al.*, 2007). In India, Saini *et al.* (2010) calculated 'b' for 14 freshwater fish species from river Betwa (a tributary of the Yamuna River) and Gomti (a tributary of the Ganga River) and found 'b' to range between 2.4 in *Mastacembelus armatus* (freshwater eel) and 3.5 in *Cirrhinus mrigala* (Indian major carp) indicating that the value of 'b' is also likely to depend on the body shape as mrigal has a much wider body in comparison to freshwater eel. Le Cren (1951) argues that maintenance of the cube law and maintenaning the value of "b" close to 3 is rather rare and values below 2 have also appeared for a number of fish species (Thomas *et al.*, 2003).

Though LWR information on good number of species is available in the literature but there is dearth of information on snakeheads particularly on *C. marulius*. Haniffa *et al.* (2006) studied LWR of 1940 specimens of *C. punctatus* of length range 15.60 cm - 27.44 cm from four rivers of Western Ghats and concluded that the 'b' value range between 2.72-3.20 exhibiting average growth of this species in these rivers.

Though he calculated these values sex-wise, but could not demarcate any difference in cube law. Singh and Ram (2011) calculated 'b' values of *C. punctatus* from riverine and pond catch from Uttrakhand State and observed that these values were highly negatively allometric and ranged between 0.078-1.713 for reservoir stock and 0.216-1.766 for pond stock. Koundal *et al.* (2014) studies LWR of *C. punctatus* collected from River Beas in Himachal Pradesh and recorded 'b' values 2.80 for male and 2.86 for female population which revealed an isometric growth amongst both the sexes.

Dayal *et al.* (2012) calculated the LWR of *C. striatus* at fry/fingerling, juveniles and adult stages and recorded 'b' to be positive allometric in size ranges studied by them. In their study the 'b' was recorded ranging between 3.9-4.1 in samples of length 35.0-45.0 mm (weight 340-650 mg), 3.0 between length 10.9-25.4 cm (weight 9-93 gm) and 3.4 in length between 22.9-42.4 cm (weight 74-476 gm). LWR of *Channa micropeltes* from Pechipparai Reservoir (T.N.) was studied by Ebanasar and Jaya Parkas (2005) for juveniles, immature, adult male and female specimens who were reported to have respective 'b' values of 2.58, 2.73, 2.18 and 2.14 and concluded that significant variations occur in this species at different stages of life. Olurin and Savage (2011) studied LWR of *Parachanna obscura* from Nigerian river basin for both the sexes during August and February months and found wide variation in 'b' values of 1.77 in male and 2.91 in female.

LWR in respect of *C. marulius* has been calculated from River Godavari (Dist. Aurangabad, Andhra Pradesh) stock during January to December 2010 for lengths ranging 22.8-37.7 cm and weight 92-473 gm by (Rathod *et al.*, 2011) in which the 'b' value was found comparatively low of 1.45 and showed negative allometric growth. On the other hand, Dua and Kumar (2006) calculated 'b' to be 2.7 from 100 samples of *C. marulius* collected from Harike Wetland (A Ramsar site) in Punjab for larger samples ranging between 37.69-85.41 cm. Khan *et al.* (2012) calculated 'b' value from a collection of 565 specimens of *C. marulius* and found to have pooled up value of 3. Bhatti (2010) calculated LWR of *C marulius* from a culture pond in Punjab Province

and recorded lengths and weights at regular monthly intervals. According to him the pooled group ranging in length from 7.37 cm to 53.80 cm (weight 2.88 gm to 1165.79 gm) showed isometric growth with 'b' value of 3.05 (r^2=0.999) though the general value of 'b' was found to range between 2.59-3.26 in different sizes and months that indicated growth difference due to temperature and maturity conditions.

Ali *et al.* (2013) compared the LWR of *C. diplogramma, C. marulius* and *C. striata* from the riverine reaches of Lake Vembanad in Kerala and observed that *C. diplogramma* and *C. marulius* showed non-isometric growth pattern in the study region, whereas, *C. striatus* showed an isometric growth pattern. The exponent 'b' was found 1.28 and 1.54 respectively for *C. diplogramma* and *C. marulius* (t = -11.76, df = 151, $p < 0.0001$) which was significantly lesser than the cubic value, whereas, in case of *C. striatus*, the exponent was 2.73 which was not significantly different from the cubic value (t = 1.43, df = 135, $p = 0.16$). They concluded that both the species showed non-isometric or negative allometric growth and hence did not grow in proportion to the length as compared to *C. striatus*.

There are also reports of conflicting values of 'b' with respect to size consideration. Devraj (1973) in his study suggested that the specimens of smaller length class (<8 cm) had an exponent of 1.18, while the specimens of larger length class (>8 cm) 3.33. Ali *et al.* (2013), however, gave opposite finding of having higher 'b' value in smaller size groups in contrast to larger and revealed that this genus has two growth stanzas. Such ontogenic variations in the cube law are also known from other fish species as well (Froese, 2006).

There are good number of citations indicating the role of food availability and its quality on the LWR of *Channa* spp. Datta *et al.* (2013) in his study with 6 types of iso-nitrogenous diets concluded that the fish showed positive allometric growth of 3.32-4.39 with only four diets which also revealed that the acceptability of formulated feed is good in this species.

2.1.3. Condition Factor

The condition factor (K) or coefficient of condition (CF), also sometimes refered as length-weight factor, has been frequently used by fish biologists as an index of well being, plumpness, fatness or relative robustness of a fish (Bagenal, 1978). The index is represented by a relationship between the weight of the fish and its length, and calculated using the formula: K = 100 * W / Lb; where 'K', condition factor; 'W', weight of the fish (g); 'L', total length of fish (cm) and 'b', value of constant obtained from LWR (Pauly, 1984). The value of the condition factor decreases with a decrease in the weight of fish in relation to its length (Bakare, 1970; Fagade, 1979).The factor has been used as an index of feeding intensity, growth (Fagade, 1979), and available environmental conditions (Bagenal and Tesch, 1978). The value of the condition factor also changes with the reproductive status of the fish, and the highest values appeared during the spawning and reproductive activity, due to accumulation of fat (Vazzoler, 1996) and development of gonads (Le Cren, 1951; Angelescu *et al.*, 1958). A higher value of the condition factor during certain seasons may indicate the state of sexual maturity and degree of nourishment (Gomiero and Braga, 2005).

Variation in the condition factor also appeared between two sexes in certain species. The value of K was reported as 0.787 and 0.859 respectivly in male and female elephant fish (*Mormrus rume*) in the river Ose, southern Nigeria (Olabode *et al.*, 2007). The condition factor was reported to be higher in late stages of maturation as compared with the earlier stages of maturation of gonads in female kutum (*Rutilus frissi kutum*) (Kousha *et al.*, 2009) indicating an increase in the size of gonads under maturation. The value of the condition factor exhibited a gradual increase with the increasing maturity of the gonads and the maximum value corresponded with the maximum development of the ovaries in kutum but the condition factor persisted at higher levels even after recession of the ovaries (Sabet *et al.*, 2009).

In case of snakeheads, the condition factor 'K' has been calculated in *C. punctatus* by Singh and Ram (2011) from natural and captive (Pond) stocks ranging from 0.995 to 1.398 and 0.705 to 1.446. These authors concluded from their study that

specimens from natural stocks have wider range than the captive stocks and monthly variation was observed in both the conditions. Koundal *et al.* (2014) calculated 'K' values for different size groups (SL 40-60 mm) of both the sexes of *C. punctatus* and revealed that the 'K' value was above 1, which indicated well being of this fish under both the conditions. They, however, noted 'K' values up to 2.32 and 2.37 in larger size groups of 40-60 mm in both the sexes and attributed to the voracious feeding behaviour. In both sexes the value of 'K' showed almost decreasing trends with increasing length, except few exeception in male where it exhibited an increasing trend after attaining 140-160 mm size. Victor *et al.* (1992) calculated 'K' value for *C. obscura* from a Nigerian pond and observed that these values were higher 2.2 for small size group (16-18 cm) in comparison to larger size (30-32 cm) having these value very low 0.05 only. Datta *et al.* (2013) evaluated 'K' value for *C. punctatus* reared in captive conditions on five types of formulated diets and observed 'K' value to range 1.09 to 1.33 that revealed well acceptance of formulated diet by the fish. Out of five diets tried by them, three diets performed much better which also confirmed that the 'K' value also depend on the quality of the diet. However, there is no report available on 'K' values for wild and culture stock and also sex-wise on *C. marulius*.

2.1.4 Morphometric and Meristic study

Morphometric and meristic studies are of great importance in the identification of inter-species and intra-species variations. Goswami *et al*. (2006) described the morphological and meristic characters of four species of snakeheads and confirmed that *C. barca, C. aurantimaculata, C. stewartii and C. bleheri*, which are endemic to Assam, are separate species and their morphometric and meristic characters are not only different from most common snakehead species like *C. punctatus*, *C. gachua*, *C. striatus* and *C. marulius* but also in the four studies species.

2.2. REPRODUCTIVE CYCLE

The range of tolerance of a species to different environmental factors is narrowest during the reproductive period and hence the reproductive activity of a fish

species, like any other organism, requires a specific set of ecological and biological controlling factors. Thus, the species adjusts its reproductive activity to the part of the year when the optimal survival of the young is ensured (Odum, 1974). Under such conditions the species is expected to show a specific set of changes in the somatic and reproductive organs to support the spawning at the set season. Various studies have exploited different variables to judge the reproductive status of the species, so that the spawning period can be defined, which may help in the management of fish in nature and / or under farmed conditions.

2.2.1. Gonadosomatic Index

The size and the activity of the gonads changes with season and the age. Gonadosomatic index (GSI) is an index indicating the relative development of the gonads (gonad weight) standardized against the total weight. The index has been frequently used to judge the relative development of the gonads (Vlaming *et al.*, 1982), with the assumption that the gonads are well developed in sexually active individuals and regress during the period of sexual inactivity. The GSI is generally calculated using the formula: GSI = (gonad weight/ body weight) X 100 (Roffda, 1983). For more accuracy and to minimize the effect of changes in the mass of gonads at various development stages, some workers, in the recent years, have tried to refine this formula as: GSI = 100 X gonad weight / (body weight–gonad weight), and have used the equation in their studies (Tracey *et al.*, 2007; Muchlisin *et al.*, 2010). The eviscerated weight can also be used to further reduce the effect of the weight of visceral contents.

Different studies have suggested seasonal variation in the value of GSI, ascribed to the corresponding changes in the reproductive activities (gonadal development) of the species. The GSI values in female kutum (*Rutilus frisil kutum*) started rising in March reaching the peak values (29.5) in April and exhibited a sharp decline in May (Sabet *et al.*, 2009; Saeed *et al.*, 2010; Kousha *et al.*, 2009). The values of GSI in male golden rabbitfish (*Siganus guttatus*) remained below 1.0 during December–April and showed significant increase in May (4.5) and June (11.1) and a

decline in July (8.3) and further in August (1.4), suggesting spawning in this fish during May–July (Rehman *et al.*, 2000). The spawning season corresponded with higher values of GSI (male 2.1, female 7.8) as compared with spent or immature stage (male 0.3, female 0.8) in *Rhabdosargus haffara* captured from the Red Sea (Abuzinadah, 2001). The study on the GSI variation in *Channa gachua* from Godavari (Aurangabad, India) revealed that the female fish maintained a low GSI (6.0-12.0) for the major part of the year. The GSI started rising in June (25.2) achieving peak value in July (55.7) and followed by a rapid decline in August (34.5) (Gaikwad *et al.*, 2009) suggesting peak reproductive activity of the species in July. Similar high peak GSI values have also been reported in male (8.0) and female (8.8) in the May sample of *Capoeta capoeta umblaand* these values remained low during June–April period (Erdouan *et al.*, 2002). The values of GSI presented in these studies are very high compared with similar values appearing in literature regarding other species.

In the two sexes of the black rockfish (*Sebastes schlegeli*), GSI values exhibited peaks during different times of the year (male, October-November; females, April-May), which has been explained by the fact that the mating in the species occurs when the male matures and sperm are stored in the female to fertilize eggs as they are produced (Mori, *et al.*, 2003). This appears to be the species adaptation, and is not probably characteristic of most teleost.

In case of snakeheads, very few literatures existing describing GSI of both the sexes. Olurin and Savage (2011) measured the GSI of *Parachanna obscura* female (size 186.71$\pm$98.88 gm) from River Oshun in Nigeria during August 2006 to February 2007 and found it to be very low 1.96$\pm$0.63, whereas they could not measure GSI of male specimens (size 227.13$\pm$49.59 gm) as the gonads stated to be very small and in want of sensitive instruments. Chakraborty, *et al*. (2007) described the GSI of female *C. striatus* females collected from West Bengal and found to range from 0.439 in October to 6.182 in May. The GSI was estimated season-wise in case of both the sexes of *C. punctatus* by Kapil, *et al*. (2011) from the ponds of Maharashtra which was found

to be 6.14±0.472, 3.69±0.775 and 18.13±0.285 in case of females during summer, rainy and winter seasons, whereas in case of males the GSI values were 4.58±0.62, 8.06±0.74 and 10.11±0.42 during same seasons. Marimuthu and Haniffa (2006) have evaluated the GSI of female *C. punctatus* from the pond located in southern India during December 1998 to February 1999 and found to range from 1.754 (size 170 mm) to 7.959 (138 mm). Tiwari, *et al*. (2014) estimated the GSI value of female *C. marulius* a year around from Son River in Madhya Pradesh and found this to be low of 8.21 percent in September which started increasing at a rapid pace from March onwards and reaching to highest 47.67 percent in May month. Recently, Siddiqui *et al.* (2015) evaluated average monthly GSI of *C. marulius* from Bangladesh in the range of 0.018-0.420 for female and 0.018-0.056 in males and the highest GSI during July in both female (0.42) and male (0.056).

2.2.2. Histological Changes

Histological studies on gonads provide more reliable and direct indications of the reproductive status of the organism and hence are more dependable for the analysis of the reproductive cycle (West, 1990). Several histological studies are available that analyze seasonal variation in the level of reproductive activities in different species (Murua and Motos, 1998; Saborido-Rey and Junquera, 1998; Kjesbu *et al.*, 2003), but barring the study of Bhatti (2010), no information is in hand on *C. marulius*.

2.2.2.1 Testes

The fish reproductive physiologists, in general have placed more emphasis on the study of histological changes in the female reproductive physiology and relatively fewer studies are available on the male reproductive physiology (Bowers and Holliday, 1961; Koya *et al.*, 2002). The testis in fishes generally appear as a pair of elongated structures, composed of a series of branched seminiferous tubules, which are embedded in the stroma in the fish. The testis mainly comprises of thin-walled tubules or lobules, which contain germ cells (spermatogonia). The spermatogonia divide in

clusters and are enclosed in a cyst. The primary spermatogonia (representing the stem cell) are present throughout the year and divide mitotically to produce the secondary spermatogonia, which finally produce the primary spermatocyst. The spermatocysts divide by meiosis to form the spermatids, which are finally transformed into spermatozoa (Fishelson *et al.*, 2005).

Generally four different stages i.e. immature, prespawning, spawning and post spawning (spent), of the testicular development are identified based upon the histological variations, which represent different stages of the reproductive activity of the individual fish (Muchlisin *et al.*, 2010; Koya *et al.*, 2002; Mori *et al.*, 2003; Rehman *et al.*, 2000). Using such histological classification of the testes, the cyclic change in the reproductive activity has been reported in golden rabbitfish (*Siganus guttatus*; Rehman *et al.*, 2000), *Rasbora tawarensis* (Muchlisen *et al.*, 2010) and black rockfish (*Sebastes schlegeli*; Mori *et al.*, 2003), etc.

2.2.2.2 Ovary

The ovaries (one or commonly two) lie in the upper part of the body cavity of the female fish, almost parallel to the kidney. The shape and the size of the ovary vary with the stages of the sexual maturity (Goswami and Sundararaj, 1971; Vitale *et al.*, 2005). Ovaries are generally bi-lobbed structures in most fishes with a short oviduct. Each lobe contains ovigerous lamellae, their number and dimensions verifying with growth and reproductive stage of the fish. Maturation of the egg is a long process that involves complex physiological and biochemical changes (Fishelson *et al.*, 2005). Maturation is accomplished in the fish oocyte, involving mitotic division, resulting in four cells and oocyte giving rise to two polar bodies. During this process, the enlarged nucleus of the primary oocytes moves towards more peripheral position, when its membrane breaks down and the first meiotic division is completed with extrusion of the polar body. The second meiotic division starts immediately, but is arrested at metaphase (Goswami and Sundararaj, 1971; Masui and Clarke, 1979). During this process, a distinct animal pole separates from vegetal pole, in some species. The yolk

also undergoes some type of maturation and becomes less opaque. The oil droplets, when present, coalesce to form one or more larger droplets. Soon after, the mature (secondary) oocyte ovulates out of the follicular envelope, and acquires a jelly coat (Goswami and Sundararaj, 1971). The ovulated eggs are then spawned in water and are almost immediately fertilized. The ovaries have been generally classified into seven stages of the reproductive activity, (virgin, developing, early maturing, maturing, spawning or ripe, spent and resting) which have been variously used in understanding the seasonal variation in reproductive activity of different fish species (Vitale *et al.*, 2005; McDermott and Lowe, 1996; Abuzinadah, 2001; Saeed *et al.*, 2010). The fecundity of snakeheads is variable amongst species and also intra-species. The fecundity of snakehead varies from 16, 350-54, 476 ova in the size range of 34.2-51.5 cm (Rahman *et al.*, 2012). Ali (1999), however, found mean absolute fecundity to range from 4326-9017 ova, whereas mean relative fecundity ranged from 10.5-36.3 ova per g. BW.

2.3 SPAWNING

The snakehead breeds in ditches, ponds and flooded paddy fields. Generally they commence breeding in ponds and rivers a little prior to or with the onset of monsoons (Bhattacharya, 1946; Chacko, 1947). According to Qasim and Qayyum (1961), the snakeheads in northern states of India breed during June to October with peak from July to August. However, this is different in southern India, where this group is reported to breed during November to January (Alikunhi, 1953). According to Jyotsna *et al.* (1995), spawning period of striped murrel is during mid monsoon from June to August. *Channa marulius* breeds naturally in ponds and brood size varied between 375 g - 3,000 g in different swamps recorded in Karnataka state, India. Murrels mature in the first year of life and *Channa marulius* attains maturity at 300-350 mm length (Arumugam, 1966). The eggs are pale red-yellow with a diameter of around 2 mm. The eggs hatch in 54 hours at 16-26 ^{o}C and in 30 hours at 28-33^{o}C (Parameswaran and Murugesan, 1976). Parents lay the eggs in nests, built with weeds

and leaves of aquatic plants (Pethiyagoda, 1991), and guard the spawn until the young reach the size of some 10 cm (Breder and Rosen, 1966). Males show a territorial behavior (Pethiyagoda, 1991).

According to Devaraj (1973) and Parameswaran (1975) the giant murrel matures sexually in two years. *Channa marulius* is a unisexual or dioceus, female is much larger than male (Alikunhi, 1957). There is generally no morphological difference between male and female. In breeding season, however, they can be recognized by observing secondary sexual characters by carefully observing the vent. The slit like vent appears pale in male, whereas female has round vent red in colour. Snakehead attains maturity in about eleven months under natural condition (Alikunhi, 1953).

In female snakehead, *Channa striatus*, urinognital opening is circular in shape while in males; it is elongated in contrast to that of the female Qasim and Qayyum (1961) observed two batches of oocytes in the maturing ovary of *C. marulius* from North Indian waters indicating that the fish is a batch spawner. Each female pair with only a single male (Parmeshwaran and Murugesan, 1976; Moitra *et al.*, 1979) and other male is rejected. The courting pair just before spawning is often seen to frequently move from one place to other for selection of spawning site and construction of nest in water in a sheltered weedy margin of river (Khan, 1924). According to Agbayani (2013), the bullseye snakehead builds floating nests of weeds and leaves where orange-yellow eggs are deposited. Parameswaran (1975) and Parameswaran and Murugesan (1976) measure the mature ovarian eggs as 1.584 mm to 1.980 mm. The typical brood size of *C. marulius* is about 500 young, and is guarded by the parents until they reach about 10 cm in length. The eggs hatch within 54 hours at 16 °C to 26 °C and 30 hours at 28 °C to 33 °C. Breeding occurs through most of the year and can vary slightly depending on location (Courtenay and Williams, 2004).

2.3.1 Induced Spawning

The use of exogenous hormones in the induction of spawning of fishes is well documented (Lam, 1982) and different doses of hormones and sex steroids have been reported to give different results (Ayinla and Nwadukwe, 1988; Zonneveld *et al.*, 1988; Salami *et al.*, 1994). Human chorionic gonadotropin (HCG) (Mollah and Tan, 1982; Zairin *et al.*, 1992; De leeuw *et al.*, 1985; Fermin, 1992) and Ovaprim, a commercial formulation of GnRH analogue and dopamine antagonist (Alok *et al.*, 1993; Francis, 1996; Haniffa *et al.*, 1996) have been successfully used for induced breeding of air breathing fishes. LHRHa have been extensively used more effectively as an ovulating agent for the induced breeding of marine fishes (Devauvchelle *et al.*, 1988; Kestemont, 1988).

According to Selvaraj and Frances (2007), HCG implantation induced faster maturation in both female and male *C. striatus*. Francis *et al.*, (2000) described use of carp pituitary extract, human chorionic gonadotropin and ovaprim as inducing agents for the breeding of *C. striatus* successfully in earthen pond conditions.

Channa striatus have been induced bred with endogenous gonadotropin-releasing hormone (GnRH) by Marimuthu *et al.* (2001). Haniffa *et al.* (2004) Successful induced breeding of *Ophiocephalus punctatus* has been reported by Benarji (1974).

2.3.2 Embryonic Development

The embryonic development of number of freshwater fishes of several groups is available on record like carps, cichlides, catfishes, etc (Haniffa *et al.*, 2007; Arockiyaraj *et al.*, 2003; Amorim *et al*., 2009; Islam, 2005; Legendre and Teugels, 1991; Meijide and Guerrero, 2000; Puvaneshwari *et al.*, 2009; Wang *et al.*, 1992, Adebiyi *et al.*, 2013). However, there is dearth of such literature on snakeheads and no such record could be traced out for *C. marulius*. Amongst the snakeheads, the embryonic and larval development of spotted snakehead, *C. punctatus* has been

presented by Haniffa *et al*. (2003), whereas that of *C. striatus* by Marimuthu and Haniffa (2007) and Thumronk *et al*. (2011). USGS (2012) described the eggs structure of *C. argus* as buoyant due to presence of a large lipid droplet that was more than three-quarters the diameter of egg and remained present even after hatching. The diameter of fertilized egg in case of *Channa* spp is generally 0.90-2.0 mm; 1.20-1.40 mm in *C. striatus* (Marumutthu and Haniffa, 2007), 1.80-1.85 mm in *C. argus* (USGS, 2012), 0.90-1.10 mm in *C. punctatus* (Haniffa *et al*., 2003). According to Marumuttu and Haniffa (2007), the egg of *C. striatus* hatched in 23.30-24.00 hrs at 29±1 ^{o}C, whereas they hatched in 28 hr 40 min at temperature range 26.5-29.0 ^{o}C (Thumronk *et al*., 2011) in the same species. *C. punctatus* also hatched within the same timing of 24 hrs at 26-28 ^{o}C (Haniffa *et al*., 2003).

2.4 LARVAL REARING

In nature, the food of *C. marulius* at larval, fry and fingerlins changes according to the size. The food of five-day old larvae was found to comprise of copepods, colonial rotifers and 300 other plankters (Alikunhi, 1953). However, this finding did not tally with the report of Mookerjee *et al*. (1948) who have referred that larvae feed almost exclusively on algae and protozoa. According to Arumugam (1966), fry of this species predominantly feed on dipteran larvae, while fingerling take insect larvae and fish fry (Alikunhi, 1957). Devraj (1973) stated that the fry of 30-49 mm size are predominantly planktophagus, Daphnia forming 70-80% and small insects and other cullicids constitute the rest of the major items. According to him, the cannibalism develops in the postlarval stage for inadequacy of the natural food. Parmeswaran (1975) also confirmed more or less similar finding as that of Devraj (1973).

Ravindranath (1988) reported that the performance of larvae of *C. striatus* is significantly much higher when reared under captive conditions in comparison to what grow in the breeding tank along with the spawned parents. However, the larval rearing in captive conditions in fishes primarily depends on water quality of the system, feed and feeding strategy and health management (Liao *et al.*, 2001). The selection of food

item depends on various factors like size of the mouth-gap, gill rakers, food preference and digestibility, etc.

In captive conditions, live feeds have been found useful for larval rearing in number of fishes (Liao *et al*., 2001; Mahmoudzadeh, *et al*., 2009). It plays an important role in the shrimp, catfish, salmon and marine fish hatcheries. Among different live feeds rotifers; *Brachionus* spp., *Moina* spp., *Artemia* spp.and tubifid worms are very popular and cheap live feed items used for feeding larvae of carnivorous and omnivorous fishes (Bucher, 1977). Live feeds have shown better results in terms of survival, growth and body composition in several of the studies carried out on rearing larvae of air-breathing catfishes like *Clarias batrachus, C. macrocephalus, C. marulius, C. lazera, Heteropneustes fossilis* (Bucher, 1977; Alam and Mollah, 1988; Mollah and Tan, 1982; Hongendoorn, 1980; Haque and Barua, 1987; Akter *et al.*, 2001; Riaz and Ahmed, 2006).

The selection of live feed in captive conditions depends on the size of mouth-gap of the rearing larvae and mechanism of digestion in the alimentary canal. In *C. striatus* (size 6-7 mm) average mouth width at first-feeding was reported 0.55 mm and it increased linearly with body length. In a laboratory trial, the larvae at this size were observed to accept only brine shrimp nauplii (BSN) when fed both with BSN and formulated diet. The larvae start consuming formulated diet when 12 days old at length of ≤ 12 mm having a mouth gap of 1.0 mm. In field and laboratory trials, snakehead diets changed as fish size increased. Larvae of *C. striatus* of length 15-20 mm (TL) have found to contain 96.5% cladocerans and copepods in their diet. Fry of 45-50 mm (TL) fed exclusively on benthic invertebrates. This shift in diet from zooplankton to benthic invertebrates is not due to reduction of zooplankton availability, but instead related to changes in gill raker structure (Qin and Fast, 1997).

Qin *et al.* (1997) in a 30-days trial with various combinations of BSN, Artemia cyst, decapsulated Artemia cysts and formulated diet concluded that the early larvae (length 6.5 mm) of *C. striatus* could be initially fed with artemia nauplii for 30 days

followed by 7-10 days mixed feeding with both Artemia and formulated feed, then switch completely to formulated feed. Thumronk *et al.* (2011) also corroborated that under the laboratory conditions; fry of *C. striatus* consumed BSN up to 11 days and thereafter switched to formulated diet only. War *et al.* (2011) advocated giving live feed up to 4 weeks to the larvae of *C. striatus* and stated that the size of live feed is very important with the age of larvae. Though BSN was found best during first two week of of rearing, cladocerans performed better during third and fourth weeks in terms of survival, growth and development of shooters. According to Abol-Munafi, *et al*. (2004), the performance of *C. striatus* larvae was best up to the age of 15 days with BSN and followed by *Moina micrura* and the larvae reared on bloodworm and formulated diet did not survive at this age. Though larvae initiated consuming blood worm and formulated diet at the age of 15-30 days but still the performance of larvae was observed to be the best on BSN up to 30 days age. Fluechter (1980) found that protein digestion enzymes in live BSN were responsible for successful rearing of white fish larvae (*Coregonus lavaretus*). Rahman (2001); Sarowar (2009) and Sarowar *et al.* (2010) also observed better survival and growth of *Ompok pabda* and *C. striatus* when fed with chopped tubificid worms in comparison to formulated diets.

The early larvae of *C. striatus* fed exclusively on formulated diet showed high rate of mortality under laboratory conditions. This poor survival might be related to the poor developed digestive enzyme system (Qin *et al.*, 1997). Dabrowski (1982) suggested that initially, digestion in these fish larvae is carried out by enzymes present in their live prey.

Some information on protein requirement of larvae of *Channa* spp is available; however, there is no information on the protein requirement of *C. marulius* (Mohanty and Samantaray, 1996; Panda *et al.*, 1999; Kumar *et al.*, 2010; Kpogue *et al.*, 2013). Mohanty and Samantaray (1996) studied the protein requirement of *C. striatus* fry (initial weight 0.552 g) for six weeks on six-types of formulated diet having CP between 350-600 g Kg^{-1} and evaluated that the best diet at this size should have CP of 55% (Gross energy 43.5 kcal/100gm) when fish meal was used as a major protein

ingredient. They also concluded that there was significant increase in carcass protein and decrease in ash contens when protein is increased in the diet. In another study with the fry of the same species, Kumar *et al.* (2010) also concluded that 55% CP is optimum for the growth. Kpogue *et al.* (2013) also observed that *C. obscura* also showed best specific growth rate, feed efficiency and productive protein value with formulated diet containing 55% protein. The combined role of protein and lipid levels on the overall permormance of growth parameters was evaluated by (Samantaray and Mohanty (1997) and it was revealed that fingerlings of *C. striatus* grew best with a diet containing 40% protein, 440 kcal energy and 13% lipid with a P / E ratio of 90.9 mg protein $kcal^{-1}$.

Aliyu-Paiko, *et al*. (2010) carried out survival and growth trials on *C. striatus* fry using various combinations of protein and lipid ratio and observed that lipid level of 6.5% gave better survival, whereas 45% protein level (highest level used in the study) along with 6.5% lipid gave better growth performance (weight gain, SGR), nutrient utilization (FCR, PER, PI) and carcass protein composition.

Qin and Fast (1996a) conducted an experiment with 0, 5, 10, 15, 20, 30 percent dried formulated feed (CP 50%) and found that the optimum feed requirement of this species is 50% and further addition had insignificant effects on survival and growth. The feed showed positive effect on survival and reduced cannibalism considerably. In another study, these authors also corroborated that addition of formulated feed reduce cannibalism from 83% to 43%. They also observed that the rate of cannibalism also depends on the size difference in a batch and 100% cannibalism occured within 5 days when the size difference ratio between smaller fish to larger was 0.35 in total length (Qin and Fast, 1996b).

2.5 GROW-OUT

The culture of snakeheads was likely to begin in the Mekong basin and Tonle Sap areas in Kampuchea and Vietnam mainly in the cages. Wee (1982) reported commercial farming practices of snakeheads in Thailand, Philippines, Vietnam, and

Cambodia. Chen (1976) described culture of *C. maculate*, which was commonly cultured in Taiwan.

In India, the culture of snakeheads began in ponds, rice fields and irrigation wells that do not support other fishes (Bardach *et al.*, 1972; Sriramlu, 1997). Snakeheads are more easily cultured in shallow water as being obligatory aerial respiratory fish and saving of energy loss by the fish due to their frequent vertical movement (Pandian and Vivekanandan, 1976). They can survive well in poor quality water with low dissolve oxygen content, and may be ideal for culture in seasonal, perennial water bodies and in backyard tanks that are not suitable for carp culture (Panda *et al.*, 1999; Haniffa and Sahthik, 2014). As being predatory, the snakeheads are often used in carp polyculture tanks for the control of unwanted small fishes (Cruz and Laudencia 1980). *Wee* (1982) recommended this species for monoculture in ponds which could be fed with tilapia. The possible use of snakehead for biological control of feral population of tilapia was studied by Yang *et al.* (2004) and concluded that a mixed-sex tilapia ratio of 1:80 (predator to prey ratio) gained the best performance by reducing overcrowding in pond culture system. This fish is highly suitable for cage culture and culture in ponds in combination with tilapia (Ebanasar and Jayaprakas 1994). Singh *et al*. (1986) recommended that *C. striatus* could be reared in high density owing to their air breathing ability which allows this fish to live in water of low oxygen tension.

Jhingran *et al.* (1985) reported that systematic culture of snakehead was attempted for the first time in India by the state fisheries department, Madras (India) at Sun-Kesula Fish farm. Later, the state fisheries departments at Hyderabad and Bombay followed this but with little success. Ravindranath (1988) conducted rearing trials with fry and fingerlings of *C. striatus* in cement tanks and earthen ponds respectively and suggested that the survival and growth rate significantly depend on stocking density and stocking size. He also suggested that frog larvae (tadpole) and trash fish are readily accepted both by the fry and fingerling.

Qin and Fast (1998) also suggested that *C. striatus* (length 13.9±1.3 cm, weight 22.9±6.9 g) can be cultured for grow-out without any difference in survival and growth up to a stocking density of 30 m^{-2}. Karl Marx (2008) reared fingerlings (length 65 mm, weight 2.5 g) of *C. striatus* in three stocking densities of 15, 20 and 30 m^{-2} in cement tanks and concluded that stocking density of 15 m^{-2} performed best in terms of survival, gain in length and weight and FCR value. Diana *et al.* (1985) cultured snakehead juveniles with a stocking densities range from 40-80 m^{-2} with 13-15% survival in 9-11 months. Rahman *et al.* (2012) evaluated stocking density for grow-out of *C. striatus* and recommended 5000 ha^{-1} as optimum for culturing in earthen ponds for better survival and growth parameters.

Though there is good piece of information on culture of *C. striatus* and *C. punctatus* but information on grow-out on *C. marulius* is almost negligible. Devraj (1973) was the first to register trial on culture of *C. marulius* in India. In 90 days culture period, he recorded an increase of 60 mm in length but with a very low survival of 19.3%. For higher survival, he has suggested stocking size of 125 mm length for culture of this species.

The reason of low survival seems due to limited information on the structure of alimentary canal, mouth structure, digestive system and feed requirements of *Channa* spp. and therefore the culture of this group of fishes have still being carried out on traditional system. Usually farmers feed the snakeheads with trash fish and cattle blood mixed with wheat flour or wastet grains and rice bran (Wee, 1982; Victor and Akpocha, 1992). Boonyaratpalin *et al.* (1985) reported that farmers of Thailand fed snakehead with trash fish mixed with rice bran, vitamins and minerals and sometime add antibiotics during first month of feeding.

The structure and morphometries of the alimentary canal of *Channa* spp in relation to their food and feeding habits has been described by Dasgupta (2000), Singh *et al*. (2013) and Choudhary and Biswas (2004) that confirm alimentary canal structure to be similar to a carnivore type. However, it was also noted that considering the

structure and morphometries of the alimentary canal, *C. striatus* was found to be the most carnivore, followed by *C. marulius*, *C. punctatus* and *C. orientalis* (Dasgupta, 2000). *Channa* spp consume number of food items including crustaceans, insects, mollusks and fishes but major food items comprises of fish; however, the rate of later may differ according to species preference and availability (Rao *et al.*, 1998; Singh, *et al.*, 2013; Reddy, 1980).

Besides being carnivorous in feeding habit, snakeheads also observe great amount of cannibalism at all stages of life and it is one of the major reasons of low survival during their culture (Ng and Lim, 1990). In the process of cannibalism although shooters are able to prey on fish measuring 2/3 in length (Diana *et al.*, 1985) or 63-80% (Qin and Fast, 1996a) to predator size in case of *C. striatus*, no information as to predator-prey ratio is available for *C. marulius*. *C. striatus* in the process of cannibalism ingested comparatively smaller numbers (more than 10%) of prey and large numbers of them die due to injury, shock and spread of diseases (Qin and Fast, 1996b). It has been demonstrated in several of the studies that application of formulated diets had improved survival greatly in fishes that observed great amount of cannibalism (Hoelzer, 1992; Kvarnemo *et al.*, 1998; Liao *et al.*, 2001; Manica, 2004; Qin and Fast, 1996b; Rohwer, 1978; Sargent, 1992). However, it is also more important that formulated feed should meet the nutritional requirement of fish in general.

Ebanasar and Jayaprakas (1995) cultured *C. striatus* in cages fed on fish, clam meat, aquatic insects and formulated diet having 45% CP revealed that though all types of above feeds have been accepted by the species but the performance was best with fish and least with formulated diet in terms of growth parameters. The muscle biocomposition also revealed that the fish fed with formulated diet had higher moisture content and less protein in comparison to other food items. Datta *et al.* (2013) reared *C. punctatus* on six-isonitrogenous diets differed in feed ingredients and reported cent percent survival with all the diets but there was significant difference in specific growth rate (SGR), LWR values, condition factors indicating that the fish readily

accept formulated diets and there is significant impact of quality of diet on growth parameters. Similar observations have also noted by Srivastava *et al.* (2012).

Arockiyaraj *et al.* (1999) evaluated the carbohydrate requirement of fingerling of *C. striatus* and concluded that carbohydrate level of 12% could be effectively utilized by this species. Dayal *et al.* (2014) evaluated the role of dietary fat on ovarian tissue of *C. striatus* using seven combinations of fat rich feed ingredients in the diet and revealed that addition of experimental fats has significant positive effect on the development of ovarian tissue and linseed oil and mixed oil could be safely used for better and /or higher follicular development and fecundity.

Most of the work carried out on nursery rearing of *Channa* spp is under the indoor conditions and there are few reports that revealed survival and growth details in open conditions. In a triplicate replication trial of 6-weeks in earthen pond system, Rahman *et al.* (2013) evaluated the role of stocking density in nursery rearing of *C. striatus* and evaluated that a stocking density of 15,000 ha^{-1} is optimum in terms of survival and growth parameters, when early larvae (length 1.17$\pm$0.18 cm, weight 0.15$\pm$0.03 g) were stocked and fed on supplementary feeding. The water temperature also plays a vital role in the growth of Chinese snakehead, *C. barca*. Liu *et al.* (1998) observed that the optimum temperature for food consumption in *C. barca* was 29.6 ^{o}C and this species lost weight at low temperature of 10 ^{o}C.

Chapter 3

STUDY AREA

Chapter 3
STUDY AREA

All study work under this thesis was carried out at Lucknow, the state capital of Uttar Pradesh (U.P.). It is India's fifth largest and most populous state, located in the north-central part of the country and spreading over a large area, and the plains of the state are quite distinctly different from the high mountains in the north. The climate of Uttar Pradesh can also vary widely, with temperatures as high as 47 °C in summer, and as low as -1 °C in winter. Geographically, it is situated between 23°52'N and 31°28'N latitudes and 77°3' and 84°39'E longitudes. The climate of the state is tropical monsoon. The average temperature varies in the plains from 3 to 4 °C in January to 43 to 45 °C in May and June. There are three distinct seasons - winter from October to February, summer from March to mid-June, and the rainy season from June to September. The major rivers are the Ganges, Yamuna, Gomti, Ghaghara, Rapti, Sarda and Ramganga that covers a length of 28,500 km (including canal system). The state is bestowed with 66 man-made reservoirs covering an area of 1.37 lakh ha, oxbow lakes covering 1.33 lakh ha rural ponds and lakes covering area of 1.62 lakh ha.

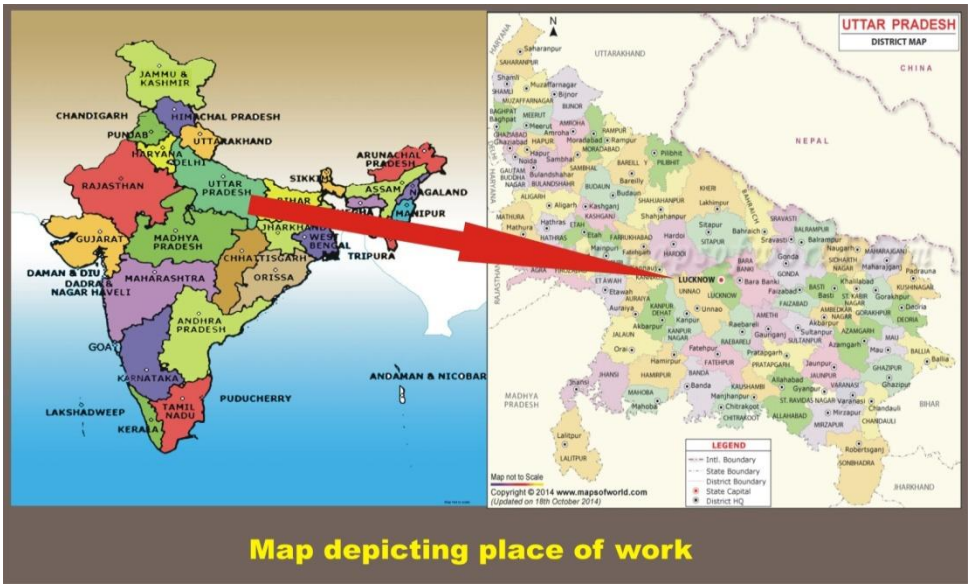


Figure 5: Map of Uttar Pradesh showing place of study area

Chapter 4

GROWTH RELATED CHANGES

Chapter 4
GROWTH RELATED CHANGES

4.1 MATERIAL AND METHODS

4.1.1 Procurement of test fish

Approximately 5000 sac-fry of the test fish were procured from a pond located at NBFGR, Lucknow with the help of a hapa, where they spawned naturally. The fry were then shifted in two cement tanks of size 14.5m X 10.0m (145 m^{-2}) and reared for 6 months so that their small-size could be easily caught during monthly sampling size by draining. Thereafter the fingerling were shifted to two earthen ponds of size 20 m x 20 m x 1.2 m (LxWxD) and reared to adult size for two years at the NBFGR fish farm so that they get better natural climatic condition for growth. The feed was provided to fish in the form of semi-moist cooked formulated diet for 6-months and thereafter switched to cut pieces of trash fishes during the remaining period. The ingredients used in the formulation of feed and their ratio are given under this para. The daily ration was adjusted according to the average weight of fish after every monthly sampling and maintained initially at 10% of the body weight and gradually brought down to 5%. The fish were harvested every month by draining the pond water and hand picked. They were brought to wet laboratory and washed with bore well water. The measurement of length-weight (LW), morphometric and meristic data were recorded. The fishes were shifted back in the same tanks by giving a short bath of potassium permanganate 1mg.L^{-1} for about 5 minutes after recording LW data; wheras fishes used for morphometric and meristic data were preserved in 10% formalin. Shooters larger in size by approximately 30% were separated after every monthly sampling and stocked in another pond for raising brood stock.

Feed ingredients used for making formulated feed:

1. Fresh fish meat
2. Hen's egg
3. Wheat flour
4. Vitamin-mineral mixture

4.1.2 Length-weight relationship (LWR)

The length and weight data was arranged in seven intervals of length groups 1-10 cm, 11-20 cm, 21-30 cm, 31-40 cm, 41-50 cm, 51-60 cm and 61-70 cm. The number of samples measured for each length interval is given in Table 2. The length was measured with a meter scale in centimeter (Figure 15) and weight by a digital balance in grams (Figure 16). Regression graphs were drawn in M.S. Excel (2007) by putting length-weight data for each interval (Figure 6, 7, 8, 9, 10, 11, 12). The length-weight relationship (LWR), Ponderal Index (K) and coefficient of determination (R^2) was calculated by the following formulae using M.S. Excel (Version 2007).

$$W = a\ L^b$$

The values of constant 'a' and 'b' were estimated from the equation of LeCren (1951) by transforming log values of length and weight, that is, linear regression equation given below:

$$\text{Log } W = \log a + b \log L$$

where, 'W' is assumed weight of fish in gram, 'a' is intercept on y-axis and 'b' an exponent between 2 and 4 and 'L' is mean length of fish in cm.

$$K \text{ (Ponderal Index)} = 100W/L^3$$

Where, W is weight in gram, L is length in centimeter

R^2 (Coefficient of determination) = Square of R (Correlation coefficient)

Where, 'R' is correlation coefficient and measured by the following formulae, x mean length (cm) and 'y' mean weight (g)

$$R \text{ (Correlation coefficient)} = \frac{n\,(\sum xy) - (\sum x)\,(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)2]\,[n\sum y2 - (\sum y)2]}}$$

Analysis

Regression graphs were plotted for comparing the LWR of different length groups. The pooled length group regression graph on $\log^{10}$ value was prepared to observe whether these groups follow straight line for predicting length and weight values for each other. The values of 'W', 'a', 'b', 'K' and 'R^2' were calculated manually using M.S. Excel (Version 2007). The data are expressed as mean $\pm$ standard deviation.

4.1.3 Morphometric analysis

Morphometric analysis of 21 samples of *C. marulius* varying in length from 3.22 cm to 19.10 cm was carried out for 24 variables. These samples were collected from a farm reared population of the NBFGR, Lucknow. The samples immediately after collection from the pond were brought to the laboratory, washed with fresh clean water and preserved in 10% formalin and kept spreaded in a tray with the help of cotton so that the samples remain in stretched condition. The morphometric data was collected irrespective of sex using divider, thread and meter scale for the following variables.

i. Total length, (TL), Distance from snout to the tip of the caudal fin.
ii. Standard length, (SL), Distance from tip of snout to the structural base of the caudal fin.
iii. Body depth, (BD), Distance from the highest point of dorsal to ventral flank.
iv. Snout length, (SL), Distance between the tip of the upper jaw and anterior margin of the orbit.
v. Eye diameter, (ED), Distance across the visible portion of the eye ball
vi. Pre orbital length, (POL), Distance from snout to beginning of eye orbit.
vii. Upper jaw length, (UJL), Distance from one end to other of upper jaw.
viii. Lower jaw length, (LJL), Distance from one end to other of lower jaw.

ix. Mouth opening, (MO), Distance of mouth width

x. Pre dorsal length, (PDL), Distance from snout to start of dorsal fin.

xi. Pre-pectoral length, (PPecL), Distance from snout to the origin of Pectoral fin.

xii. Pre-pelvic length, (PPelL), Distance from snout to the origin of the pelvic fin.

xiii. Pre anal length, (PAL), Distance from snout to the origin of the anal length.

xiv. Head length, (HL), From the tip of the snout to the end of the opercular bone with both the jaws closed

xv. Head width, (HW), Distance from highest dorsal flank to ventral in head region.

xvi. Head diameter, (HD), Distance from one lateral flank to other.

xvii. Dorsal fin-base length, (DFBL), Distance from the origin to end of the dorsal fin at dorsal flank.

xviii. Anal fin-base length, (AFL), Distance from origin to end of anal fin at ventral flank.

xix. Pelvic fin-base length, (PelFL), Distance from extreme base of rays to the farthest of pelvic fin.

xx. Pectoral fin-base length, (PecFL), Distance from extreme base of the rays to farthest tip of pectoral fin.

xxi. Caudal fin-base length, (CFL), Distance from the structural base of the caudal fin to the tip of the caudal fin.

xxii. Caudal peduncle length, (CPL), the straight-line distance from the posterior end of the anal fin base to the caudal base

xxiii. Caudal peduncle depth, (CPD), the greatest depth (straight-line distance from dorsal to ventral surface) of the caudal peduncle.

xxiv. Caudal fin height, (CFH), Distance from dorsal to ventral flank at maximum point.

xxv. Dorsal spine length, (DSL), Distance from the extreme base of the largest fin spine to its farthest end.

xxvi. Dorsal fin rays, (DFR), Number of dorsal fin rays

xxvii. Anal fin rays, (AFR), Number of anal fin rays

xxviii. Pelvic fin rays, (PelFR), Number of pelvic fin rays

xxix. Pectoral fin rays, (PecFR), Number of pectoral fin rays

xxx. Caudal fin rays, (CFR), Number of caudal fin rays

xxxi. Number of rosettes.

Analysis

The data on the each length variable of sampled fishes was pooled to calculate different statistics, i.e., mean, standard error of mean (SEM), range, coefficient of variation, and 95% confidence limits of mean (95% CL) using Microsoft Excel (Version 2007).

4.1.4 Morphomeristic analysis

The fish samples used for the morphometric variables were also used for the study of 5 morphomeristic variables viz. 1. Dorsal fin rays, 2. Anal fin rays, 3. Pelvic fin rays, 4. Pectoral fin rays, 5. Caudal fin rays in order to see any significant change. The variables were manually counted with the help of needles, forceps and a hand lens.

Analysis

The data on the each morphomeristic variable of sampled fishes was pooled to calculate different statistics, i.e., mean, standard error of mean (SEM), range, coefficient of variation, and 95% confidence limits of mean (95% CL) using Microsoft Excel (Version 2007).

GROWTH RELATED CHANGES

4.2 RESULTS AND DISCUSSION

4.2.1 Length-weight relationship

The data recorded and calculated on mean length, standard deviation in mean length, minimum and maximum lengths, mean weight, standard deviation in mean weight, minimum and maximum weights, a, b and log w values, ponderal index (K) and coefficient of determination (R^2) is given in Table 2 and Fig. 6, 7, 8, 9, 10, 11, 12 It was observed that this species in the present study strictly followed Lecren's Cube law with 'b' values of 3 in all the length intervals. The log w value (0.4868) was found lowest for smallest length group (1-10 cm), which subsequently increased to 1.3767 in length group 11-20 cm, 2.1551 in length group 21-30 cm, 2.4871 in length group 31-40 cm and 2.7584 in length group 41-50 cm. A significant drop in log w value was observed in higher length groups of 51-60 cm and 61-70 cm in comparison to small length groups with respective log w values of 2.1982 and 2.2768. Though the sampling of fish was done after complete harvesting on monthly basis when shooters larger in size by approximately 30% were separated from the experimental fishes, a good number of outlawyers (shooters) were visible all through the culture period though the difference in their size was lowered down with increase in size (Figures 6, 7, 8, 9, 10, 11, 12). The pooled LWR data for all length groups (1-10 cm to 61-70 cm) plotted on $\log^{10}$ values depicted straight line (Figure 13) indicating that the species followed cube law in the present study when they were reared under the captive conditions.

The Ponderal Index (K) was found in all the length groups under 1, indicating negative allometric growth in the present study. However, these values were higher in small size length groups in comparison to large size length groups. K value of 0.92 in length group of 1-10 cm suggest that at this size, plumpness in the fish is highest which was found to reduce to 0.73 in length group 11-20 cm, 0.78 in length group 21-30 cm,

0.69 in length group 31-40 cm, 0.66 in length interval 41-50 cm, 0.58 in length interval 51-60 cm and 0.70 in length group 61-70 cm (Table 2).

The values of Coefficient of determination (R^2) were found to follow similar trend as that of K values showing negatively allometric growth in this species i.e. K less than 1. Highest R^2 value of 0.86 in length group of 1-10 cm suggest that at small size, the fish followed strong LWR relationship in the present study in comparison to larger size groups with R^2 values of 0.68 in length group 11-20 cm, 0.76 in length group 21-30 cm, 0.51 in length group 31-40 cm, 0.67 in length interval 41-50 cm, 0.67 in length interval 51-60 cm and 0.6 in length group 61-70 cm (Table 2).

The fish biologist have always remained attracted towards the length-weight relationship as length being treated as a predictor variable, which may act as an indicator of weight and age, with certain limitations. The LWR, K and R^2 values are also strong parameter to compare the growth of any species in a new ecosystem like pond system with that of natural open water system which may also help in determining the suitability of the species for aquaculture Many previously available studies (Wootton, 1990; Pauly, 1993; Petrakis *et al.*, 1993; Ozaydin *et al.*, 2007; Moutopoulos and Stergiou, 2002; Froese, 2006) also suggested that the analysis of length-weight relationship is important for fish biologists and managers as it allows: (a) conversion of growth in length to growth in weight, (b) estimation of biomass from length observations, (c) estimate of the condition of the fish, and (d) between region comparisons of life histories of certain fish species. Such studies have also been considered important for the analysis of comparative growth (Froese and Pauly, 1998). The length-weight relationship is population specific, though this relationship changes with developmental stages of life, like, metamorphosis, growth and onset of the maturity (Thomas *et al.*, 2003).

The length-weight relationship is based upon the cube law, i.e. $W = a.L^3$, where ‘a’ is a species specific constant; ‘W’, the weight of fish and ‘L’, length of fish. Various studies suggested that the cube law is not applicable to all the fish species, and

there are inter-specific or even inter-population variations, and hence the relation is now expressed as $W = a L^b$, where 'W', weight of fish (g); 'L', standard length, (Pauly, 1983) or total length of fish (cm) (Abowei, 2009) and 'a', constant (intercept), and 'b', length exponent (slope). Under the cube law the value of 3 for 'b' suggests isometric growth of weight in relation to length, while values lower than 3 indicate a negative allometric growth and conversely values higher than 3 indicate a positive allometric growth in the fish (Gayanilo and Pauly, 1997). In the present study, the b values have been recorded to be 3 in all the group intervals, which indicate that the growth in the captive conditions in the present study was isometric. The value of 'b' primarily depends upon the shape and fatness of the individuals, controlled by a number of different factors, including environmental factors, like, temperature, food availability, etc. (Pauly, 1984), which may vary seasonally (Bagenal and Tesch, 1978; Ozaydin *et al.*, 2007), gonadal maturity, sex, stomach fullness, health, preservation techniques, season and habitat (Cherif *et al.*, 2008).

The value of 'b' calculated on 36 species captured from Aegean Sea (Greece) ranged between 2.27 and 3.70, though in one of the species (*Citharus linguatula*) it was as low as 1.29 (Froese and Pauly, 1998). No significant seasonal variation in value of 'b' was reported for the species for which seasonal data was available (Moutopoulos and Stergiou, 2002). The comparison of these result with those available previously (Petrakis and Stergiou, 1995) suggested a difference, which was attributed to difference in sample size, sampling area / season and length range (Mountopoulos and Stergiou, 2002). In another study on 11 marine fish species from the Gulf of Tunisia (Mediterranean Sea) the values of b ranged between 2.67 and 3.36 (Cherif *et al.*, 2008). Some low values of b have been suggested for elephant fish (*Mormyrus rume*; male =1.69, female = 2.13, combined = 1.99), in the river Ose (southwestern Nigeria), showing a strong negative allometric growth, though the species was still able to survive in the freshwater environment (Odedeyi *et al.*, 2007). In India, Saini *et al.* (2010) calculated 'b' for 14 freshwater fish species from river Betwa (a tributary of the Yamuna River) and Gomti (a tributary of the Ganga River) and found 'b' to range

between 2.4 in *Mastacembelus armatus* (freshwater eel) and 3.5 in *Cirrhinus mrigala* (Indian major carp) indicating that the value of 'b' is also likely to depend on the body shape as mrigal has a much wider body in comparison to freshwater eel. Le Cren (1951) argues that maintenance of the cube law value "b" close to 3 is rather rare and values below 2 have also appeared for a number of fish species (Thomas *et al.*, 2003).

Though LWR information on good number of species is available in the literature but there is dearth of information on snakeheads particularly on *C. marulius* reared under captive conditions. Haniffa *et al.* (2006) studied LWR of 1940 specimens of *C. punctatus* of length range 15.60 cm - 27.44 cm from four rivers of Western Ghats and concluded that b value range 2.72-3.20, exhibiting average growth of this species in these rivers. Though they calculated these values sex-wise, but could not demarcate any difference in cube law. Singh and Ram (2011) calculated 'b' values of *C. punctatus* from riverine and pond catch from Uttrakhand State and observed that these values were highly negatively allometric and ranged between 0.078-1.713 for reservoir stock and 0.216-1.766 for pond stock. Koundal *et al.* (2014) studies LWR of *C. punctatus* collected from River Beas in Himachal Pradesh and recorded 'b' values 2.80 for male and 2.86 for female population which revealed almost an isometric growth amongst both the sexes.

Dayal *et al.* (2012) calculated the LWR of *C. striatus* at fry/fingerling, juveniles and adult stages under the captive conditions and recorded 'b' to be positively allometric in size ranges studied by them. In their study, the b was recorded ranging 3.9-4.1 in samples of length 35-45 mm (weight 340-650 mg), 3.0 between length 10.9-25.4 cm (weight 9-93 gm) and 3.4 in length between 22.9-42.4 cm (weight 74-476 gm). LWR of *Channa micropeltes* from Pechipparai Reservoir (T.N.) was studied by Ebanasar and Jaya Parkas (2005) for juveniles, immature, adult male and female specimens who were reported to have respective 'b' values of 2.58, 2.73, 2.18 and 2.14 and concluded that significant variations occur in this species at different stages of life. Olurin and Savage (2011) studied LWR of *Parachanna obscura* from Nigerian river

basin for both the sexes during August and February months and found wide variation in 'b' values of 1.77 in male and 2.91 in female.

LWR in respect of *C. marulius* has been calculated from River Godavari (Dist. Aurangabad, Andhra Pradesh) stock during January to December 2010 for lengths ranging 22.8-37.7 cm and weight 92-473 gm by (Rathod *et al.*, 2011) in which the 'b' value was found comparatively low of 1.45 and showed negative allometric growth. On the other hand, Dua and Kumar (2006) calculated 'b' to be 2.7 from 100 samples of *C. marulius* collected from Harike Wetland (A Ramsar site) in Punjab for larger samples ranging between 37.69-85.41 cm. Khan *et al.* (2012) calculated 'b' value from a collection of 565 specimens of *C. marulius* and found to have pooled up value of 3. Bhatti (2010) calculated LWR of *C marulius* from a culture pond in Punjab Province and recorded lengths and weights at regular monthly intervals. According to him the pooled group ranging in length from 7.37 cm to 53.80 cm (weight 2.88 gm to 1165.79 gm) showed isometric growth with 'b' value of 3.05 (r^2=0.999) though the general value of 'b' was found to range between 2.59-3.26 in different sizes and months that indicated growth difference due to temperature and maturity conditions. Therefore, 'b' value of 3 in all the length groups in the present study indicated that the growth of this species was optimum in the captive conditions which may be due to regular feeding to fish on supplementary diet.

Ali *et al.* (2013) compared the LWR of *C. diplogramma, C. marulius* and *C. striata* from the riverine reaches of Lake Vembanad in Kerala and observed that *C. diplogramma* and *C. marulius* showed non-isometric growth pattern in the study region, whereas, *C. striatus* showed an isometric growth pattern. The exponent 'b' was found 1.28 and 1.54 respectively for *C. diplogramma* and *C. marulius* ($t = -11.76$, $df = 151$, $p < 0.0001$) which was significantly lesser than the cubic value, whereas, in case of *C. striatus*, the exponent was 2.73 which was not significantly different from the cubic value ($t = 1.43$, $df = 135$, $p = 0.16$). They concluded that both the species showed

non-isometric or negative allometric growth and hence did not grow in proportion to the length as compared to *C. striatus*.

There are also reports of conflicting values of 'b' with respect to size consideration. Devraj (1973) in his study suggested that the specimens of smaller length class (<8 cm) had an exponent of 1.18, while the specimens of larger length class (>8 cm) 3.33. Ali *et al.* (2013), however, gave opposite finding of having higher 'b' value in smaller size groups in contrast to larger and revealed that this genus has two growth stanzas. Such ontogenic variations in the cube law are also known from other fish species as well (Froese, 2006).

There are good number of citations indicating the role of food availability and its quality on the LWR of *Channa* spp. Datta *et al.* (2013) in his study with 6 types of iso-nitrogenous diets concluded that the fish showed positive allometric growth of 3.32-4.39 with only four diets which also revealed that the acceptability of formulated feed was good in this specie and hence suggested suitability of this species under captive conditions on formulated diets.

From the above studies on murrels, it is evident that the value of b varies largely and may be negatively allometric to positively allometric depending on climatic conditions and rearing systems. However in most of the above cases, the b value collected from the open water systems was generally found negatively allometric, whereas the same was recorded near isometric to positively allometric in case of fishes collected from the captive conditions. Therefore, the present study revealed that the species is highly suitable for growth in captive conditions when grown on supplementary diets and the fish gained weight in sincronization with length.

4.1.4 Condition Factor

The condition factor (K) or coefficient of condition (CF), also sometimes refered as length-weight factor, has been frequently used by fish biologists as an index of well being, plumpness, fatness or relative robustness of a fish (Bagenal, 1978; Pauly, 1984). The value of the condition factor decreases with a decrease in the weight

of the fish in relation to its length (Bakare, 1970; Fagade, 1979).The factor has been used as an index of feeding intensity, growth (Fagade, 1979), and available environmental conditions (Bagenal and Tesch, 1978). The value of the condition factor also changes with the reproductive status of the fish, and the highest values appeared during the spawning and reproductive activity, due to accumulation of fat (Vazzoler, 1996) and development of gonads (Le Cren, 1951; Angelescu *et al.*, 1958). A higher value of the condition factor during certain seasons may indicate the state of sexual maturity and degree of nourishment (Gomiero and Braga, 2005).

Variation in the condition factor also appeared between two sexes in certain species. The value of K was reported as 0.787 and 0.859 respectivly in male and female elephant fish (*Mormrus rume*) in the river Ose, southern Nigeria (Olabode *et al.*, 2007). The condition factor was reported to be higher in late stages of maturation as compared with the earlier stages of maturation of gonads in female kutum (*Rutilus frissi kutum*) (Kousha *et al.*, 2009) indicating an increase in the size of gonads under maturation. The value of the condition factor exhibited a gradual increase with the increasing maturity of the gonads and the maximum value corresponded with the maximum development of the ovaries in kutum but the condition factor persisted at higher levels even after recession of the ovaries (Sabet *et al.*, 2009).

In case of snakeheads, the condition factor 'K' has been calculated in *C. punctatus* by Singh and Ram (2011) from natural and captive (Pond) stocks ranging from 0.995 to 1.398 and 0.705 to 1.446. These authors concluded from their study that specimens from natural stocks have wider range than the captive stocks and monthly variation was observed in both the conditions. Koundal *et al.* (2014) calculated 'K' values for different size groups (SL 40-60 mm) of both the sexes of *C. punctatus* and revealed that the 'K' value was above 1, which indicated well being of this fish under both the conditions. They, however, noted 'K' values up to 2.32 and 2.37 in larger size groups of 40-60 mm in both the sexes and attributed to the voracious feeding behaviour. In both sexes the value of 'K' showed almost decreasing trends with increasing length, except few exeception in male where it exhibited an increasing trend

after attaining 140-160 mm size. Victor *et al.* (1992) calculated 'K' value for *C. obscura* from a Nigerian pond and observed that these values were higher 2.2 for small size group (16-18 cm) in comparison to larger size (30-32 cm) having these value very low 0.05 only. Datta *et al.* (2013) evaluated 'K' value for *C. punctatus* reared in captive conditions on five types of formulated diets and observed 'K' value to range 1.094 to 1.334 that revealed well acceptance of formulated diet by the fish. Out of five diets tried by them, three diets performed much better which also confirmed that the'K' value also depend on the quality of the diet. In the present study the highest condition factor (0.92) was observed in the smallest size group of 1-10 intervals and which gradually decreased with the advancement of size and age which confirm the findings of Victor *et al*. (1992). The current low K values also indicate that *C. marulius* exhibit comparatively low values of K in comparison to carps and other snakeheads. The poor K values may also be due to poor weight gain in the fish as against length and further the species observe very poor GSI in comparison to C. striatus and C. punctatus.

Table 2: Growth related parameters in different groups of *C. marulius*

Parameter	Group I (1-10 cm)	Group II (11-20cm)	Group III (21-30cm)	Group IV (31-40cm)	Group V (41-50cm)	Group VI (51-60cm)	Group VII (61-70cm)
No. of samples	72	306	141	268	72	27	09
Mean length (cm)	6.92 SD±1.97	14.81 SD±1.86	26.375 SD±2.72	35.42 SD±2.64	44.27 SD±2.79	54.56 SD±2.48	63.11 SD±2.42
Max length (cm)	9.8	20.0	30.0	40.5	50.0	60.0	69.0
Min length (cm)	2.5	10.5	20.3	30.5	40.0	51.0	61.0
Mean weight (gm)	3.07 SD±2.2	23.81 SD±10.6	142.92 SD±47.1	307.03 SD±76.3	573.42 SD±125.4	949.30 SD±2.5	1761.1 SD±256.4
Max weight (gm)	8.2	81.0	320.0	600.0	1000.0	1500.0	2085.0
Min weight (gm)	0.5	9.7	47.5	160.0	394.0	590.0	1365.0
A	0.00924	0.00733	0.00779	0.00690	0.00661	0.00585	0.00701
B	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Log‘ w’ values	0.4868	1.3767	2.1551	2.4871	2.7584	2.1982	2.2768
K	0.92	0.73	0.78	0.69	0.66	0.58	0.70
R^2	0.84	0.68	0.76	0.51	0.67	0.67	0.60

a = constant (intercept), b = length exponent (slope), K = Ponderal Index, R^2 = Coefficient of determination

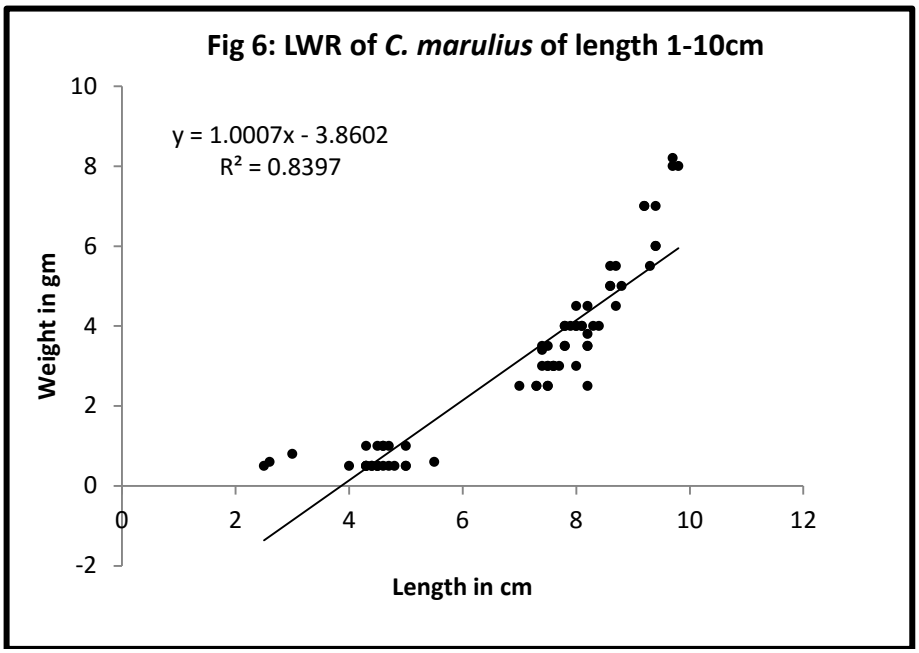


Fig 6: LWR of C. marulius of length 1-10cm
y = 1.0007x - 3.8602
R² = 0.8397
Weight in gm
Length in cm
10
8
6
4
2
0
-2
0
2
4
6
8
10
12

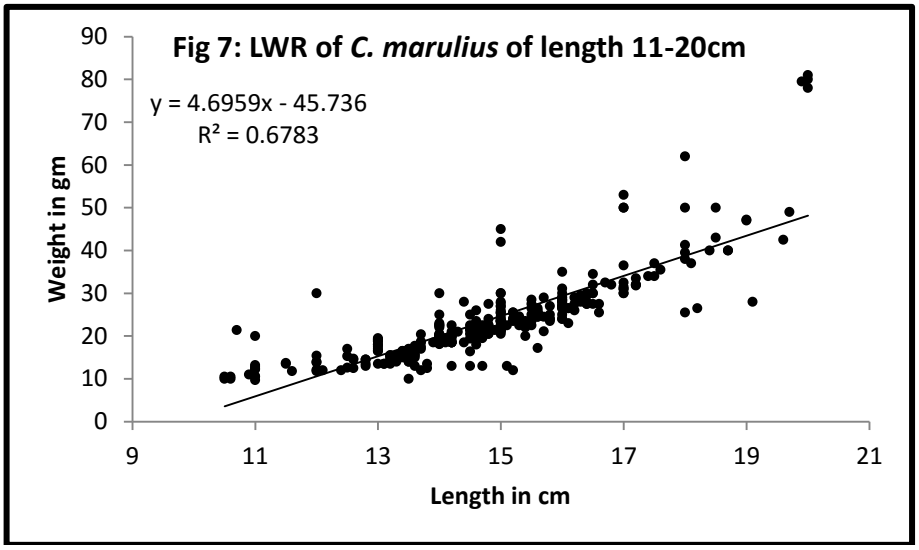


Fig 7: LWR of C. marulius of length 11-20cm
y = 4.6959x - 45.736
R² = 0.6783
Weight in gm
Length in cm
90
80
70
60
50
40
30
20
10
0
9
11
13
15
17
19
21

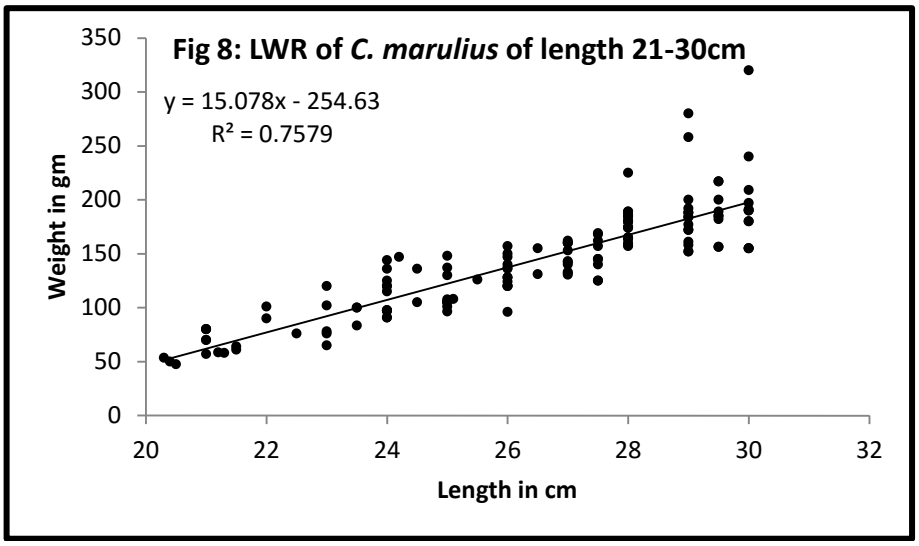


Fig 8: LWR of C. marulius of length 21-30cm
y = 15.078x - 254.63
R² = 0.7579
Weight in gm
Length in cm
350
300
250
200
150
100
50
0
20
22
24
26
28
30
32

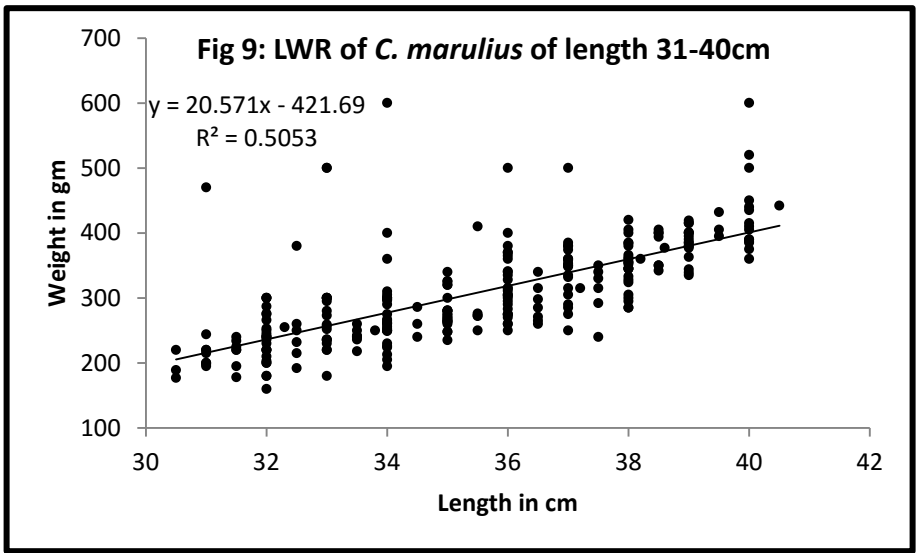


Fig 9: LWR of C. marulius of length 31-40cm
y = 20.571x - 421.69
R² = 0.5053
Weight in gm
Length in cm
700
600
500
400
300
200
100
30
32
34
36
38
40
42

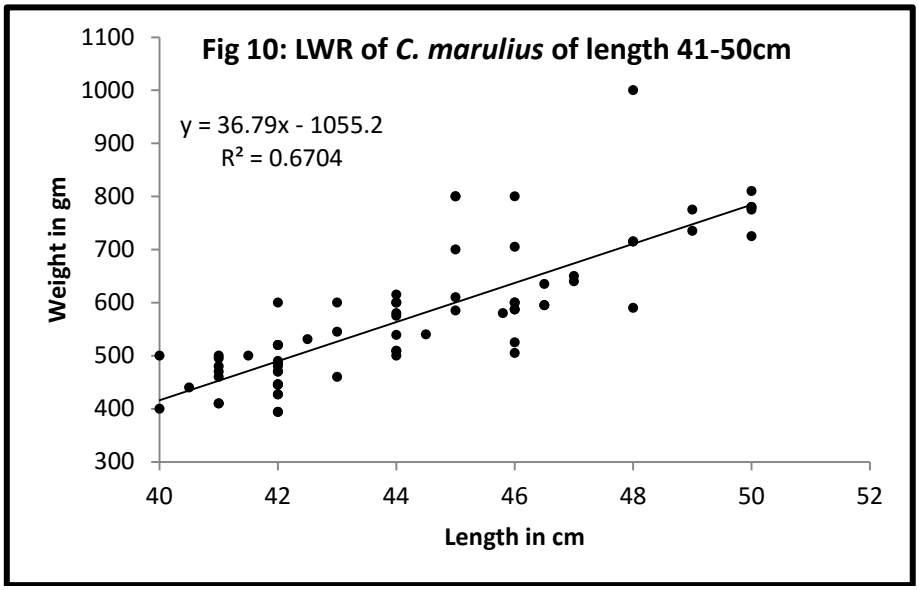


Fig 10: LWR of C. marulius of length 41-50cm
y = 36.79x - 1055.2
R² = 0.6704
Weight in gm
Length in cm
1100
1000
900
800
700
600
500
400
300
40
42
44
46
48
50
52

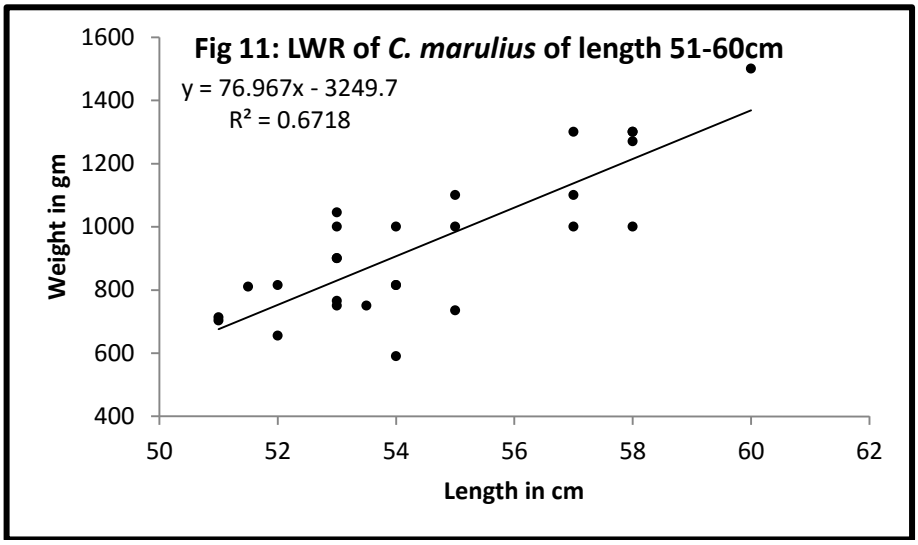


Fig 11: LWR of C. marulius of length 51-60cm
y = 76.967x - 3249.7
R² = 0.6718
Weight in gm
Length in cm
1600
1400
1200
1000
800
600
400
50
52
54
56
58
60
62

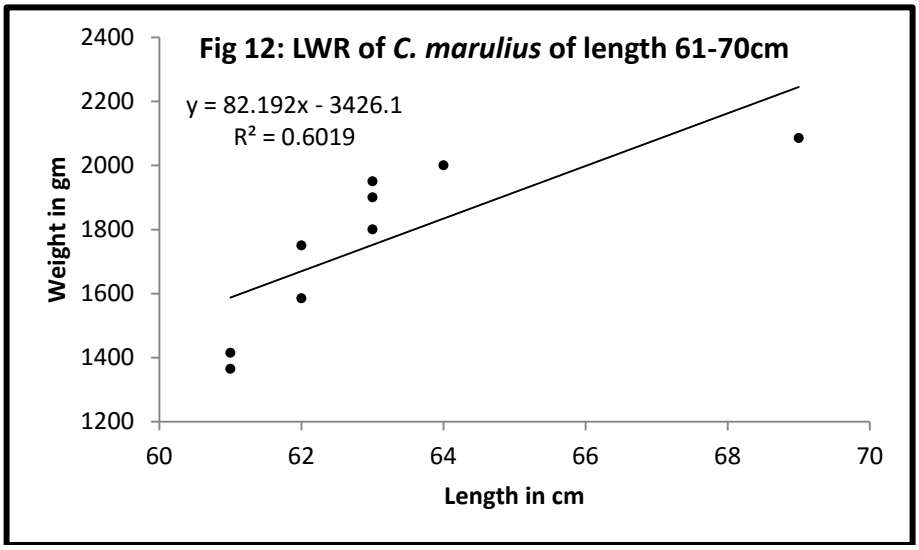


Fig 12: LWR of C. marulius of length 61-70cm
y = 82.192x - 3426.1
R² = 0.6019
Weight in gm
Length in cm
2400
2200
2000
1800
1600
1400
1200
60
62
64
66
68
70

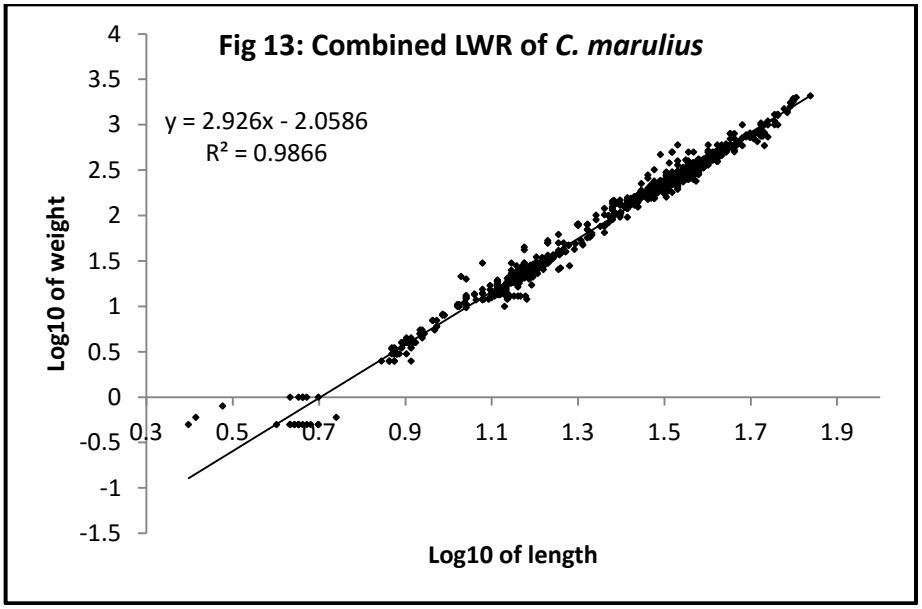


Fig 13: Combined LWR of C. marulius
y = 2.926x - 2.0586
R² = 0.9866
Log10 of weight
Log10 of length
4
3.5
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
0.3
0.5
0.7
0.9
1.1
1.3
1.5
1.7
1.9

Figure 14: Measuring length of *C. marulius* with measuring scale for LWR

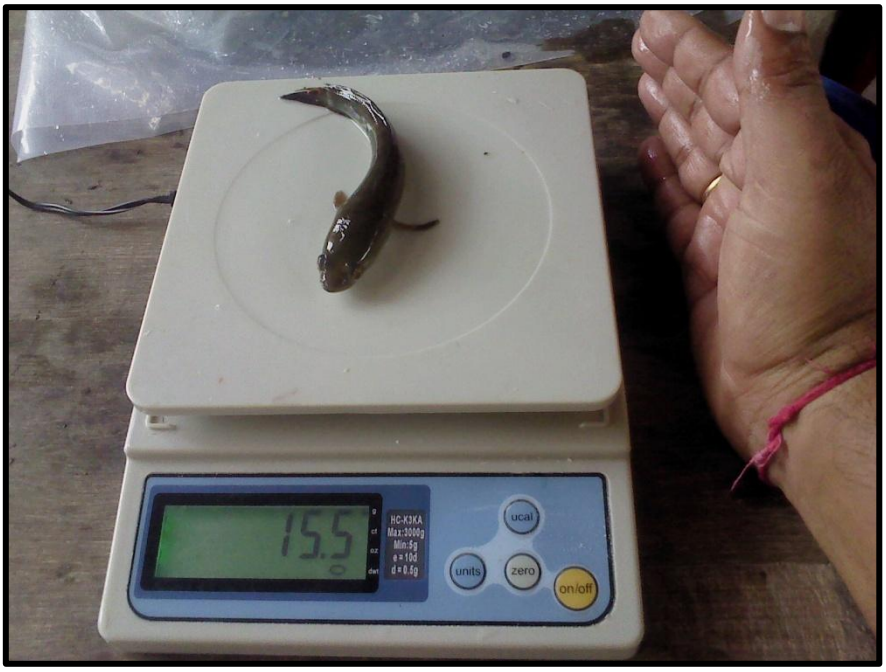


Figure 15: Measuring length and weight of *C. marulius* with electronic balance for LWR

4.2.2 Morphometric relationship

The summary of the statistical analysis for 24 morphometric variable of *C. marulius* of size range 3.22 cm to 19.10 cm is given in Table 3. The values of coefficient of variation (CV) were found >30% for all the parameters of the study when compared with the total length. The highest CV was observed for caudal fin length (71.43%) followed by head depth (51.50%), dorsal fin length (50.44%), pre-orbital length (49.47%), standard length (49.26%), forked length (48.10%) and upper jaw length (47.79%). The lowest value of CV was observed for dorsal fin spine length (32.42%), followed by head width (36.61%), pre-pectoral length (38.51%), eye diameter (38.56%) and caudal fin height (38.98%). The present results were, however, quiet different from the study of Bhatti (2010) on the same species, which correlated the CV values with total wet weight of the fish with different body parts lengths. The maximum CV found by him was for dorsal fin length (37.10%) followed by caudal fin length (37.08%), pectoral fin length (34.95%) and ventral fin length (33.51%), whereas the lowest values were observed for dorsal fin distance from mouth tip (6.59%), pectoral fin distance from mouth tip (13.29%), pelvic fin distance from mouth tip (14.14%) and eye diameter (18.48%). Therefore the CV values were found comparatively low when different lengths were compared with that of total wet weight of the fish. However, most significant changes were recorded for dorsal fin length and caudal fin length in both the studies.

The analysis on 5 different meristic variables (fins) of *C. marulius* fins under the present study (Table 4) suggested a relatively higher variability for all the variables as indicated by high CV in comparison to the study of Bhatti (2010). The difference in the CV in the present study ranged 8.92% to 18.80% in comparison to 2.22% to 9.25% obtained by Bhatti (2010). The highest variability in the present study was found with the anal fin rays (18.80%) followed by pectoral fin rays (15.69%), dorsal fin rays (12.15%), caudal fin rays (8.98%) and pelvic fin rays

(8.92%), which was observed 2.59%, 6.70%, 2.22%, 6.02% and 9.25% for the same parameters by Bhatti, (2010).

The morphometric and meristic variables are species-specific, depending upon its genomic structure, and vary with age and sex. These variables are therefore important from the taxonomic points of view and used for identification of the species as well in determining inter-specific differences. However, changes in the values of these variables also relate to the environmental conditions, like, food, temperature, rearing conditions, seasons, etc. (Beverton and Holt, 1959; Jakupsstovu and Haug, 1988; Bromley, 1989). The morphometric variables exhibit wider variations as compared with the meristic variables, the later (meristic variables) having a higher taxonomic value, while the former are more under the control of age and other environment related factors.

Goswami *et al*. (2006) described the morphological and meristic characters of four species of snakeheads and confirmed that *C. barca, C. aurantimaculata, C. stewartii and C. bleheri*, which are endemic to Assam, are separate species and their morphometric and meristic characters are not only different from most common snakehead species like *C. punctatus*, *C. gachua*, *C. striatus* and *C. marulius* but also in the four studied species.

Talwar and Jhingran (1992) have described the meristic characterization of *C. marulius* as dorsal fin rays 45–55, pectoral fin rays 16–18, pelvic fin rays 6, caudal fin rays 13-16 and anal fin rays 36. Talwar and Jhingran (1992) with having dorsal fin rays 43-63, pectoral fin rays 15-20, caudal fin rays 13-20, pelvic fin rays 5-9, however, there was too much variation in number of anal fin rays which were counted 32-40 in comparison to 6 recorded by him. Bhatti (2010) also enumerated the meristic characteristics of *C. marulius* and recorded this to be as dorsal fin rays 51-55, caudal fin rays 13-15, anal fin rays 32-35 and the numbers of the pectoral fin rays very low 4–6. Therefore, the population of the present study seems to be different from both Talwar and Jhingran (1992) in respect of wide difference in the

number of anal fin rays and with Bhatti (2010) on account of large difference in the number of pectoral fin rays.

Therefore, the present study can act as a base line reference point for the future studies for a detailed description of this species with a care that the data has been procured from single pond.

Table 3: Summary of morphometric variables of fingerling of pond reared *C. marulius* at total lengths 3.22-19.10 cm, n = 21 for all variables.

Variables	Mean ± SEM	Range (cm)	95% CL of Mean	C V (%)
1. Total length (cm)	12.05 ± 2.78	3.22 - 19.10	1.20	120.12
2. Standard length (cm)	7.50 ± 0.71	2.96 - 16.80	0.49	49.26
3. Fork length (cm)	8.80 ± 0.81	3.00 - 19.10	0.48	48.10
4. Pre anal length (cm.)	4.15 ± 0.34	8.00 - 6.00	0.44	43.29
5. Pre pelvic length (cm)	2.63 ± 0.20	1.15 - 5.20	0.39	39.85
6. Pre pectoral length (cm)	2.42 ± 0.18	1.12 - 4.80	0.38	38.51
7. Caudal peduncle length (cm)	0.63 ± 0.05	0.17 - 1.20	0.47	44.08
8. Caudal peduncle depth (cm)	0.65 ± 0.05	0.22 - 1.26	0.41	41.66
9. Body depth (cm)	1.25 ± 0.10	0.49 - 2.51	0.43	43.64
10. Head length (cm)	1.92 ± 0.13	0.84 - 3.35	0.39	37.64
11. Head width (cm)	1.31 ± 0.09	0.55 - 2.55	0.39	38.60
12. Head depth (cm)	1.10 ± 0.07	0.50 - 1.96	0.37	36.61
13. Dorsal fin length (cm)	4.68 ± 0.46	1.57 - 10.60	0.52	51.50
14. Anal fin length (cm)	2.91 ± 0.28	1.08 - 7.00	0.51	50.44
15. Pelvic fin length (cm)	0.83 ± 0.05	0.26 - 1.60	0.37	37.24
16. Pectoral fin length (cm)	1.20 ± 0.09	0.39 - 2.54	0.41	41.09
17. Caudal fin length (cm)	1.38 ± 0.10	0.58 - 2.90	0.41	41.02
18. Caudal fin height (cm)	1.15 ± 0.15	0.40 - 4.40	0.71	71.43
19. Pre dorsal length (cm)	2.53 ± 0.19	0.90 - 4.56	0.39	38.98
20. Dorsal spine length (cm)	0.43 ± 0.03	0.20 - 0.82	0.42	42.07
21. Eye diameter (cm)	0.53 ± 0.03	0.20 - 1.10	0.32	32.42
22. Pre orbital length (cm)	0.59 ± 0.04	0.20 - 1.10	0.39	38.56
23. Upper jaw length (cm)	0.87 ± 0.08	0.30 - 1.86	0.49	49.47
24. Lower jaw length (cm)	0.91 ± 0.08	0.30 - 1.87	0.48	47.79
	0.76 ± 0.05	0.46 - 1.40	0.35	35.46

SEM: Standard error of mean
CV: Coefficient of variation
CL: Confidence limit

4.2.3 Morphomeristic relationship

Table 4: The summary of different meristic variables of pond reared fingerling of *C. marulius* at NBFGR, Lucknow (n = 21 for all variables)

Variables	Mean ± SEM	Range	95% CL of Mean	C V
1. Dorsal fin rays	52.3 ± 00.83	43.0 - 63.0	0.0823	12.1546
2. Anal fin rays	33.8 ± 00.35	32.0 - 40.0	0.0532	18.8045
3. Pelvic fin rays	07.0 ± 00.15	05.0 - 09.0	0.1121	8.9232
4. Pectoral fin rays	18. 3 ± 00.22	15.0 - 20 .0	0.0637	15.6960
5. Caudal fin rays	16.2 ± 00.34	13.0 - 20.0	0.1112	8.9896

Figure 16: Samples used for the measurement of morphometric and meristic characters of *C. marulius*

Chapter 5

REPRODUCTIVE CHANGES

Chapter 5

REPRODUCTIVE CHANGES

5.1. MATERIAL AND METHODS

5.1.1 Brood stock rearing

One hundred and five, 2-3 years age group brooders of of *C. marulius* from existing stock of the NBFGR farm facility were procured and stocked in an earthen pond of size 0.04 ha (20m x 20m x 1.5m, L x W x D) for the procurement of samples for this study. Before stocking, the pond was drained out and cleaned from all vegetation in the month of September 2014. The water was filled up to 4.5 feet depth and around 4 kg of mustared oil cake and 0.5 kg of Diammonium phosphate (DAP) fertilizer was added for developing planktonic turbity as well for the production of sufficient plankton to serve as live food to the carp minnows also to be developed in the pond to serve as prey to *C. marulius*. Around four kilogrammes of live carp minnows were introduced 7-days after manuring in this pond. The brooders of *C. marulius* were stocked in the pond one month after stocking of carp minnows so that they can acclimatize well to pond water conditions. The fishes were also fed cut pieces of fish at least twice in a week. The brooders were collected from this pond by draining the pond with a portable diesel pump, hand picking them and transporting in a canvass carry bag along with water to the laboraotry.

5.1.2 Sampling for morphological identification

Healthy looking adults of *C. marulius* (length 49.4 cm to 95.06 cm, weight 0.8 kg to 5.4 kg) were collected from the above pond during January, February, March and April months. As March and April being the best spawning period of this species, majority of the gonadal changes occur during February-April. Initially, sexes were identified based on morphological differences and secondary sexual characters which are elaborated in the subsequent paras.

5.1.3 Sampling for gonado-somatic index

Wet weight (using top loading balance, Shimadzu 120 HA, Germany, minimal count 0.1 g) and length using measuring scale (minimal count 1 mm) were recorded in live condition in the laboratory for both the sexes. As the terminal part of gonads (vent side) remain adhered to the ventral mesovarium for quite a good distance, each sample was dissected carefully by making an incision with scissors about one centrimeter away from vent towards the tail end. The incision was further moved on the fish's lateral side up to the base of pectoral fin so that the whole of the coelome could be easily opened without cutting the mid ventral margin, where initial part of the gonads remain attached, and the gonads could be easily traced out intact.

Gonads were carefully separated from the rest of the visceral mass and weighed with the portable digital mini-balance (Shimadzu 120 HA, Germany; minimum count 0.1 g). Samples of gonads were then preserved in 10% buffer formalin in glass bottles and kept for further processing for histological studies. A sample size of more than seven (7) from both sexes was taken for each calendar month to give 99% confidence to the sampling.

5.1.4 Sampling for histological study

The fixed tissue of the gonads were taken out of the 10% buffer formalin and processed for histological studies. The tissues were than passed through ascending grades of alcohol, and given repeated washes with the absolute alcohol to ensure complete removal of water from the tissue. The dehydrated tissues were then cleared with xylene (Merck). The tissues were then embedded in the paraffin wax (Merck) (melting point 58-60^{o}C).

Sections of the embedded tissues were cut (5 μm thick) using microtome. The sections were placed on a clean glass slide; wax removed by mild heating and placing in xylene. The tissues were further processed through grades of alcohol for differential double staining (eosine and haemotoxyline) and preparation of permanent slides using

DPX. The permanent slides were then studied for different histological features under the light microscope (Labomad Model D2). Photomicrographs of the slides were prepared for further detailed examination using light microscope.

Analysis

The reproductive changes were analyzed separately with different variables. Gonadosomatic index (GSI = wt of gonad / body weight of fish X 100) were calculated for individual fish. The individual values for different specimen collected during different sampling calendar months were pooled and different statistic constants for range, mean, 95% confidence limits of mean and standard error of mean, were calculated for different variables for each calendar month using M.S. Excel version 2007.

5.2 RESULTS AND DISCUSSION

5.2.1 Morphological changes

Morphological sex determination in *C. marulius* is difficult both during spawning and non spawning season and that create problem in the identification of well matured specimens during captive breeding programmes. Therefore, efforts were made to demarcate this problem with more reliable characters and based on the experience of earlier workers and this study. Now, the sex and maturity can be identified comparatively with much ease in both the sexes. The details of which are given below sex-wise.

Female

The females were found to have either no distance or little distance between the tail end margin of pectoral fin and the first rosette of the lateral line (Figure 17a). Further, there is comparatively very little distance between the vent and the start point of anal fin (Figure 17b). These two points are very well observed even during non breeding period. During spawning season, the belly was found slightly swollen below the base of pectoral fins but this character was only pronounced in a good fecund fish and can be judged well when the fish is kept in container in clear water. The females have round vent and during spawning season, slightly fleshy and deep red (Figure 17b). In no case, ova protruded out from the vent when mild pressure over the abdomen is applied as in the other fish species.

Male

It was observed in the present study that the males were found to have larger distance between the tail end of the pectoral margin and the first rosette of lateral line in comparison to females and this character exists in both immature and mature specimens and during spawning as well non spawning seasons (Figure 18a). Another character which could be observed both during spawning and non spawning season is larger distance between the vent and the start point of anal fin (Figure 18b). During

spawning season, the colour of males is generally darker in comparison to females, vent pale in colour, slit like. A good and robust brooder also has small but distinct papilla on the downside of the vent (Figure 18b). A good mature male was found to exude transparent milt from the vent when pressure is applied on the abdomen.

The morphological sex-identification characters have been elucidated by several authors in the past but they are generally defined for secondary sexual characters which develop on attaining maturity. Parmeswaran and Murugesan, 1976 and Devraj, 1973 have suggested characters of identification for both the sexes but that were not much helpful to make differentiation between mature and immature specimens of both the sexes' particularly a mature male brooder. Hossain *et al*. (2008) described that mature females have bulging soft abdomen, oval shaped reddish vent and smooth pectoral fins, which is different from the present description. The similar workers also described that males were identified by them by pale reddish vent slit and rough pectoral fins; however, the later character was not observed in any of the specimens and also not reported by any other author. According to Bilal *et al*. (2013), the vents is pale, slit-like and have anal papilla like structure with pointed tip in male fish which was also observed in the present study. These authors further narrated that the female fish oozes egg when pressure is applied on the abdomen, whereas males did not spill-out milt. These later two findings in case of both female and male fish was not observed in the present study and in fact even a good fecund female never oozed out eggs on applying mild pressure over the abdomen as in case of other fishes and good matured males were observed to spill-out transparent milt on applying mild pressure on the abdomen.

Considering the difficulty observed in the morphological sexual differentiation in the *C. marulius,* the characteristics suggested for the identification of sex in the present study, are more elaborate and can be utilized in a better way during captive breeding programmes in comparison to what have been suggested by the earlier workers. This will also help in the morphological identification of sex in other species of *Channa* group.

Figure 17: A female specimen having (a) first rosette close to tail end of the pectoral fin, (b) vent slightly bulging, red and the distance between vent and base of anal fin closure.

Figure 18: A male specimen showing (a) large distance between the first rosette and the tail end of the pectoral fin, and (b) a fully mature male showing distinct papilla and larger distance between vent and the base of pelvic fin.

5.2.2 Anatomical changes

Ovaries

The ovaries of *C. marulius* were paired organs and situated in the peritoneal cavity and suspended on either side of the mid-line by a mesovarium. The ovaries were spindle shaped and vary in size with left lobe larger in length than the right lobe. Initially it is small, and looks like a muscular mass reddish in colour and without oocytes (Figure 19A). With advancement of maturity, the size of the ovary increases both in length and diameter and oocytes become visible in the ovarian follicle (Figure 19B). Initially, oocytes are pale yellow in colour. With further ovarian development, the length and size of both lobes of ovary increases and the ovarian follicles were found to be filled entirely with the ova which were pale yellow in colour. The ovarian follicles at this stage had large network of blood capillaries over its outer surface (Figure 19C, D, and E). With further advancement in the maturity, the ovarian follicles were found to increase more in diameter than the length due to increase in the number and size of oocytes and both lobes of ovarian follicles become flaccid. The colour of ova now turns to orange colour and there was reduction of network of blood capillaries over the ovarian follicles, which marked attaining of complete maturity in the fish (Figure 19F)

Though majority of the samples (>80%) were found to have ovary in immature phase during February, the rest had the ovary in maturing phase but none of the sample was found to have ovary in complete maturity phase. The complete maturity phase was observed during March and April when ovarian follicles were found highly flaccid, filled with orange colour ova. However, it was surprising that around 60% of the samples dissected were found to have ovary in full maturity phase, whereas rest were found to have ovary both in immature and maturing phase.

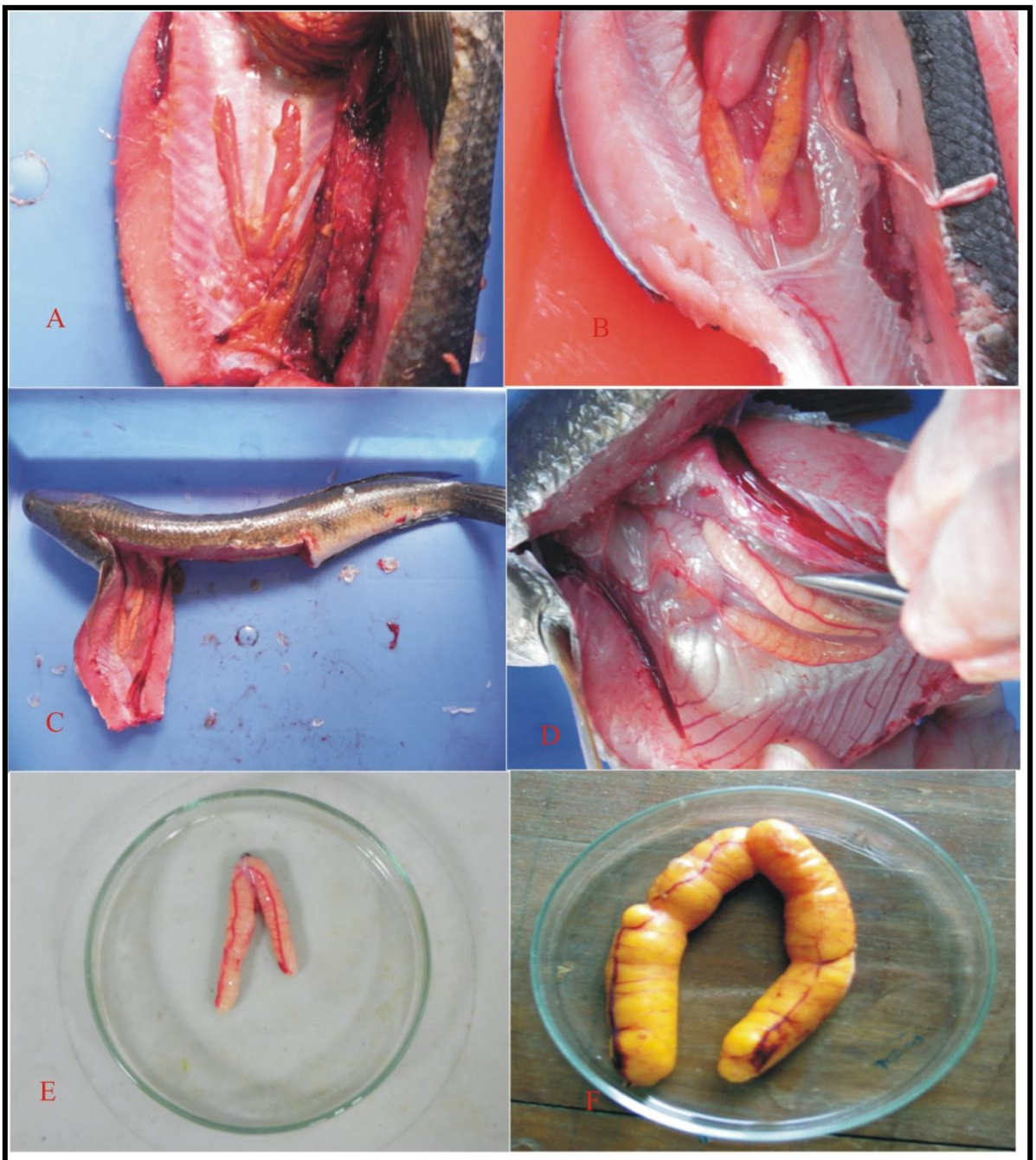


Figure 19: Showing different maturity stages of ovary of *C. marulius* (A) Immature fleshy ovary, (B) Early maturing stage containing little ova, (C) Maturing stage containing larger number of ova, (D) Maturing stage having large network of blood capillaries in *situ*, (E) Maturing stage *ex situ* and (F) Fully mature stage.

Testis

The testes of *C. marulius* are paired lobular type structure, situated in the peritoneal cavity and suspended on either side of the mid-line by a mesovarium. At initial phase of development, the testes remain attached completely over the ventral mesovarium and with advancement of maturity the cranial part was found to invade both side of the alimentary canal. Like ovary, the left lobe of the testis is also larger in size than right. Both the testis lobes were comparatively very small in length and diameter in comparison to carps. At immature stage which was observed in all three months of sampling (February – April), the testes were very minute; ribbon-shaped, flat and pale pinkish structures embedded on the ventral mesovarium and had no spermatic fluid within (Fig.20A, B). Though GSI of male specimens in case of snakeheads has been described by some of the earlier workers which differ so much in size of testes in this species and none of the authors described the difficulty they faced in the extraction of the testes. In the begening of the work, it became difficult to extract testes from the fish following the routine methodology used in case of carps and catfishes. Therefore, an entirely different methodology was developed for the extaction of the testes which is more or less similar as recently described by Newhard (2015) in the case of *C. argus* as being the first report. The maturing and mature stages of testes were, however, observed both in March and April months as this being the spawning season of *C. marulius* in north India. As the maturity advances in the testis, both lobules of the testis increases in size and turns to tubular membranous structure lighy pink in colouration (Figure 20C, D). At this stage, both the lobes of the testis start innervating the coelomic cavity and expand along with the alimentary canal towards cranial part. The size of both the lobes further increased with advancing of the maturity condition and a fully mature male was observed to have testis comprises of two cylindrical lobes (right lobe being larger) which terminate into a small seminal vesicle near the vent. The testis lobes were membranous, light pink in colour and filled with transparent spermatic fluid which exuded out as transparent syrup on cutting the testis lobes (Figure 20E, F).

Majority of the samples (>80%) dissected were found to have small immature testes with little spermatic fluid even during March and April months though this being the major spawning period of this species. The fully mature testes were found only in few specimens who were larger in size and darker in body colouration, which was unlike to other fish species.

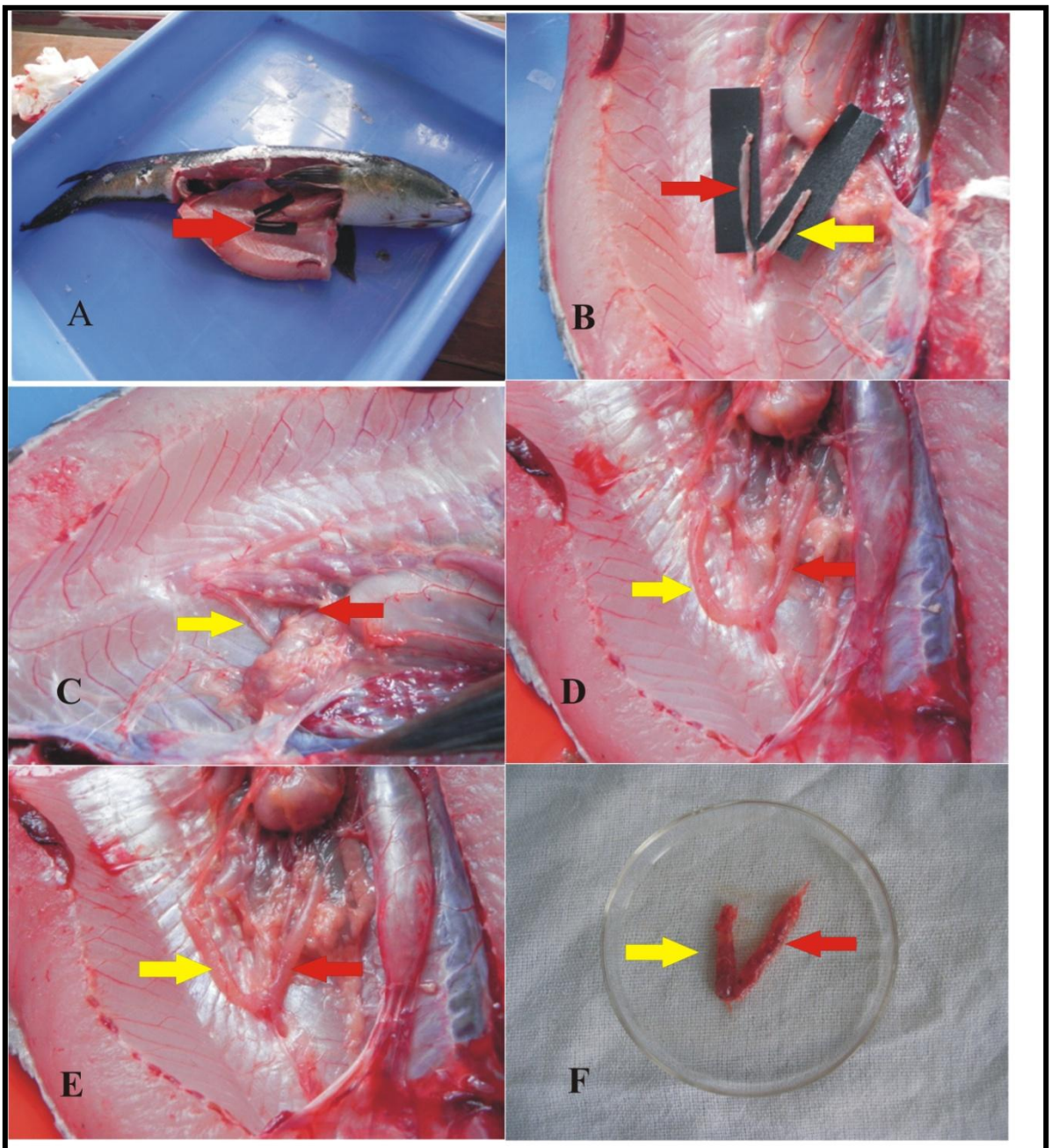


Figure 20: Showing maturity stages of testis of C. Marulius, (A) Immature testis in situ, (B) Immature testis in situ in close-up, (C) Early maturing stage testis, (D) Late maturing stage testis, (E) Matured testis in situ and (F) Matured testis ex situ in a watch glass. Yellow arrow shows small right lobe and red arrow left larger lobe of testis.

5.2.3 Gonadosomatic Index

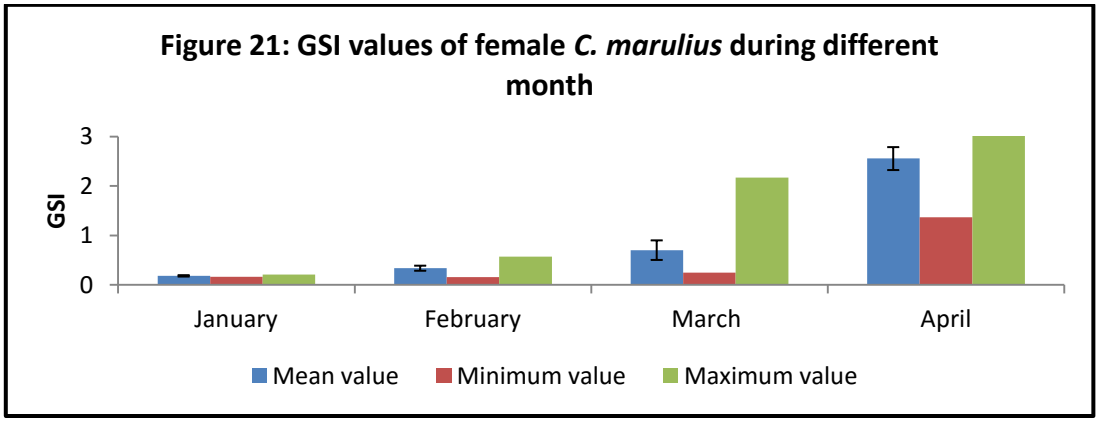
The length and weight of the fishes used for the assessment of GSI was 49.4 cm to 81.8 cm and weight 0.8 kg to 3.5 kg in case of females and 51.4 cm to 95.04 cm and weight 0.8 kg to 4.9 kg in case of males.

Female GSI

Variations in the values of the gonadosomatic index (GSI) of the female *C. marulius* during Janurary to April months are presented in Table 5 and Figure 21. The mean value of GSI was lowest in January (0.184 ± 0.010), which showed gradual rise in February (0.340 ± 0.051) and March (0.705 ± 0.199) months and reached maximum in April (2.556 ± 0.233). The GSI values were found highest during April (1.37-3.69) but comparatively larger differences in minimum and maximum values in GSI were observed during March (0.25-2.17). However, standard error of mean was observed lowest in January with gradual rise subsequently reaching highest in April.

Table 5: Gonadosomatic index of female *C. marulius* during January to April

Month	No.	Mean ± SEM	Min.	Max.	95% Confidence Interval for Mean	
					Lower bound	Upper Bound
January	4	0.184 ± 0.010	0.17	0.21	0.151	0.217
February	9	0.340 ± 0.051	0.16	0.58	0.221	0.458
March	9	0.705 ± 0.199	0.25	2.17	0.246	1.163
April	9	2.556 ± 0.233	1.37	3.69	2.019	3.092



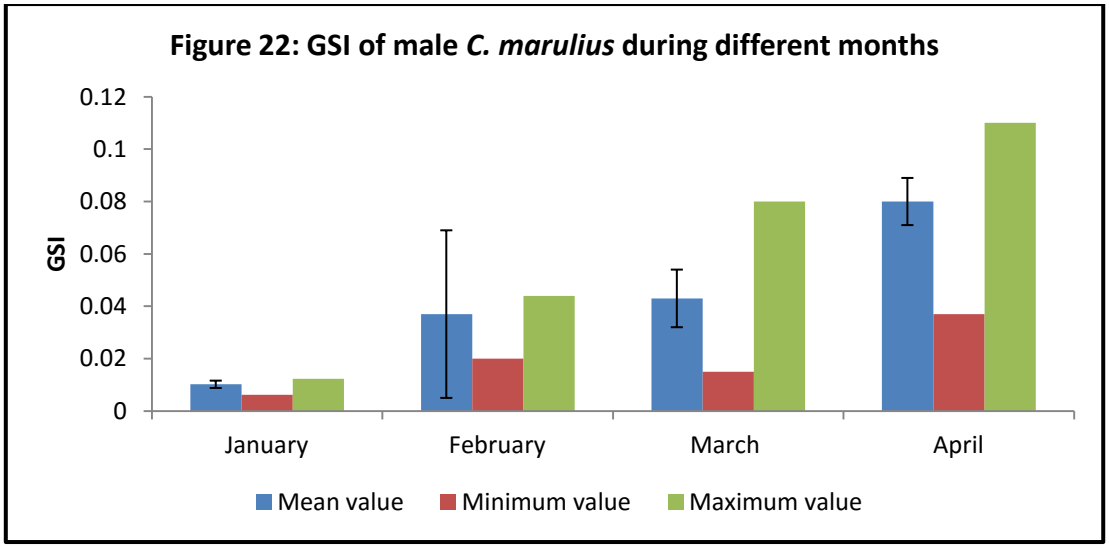
Male GSI

Variations in the values of the gonadosomatic index (GSI) of the male *C. marulius* during Janurary to April months are presented in Table 6 and Figure 22.

The mean value of GSI were the lowest both in January and February months (0.038 ± 0.029 and 0.037 ± 0.032), which showed gradual rise in March (0.043 ± 0.011) and reaching maximum in April (0.080 ± 0.009). Similar to females, the males of *C. marulius* also exhibited greater differences in the minimum and maximum values of GSI during March (0.015-0.080) in comparison to other months. There was no synchronization in the values of standard error of mean during different months.

Table 6: Gonadosomatic index of male *C. marulius* during January to April

Month	No.	Mean ± SEM	Min.	Max.	95% Confidence Interval for Mean	
					Lower bound	Uper bound
January	4	0.010 ± 0.001	0.006	0.0123	-0.0545	0.13244
February	4	0.037 ± 0.032	0.002	0.044	0.02407	0.04714
March	6	0.043 ± 0.011	0.015	0.08	0.01329	0.07338
April	8	0.080 ± 0.009	0.037	0.11	0.0585	0.10225



The Gonadosomatic Index (GSI) is an index indicating the relative development of the gonads (gonad weight) standardized against the total weight. The index has been frequently used to judge the relative development of the gonads (Vlaming *et al.,* 1982), with the assumption that the gonads are well developed in sexually active individuals and regress during the period of sexual inactivity. The GSI depends on several factors like age, season and more particularly on the maturity status of a fish.

The GSI values in number of fishes have been described as this being an important parameter relating to predicting spawning season of any fish. In female kutum (*Rutilus frisil kutum*) GSI started rising in March reaching the peak values (29.5) in April and exhibited a sharp decline in May (Sabet *et al.,* 2009; Saeed *et al.,* 2010; Kousha *et al.,* 2009). The values of GSI in male golden rabbitfish (*Siganus guttatus*) remained below 1.0 during December–April and showed significant increase in May (4.5) and June (11.1) and a decline in July (8.3) and further in August (1.4), suggesting spawning in this fish during May–July (Rehman *et al.,* 2000). The spawning season corresponded with higher values of GSI (male 2.1, female 7.8) as compared with spent or immature stage (male 0.3, female 0.8) in *Rhabdosargus haffara* captured from the Red Sea (Abuzinadah, 2001). Similar high peak GSI values have also been reported in male (8.0) and female (8.8) in the May sample of *Capoeta capoeta umblaand* these values remained low during June–April period (Erdouan *et al.,* 2002). In the two sexes of the black rockfish (*Sebastes schlegeli*), GSI values exhibited peaks during different times of the year (male, October-November; females, April-May), which has been explained by the fact that the mating in the species occurs when the male matures and sperm are stored in the female to fertilize eggs as they are produced (Mori, *et al.,* 2003). This appears to be the species adaptation, and is not probably characteristic of most teleost.

In case of snakeheads, very few literatures existing describing GSI of both the sexes. Olurin and Savage (2011) measured the GSI of *Parachanna obscura* female

(size 186.71±98.88 gm) from River Oshun in Nigeria during August 2006 to February 2007 and found it to be very low 1.96±0.63, whereas they could not measure GSI of male specimens (size 227.13±49.59 gm) as they record that the gonads were very small and in want of sensitive instruments. Chakraborty, *et al*. (2007) described the GSI of female *C. striatus* females collected from West Bengal and found to range from 0.439 in October to 6.182 in May. The GSI was estimated season-wise in case of both the sexes of *C. punctatus* by Kapil *et al*. (2011) from the ponds of Maharashtra which was found to be 3.69±0.775, 6.14±0.472 and 18.13±0.285 in case of females during winter, summer and rainy seasons, whereas in case of males the GSI values were 4.58±0.62, 8.06±0.74 and 10.11±0.42 during same seasons. Marimuthu and Haniffa (2006) have also evaluated the GSI of female *C. punctatus* from the pond located in southern India during December 1998 to February 1999 and found to range from 1.754 (size 170 mm) to 7.959 (138 mm). Tiwari *et al*. (2014) estimated the GSI value of female *C. marulius* a year around from Son River in Madhya Pradesh and found this to be low of 8.21 percent in September which started increasing at a rapid pace from March onwards and reaching to highest 47.67 percent in May month. The recording of Tiwari *et al*. (2014) with respect to GSI do not seems to be correct as so much of GSI is normally never encountered in most of the species and further the size of gonad is very small in comparison of its body size.

The low values of GSI in case of *C. marulius* recorded for December started showing an increase in February. The trend of increasing values of GSI continued so that peak values appeared in April. GSI exhibited a rapid decline in subsequent calendar months touching the lowest values in September which were maintained till December (Bhatti, 2010). The size of the testes starts increasing some where between December and February and maximum size achieved in April, when testis start receeding and the minimum size is maintained from June till December.

The changing values of GSI indicate the changes in size of the gonads standardized to the total body weight, and work as a good indicator of the reproductive status of the testis of the individual (Vlaming *et al.,* 1982). Higher values of GSI are

frequently regarded as indicative of spawning season (Abuzinadah, 2001). However, value of GSI is affected by the weight through temporary changes in the visceral mass caused by feeding. Different workers have tried to address this problem and have been subtracting gonad weight from the body weight (Tracey *et al.,* 2007; Muchlison *et al.*, 2010) or using eviscerated body weight.

5.2.4 Histological study

Ovaries

The histological study of ovarian development in the fish samples collected during February to April 2015 indicated different developmental stages of oocytes all through the study period and thus revealed that the ovarian development in this species is asynchronous and this species spawn many times during a prolonged breeding season. Each oocyte during its early development becomes surrounded by a layer of follicle cells and then develops to a full size ovum through processes of oogenesis. Eight stages of oocyte development defined cytologically by size, appearance of nucleus and nucleolus, and the type and localization of cytoplasmic inclusions were observed. They were chromatin-nucleolus stage, perinucleolus stage (subdivided into early and late stages), oil drop stage, primary yolk stage, secondary yolk stage, tertiary yolk stage, and maturation stage or migrating nucleus stage.

Ovarian development during February

Four stages of oocyte development were noticed during February viz; chromatin nucleolus stage (CNS), perinuclear stage (early perinuclear stage, EPS and late perinuclear stage, LPS), primary yolk vesicle stage (PYVS) and secondary yolk vesicle stage (SYVS) (Figs 23, 24, 25, 26). These stages revealed that the maturity in female *C. marulius* advances from immature to maturing stage in February.

Ovarian development during March

All six stages of oocyte development were observed in this month viz; CNC, EPS/LPS, PYVS, SYVS, Tertiary yolk vesicle stage (TYVS) and Yolk migrating stage (YMS) (Figs 27, 28, 29, 30). The presence of all six maturity stages in the female

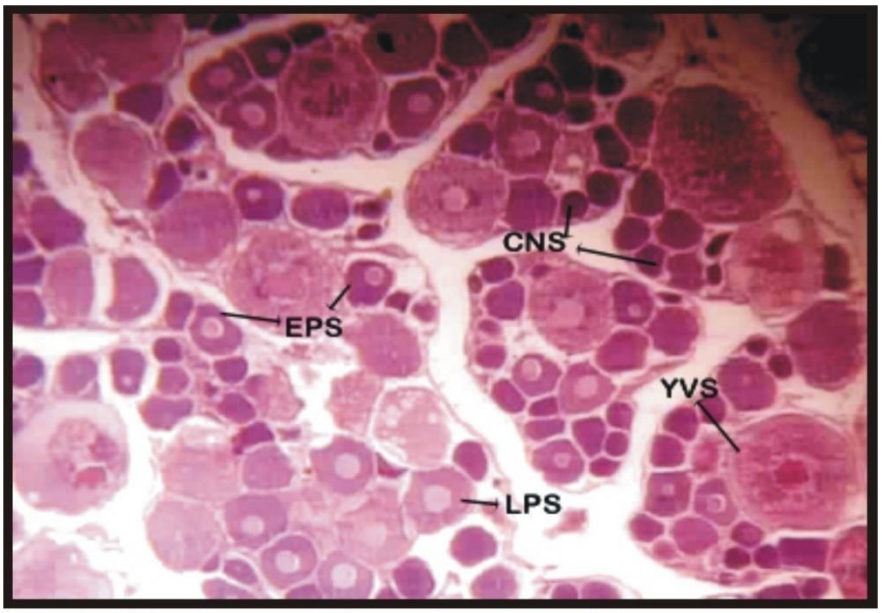


Fig 23: T.S. of ovary of *C. marulius* during February 2015. Four stages of oocyte development, chromatin necleolus stage (CNS), Early perinucleolus stage (EPS), Late perinucleolus stage (LPS), Yolk vesicle stage (YVS) are visible indicating immature stage of ovary of *C. marulius* (magnification 40X)

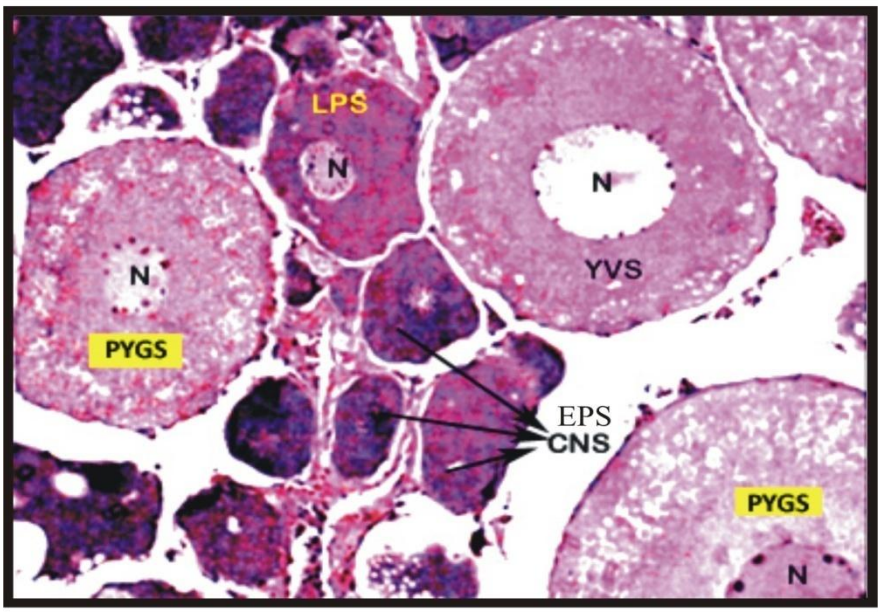


Fig 24: T.S. of ovary of *C. marulius* during February 2015. Four stages of oocyte development, chromatin necleolus stage (CNS), Early perinucleolus stage (EPS), Late perinucleolus stage (LPS), Primary yolk vesicle stage (PYVS) are visible indicating immature stage of ovary of *C. marulius* (magnification 100X), N-Nucleus

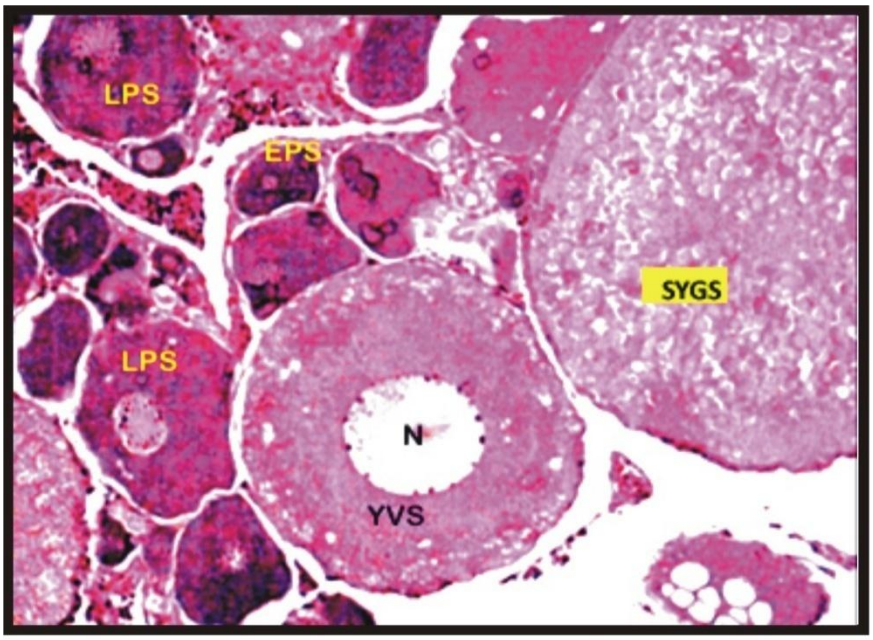


Fig 25: T.S. of ovary of *C. marulius* during February 2015. Four stages of oocyte development, chromatin necleolus stage (CNS), Early perinucleolus stage (EPS), Late perinucleolus stage (LPS), Yolk vesicle stage (YVS) are visible indicating early maturing stage of ovary of *C. marulius* (magnification 100X)

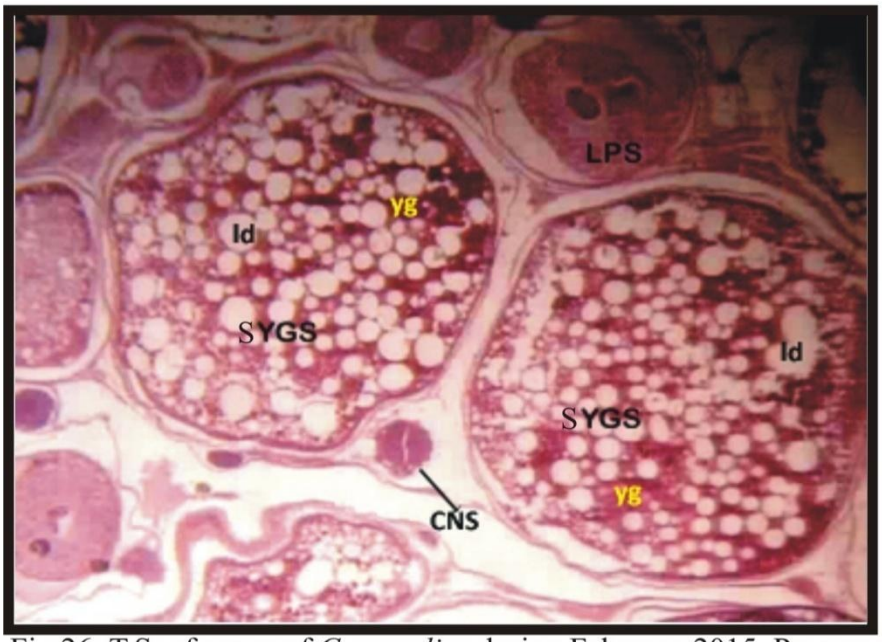


Fig 26: T.S. of ovary of *C. marulius* during February 2015. Presence of Secondary yolk globular stage (SYGS) showing maturing stage of oocyte development. Few oocyte showing chromatin nucleolus stage (CNS), early perinucleolus stage (EPS), late perinucleolus stage (LPS) are also visible (magnification 100X). Yg-yold granule and ld-lipid droplets.

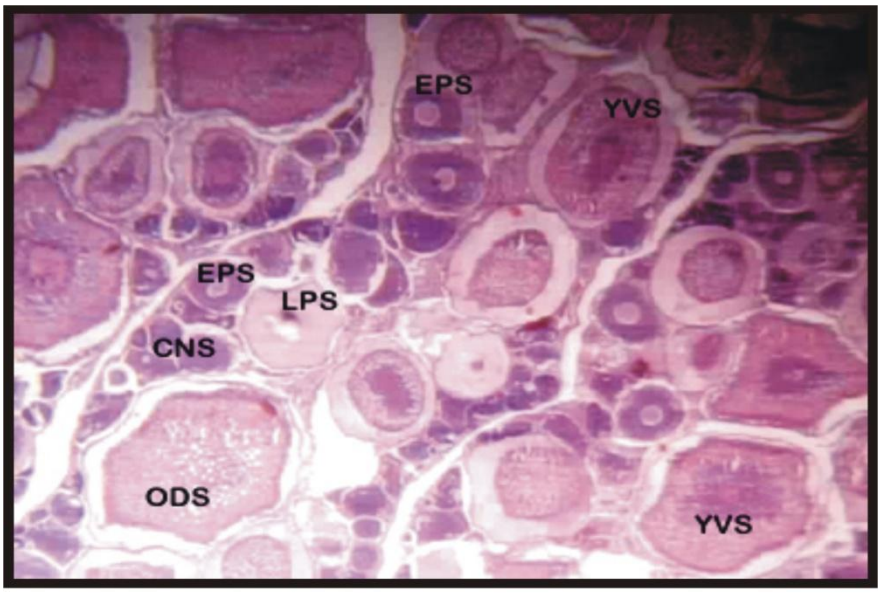


Fig 27: T.S. of ovary of *C. marulius* during March 2015. Four stages of oocyte development, chromatin necleolus stage (CNS), Early perinucleolus stage (EPS), Late perinucleolus stage (LPS), Yolk vesicle stage (YVS) are visible indicating early maturing stage of ovary (magnification 40X)

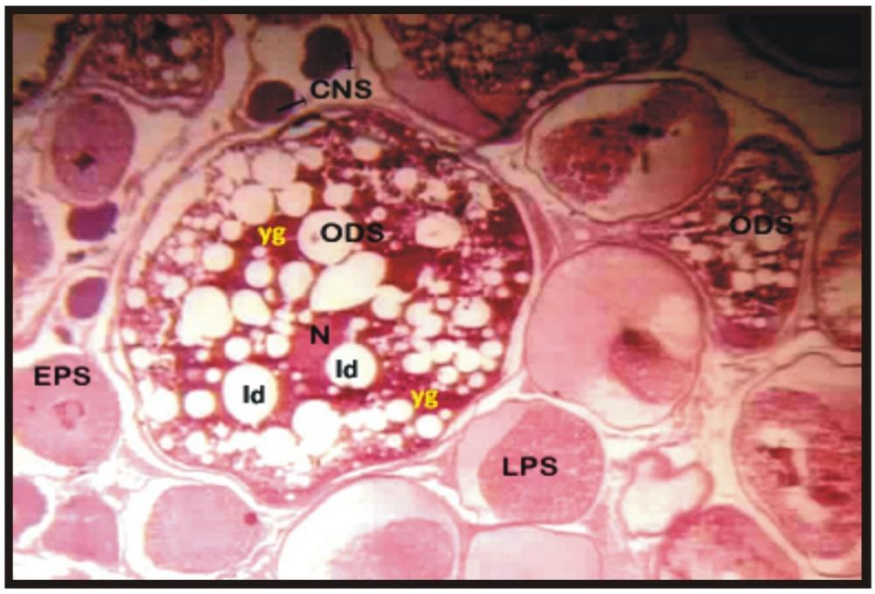


Fig 28: T.S. of ovary of *C. marulius* during March 2015. Four stages of oocyte development, chromatin necleolus stage (CNS), Early perinucleolus stage (EPS), Late perinucleolus stage (LPS), Yolk vesicle stage (YVS) are visible indicating early maturing stage of ovary (magnification 100X)

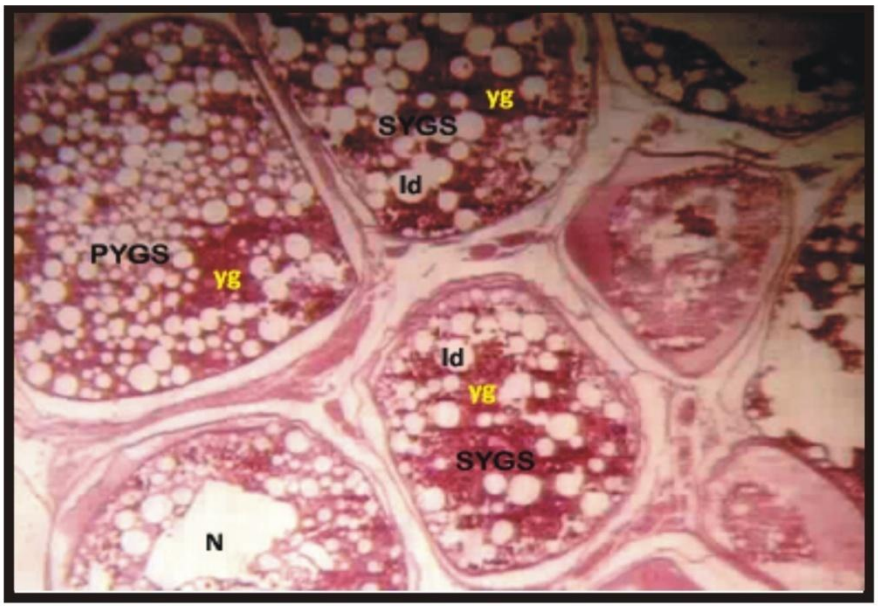


Fig 29: T.S. of ovary of *C. marulius* during March 2015 showing primary (PYGS) and secondary yolk globule stages (SYGS) indicating maturing stages of oocyte development. (magnification 100X)

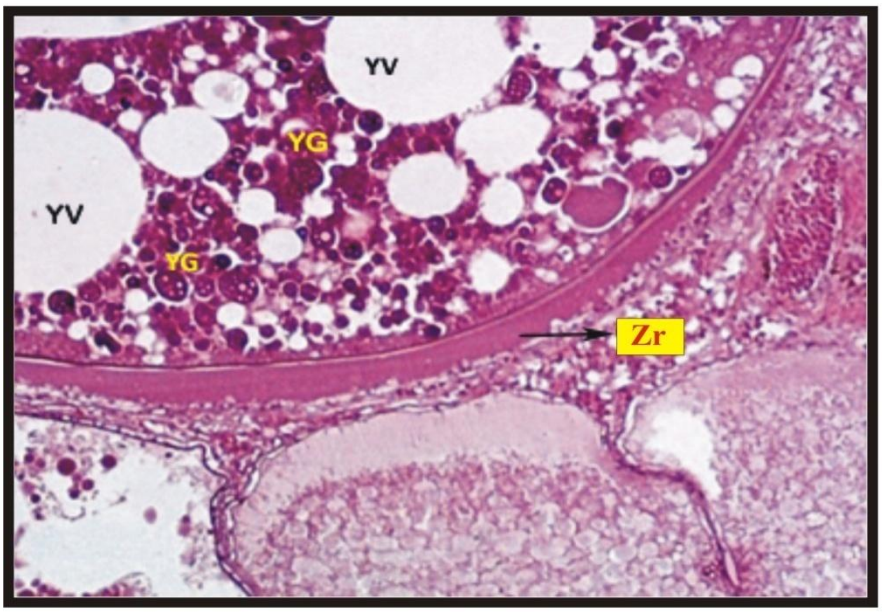


Fig 30: T.S. of ovary of *C. marulius* during March 2015 showing a portion of oocyte in secondary yolk globule stage depicting presence of lipid droplets (ld), yolk globule (yg) and zona radiata (zr). (magnification 100X)

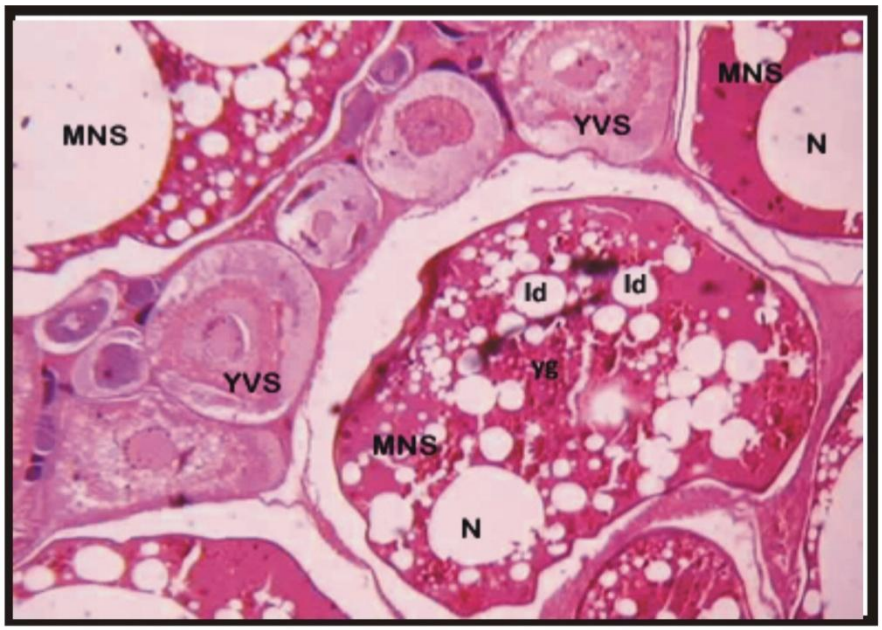


Fig 31: T.S. Of ovary of C. *marulius* during April 2015. A good number of yolk globular stages of oocytes are visible along with other immature stages of oocytes indicating mature stage of ovary. All type of yolk globular stages including migrating nuclear stage (MNS) are visible (magnification 100X)

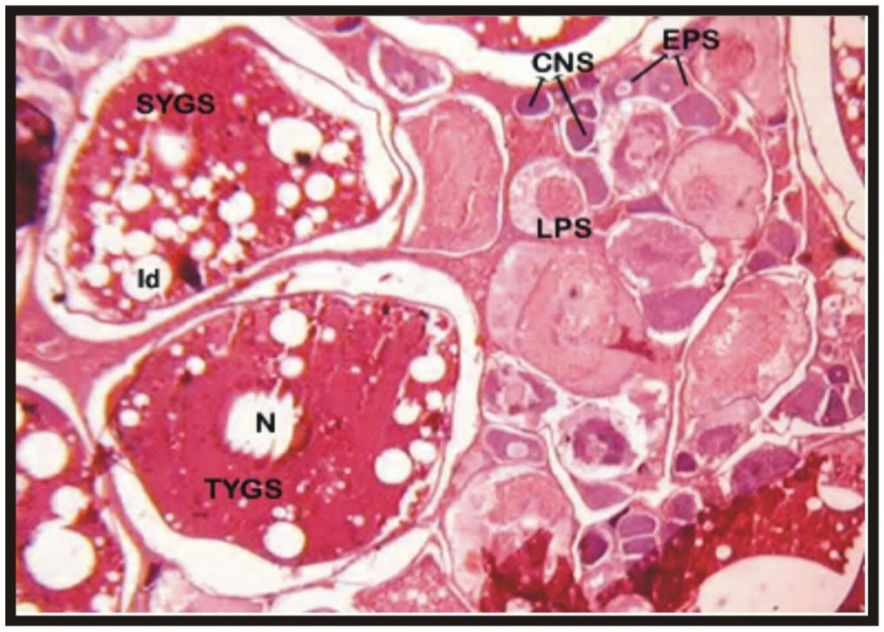


Fig 32: T.S. Of ovary of *C. marulius* during April 2015. All stages of oocyte development along with advance stages of secondary and tertiary yolk globular stages indicating mature stage of ovary (magnification 40X).

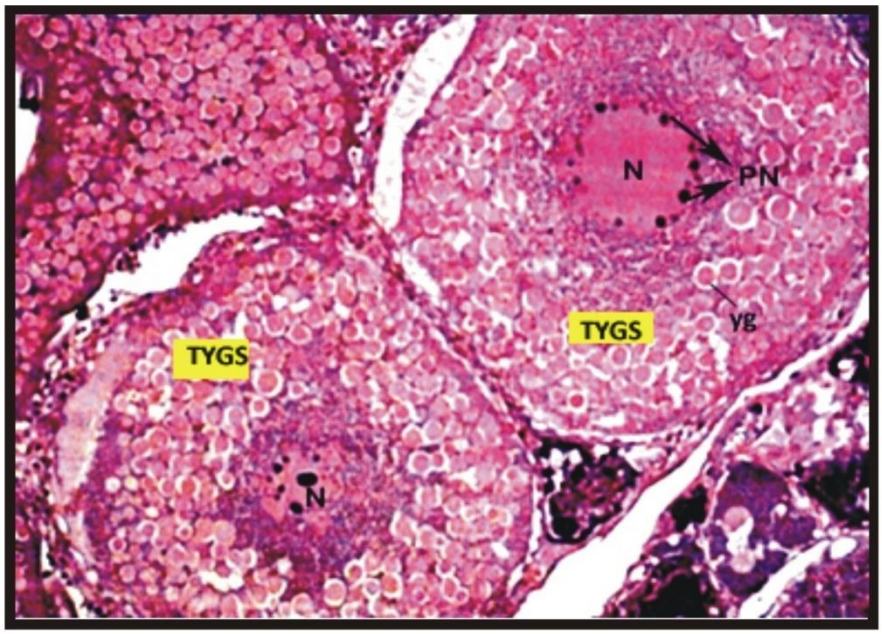


Fig 33: T.S. of ovary of *C. marulius* during April 2015. The oocytes in the tertiary yolk globular stages showing mature oocyte stage of thee ovary (magnification 100X)

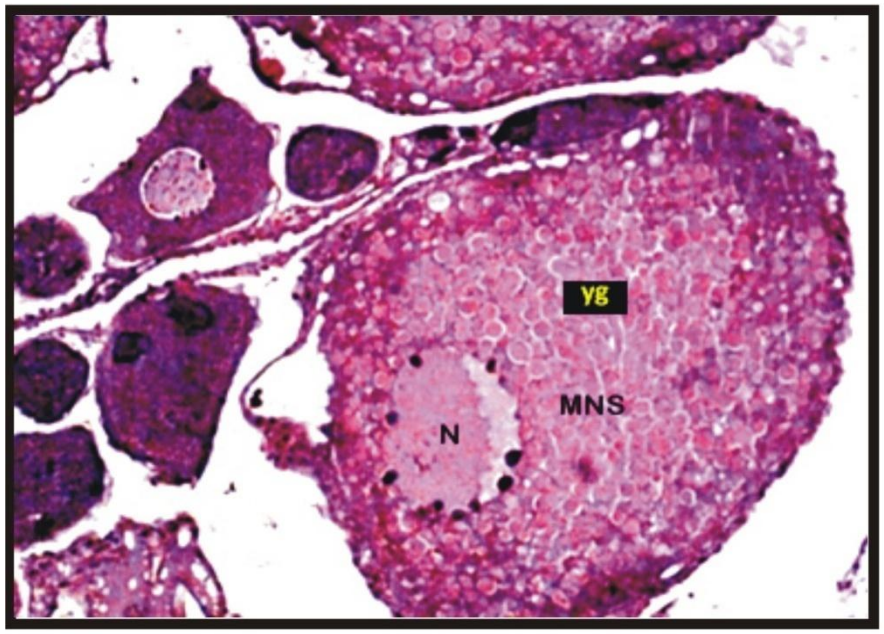


Fig 34: T.S. of ovary of *C. marulius* during April 2015. A view of migrating nucleolar stage (MNS) along with yolk globule oocyte indicating mature stage of ovary (magnification 100X)

Specimens in this month confirmed that the spawning season of this species in the area of experiment starts from March and this was also confirmed from natural spawning in ponds at the experimental site. However, all six stages of oocyte developments were found only in 66% cases, whereas others have lesser number of oocyte developmental stages. The presence of all six stages of oocyte development in March indicated that the spawning season in this species commence in this month.

Ovarian development during April

Around 80% of the dissected specimens observed all six stages of oocyte development in this month with comparatively smaller number of oocytes having early developmental stages and higher numbers having advance developmental stages (Fig 31, 32, 33, 34). The presence of all six stages of oocyte development in 80% samples in April revealed that April is the best season of spawning in this species.

Testes

The testes of *C. marulius* are lobular type, which is typical of most teleost and is composed of numerous lobules which are separated from each other by a thin layer of fibrous connective tissue. Testes are surrounded by thin tunica albuginea and consisted of dense collagenous connective tisse with few fine elastic fibers; they extend inside the testis to give connective tissue septa. The tunica albuginea was covered externally by mesothelium. The bulk of the testis is composed of seminiferous tubules which are oriented at right angles to the long axis of the testis and are ended blindly at its periphery. Within the lobules, primary spermatogonia undergo numerous mitotic divisions to produce cysts containing several spermatogonial cells. Each spermatogenic cell (germ cell) passes through several developmental stage viz. spermatogonia (spermatogonia type A and spermatogonia type B), primary spermatocyte, secondary spermatocyte, spermatid and spermatozoa stages. *C. marulius* observed more than one stages of development at a time and thus the development is asynchronous. During maturation, all of the germ cells within one cyst were at approximately the same stage of development. The maturation process starts from the periphery of the cysts and the developing stages migrates to central lumen with

advancement of maturity. As spermatogenesis and then spermiogenesis proceed, the cysts expand and eventually rupture, liberating sperm into the lobular lumen which is continuous with the sperm duct.

Testicular development during February

During February, the testis were found full of seminiferous tubules arranged in a well developed architecture fashion having spermatogonia of both Type A and Type B and even some in the primary and secondary spermatocyte stages of development (Figs 35, 36).

Testicular development during March

During March, this species depicted seminiferous tubules with different stages of development ranging from immature to fully mature condition of testis. The fully mature specimens exhibited all stages of development in the seminiferous tubules viz. spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa and they depicted the structure similar to Figs 37, 38.

Testicular development during April

In April month also, all developmental stages of testes were observed with comparatively higher numbers of spermatogonia having all stages of complete maturation (Figure 39, 40, 41, 42).

Different reports on the breeding season of this fish species suggested that Sol breeds almost 10 months in a year depending on the climatic conditions. They breed in June-August in the Southern Nepal (Shrestha, 1990), rainy season (Khan, 1924), and April-June in Pakistan (Mirza and Bhatti, 1993). Srivastava (1980) suggested that in Andhra Pradesh (India) the species has two separate reproductive periods, i.e. May and November-December. Bhatti (2010) partly goes in line with the suggestion of Srivastava (1980) though he did not find an indication of breeding after early May. In the present study, *C. marulius* was found to start spawning from late March to end of May profusely but the activity slowed down subsequently though it did not stopped till end of September. Different repots at different places of work therefore suggests that

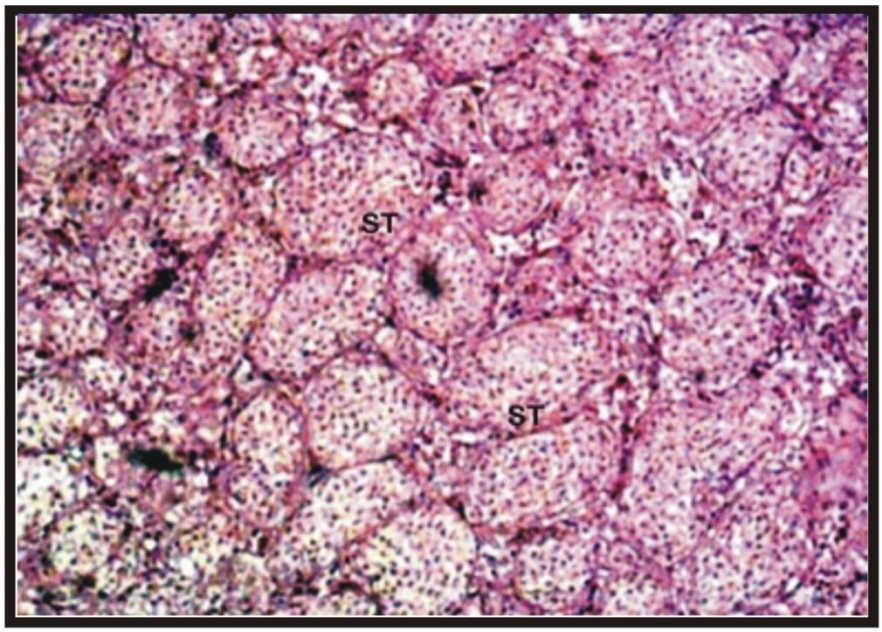


Fig. 35: T.S. of testis of *C. marulius* from cranial region during February 2015. The seminiferous tubules are well organized and containing spermatocytes in primary and secondary developmental stages. (Magnification 40X)

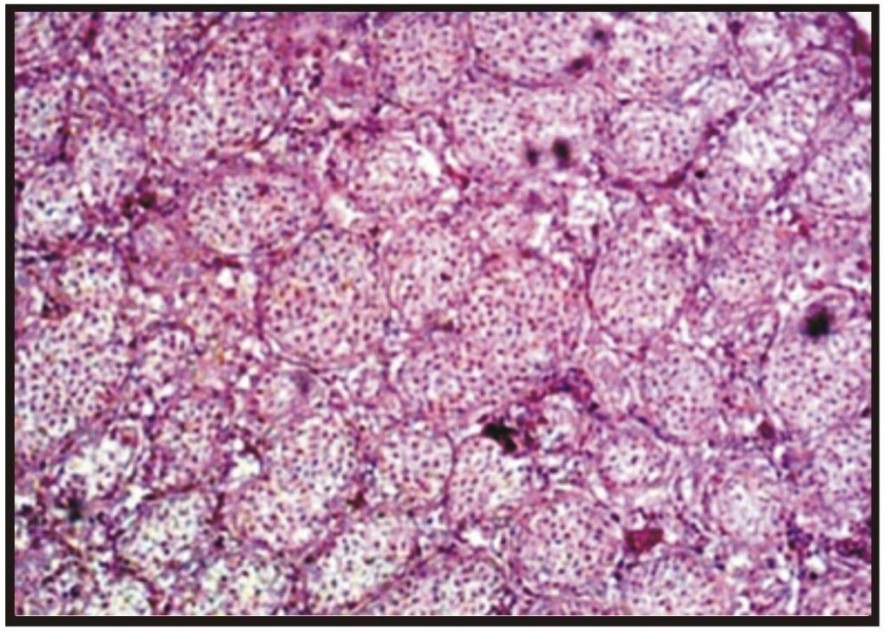


Fig. 36 : T.S. of testis of *C. marulius* from tail side during February 2015. The seminiferous tubules are well organized and containing spermatocytes in primary and secondary developmental stages. (Magnification 40X)

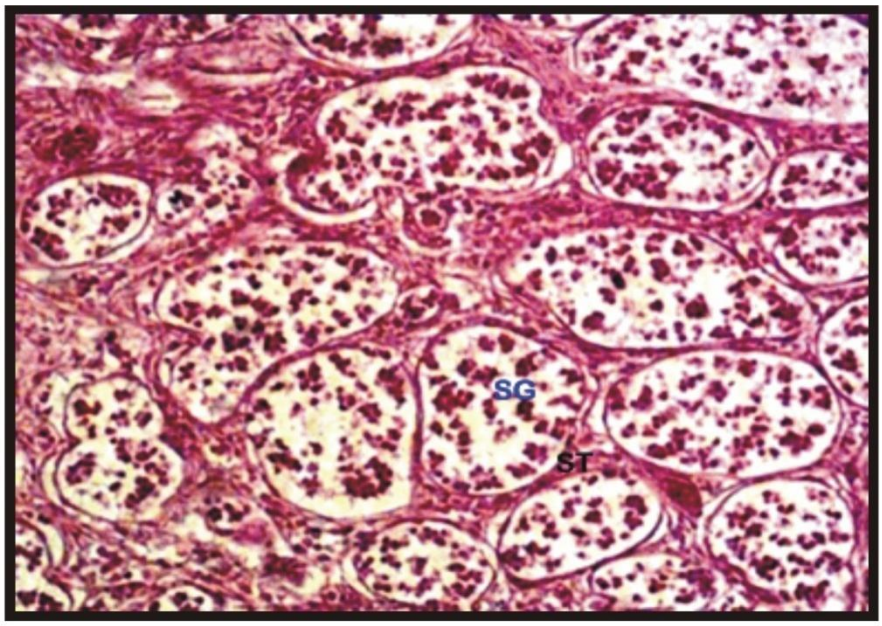


Fig. 37: T.S. of testis of *C. marulius* during March 2015 showing advance stage of spermatogenesis inside the seminiferous tubules where spermatocytes have been disintegrated from the Leydig cell. Magnification X40

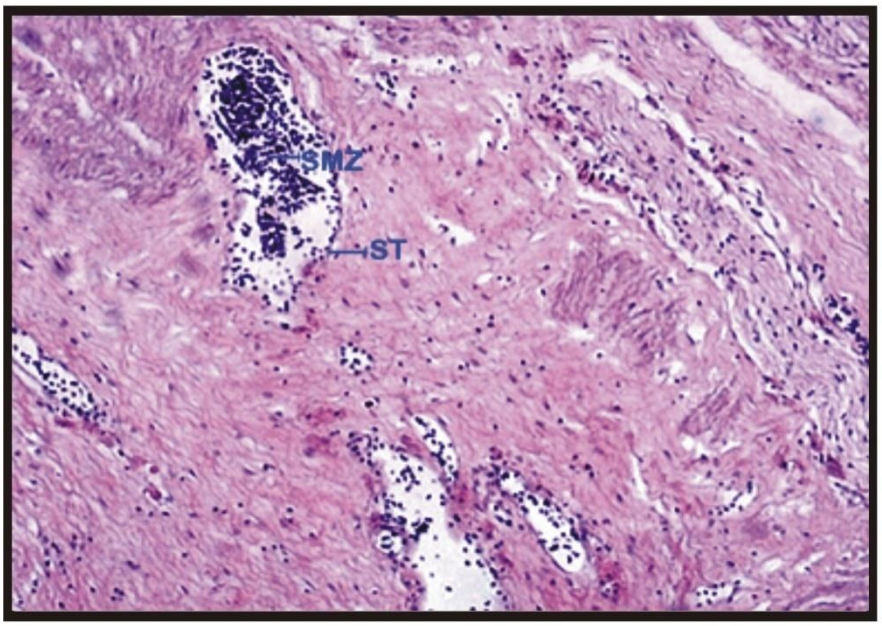


Fig. 38: T.S. Of testis of *C. marulius* during March 2015 showing well differentiated seminiferous tubes (ST) both filled and released spermatozoa (SMZ) stages. On right side, the spermamatogenic tubule is visible.

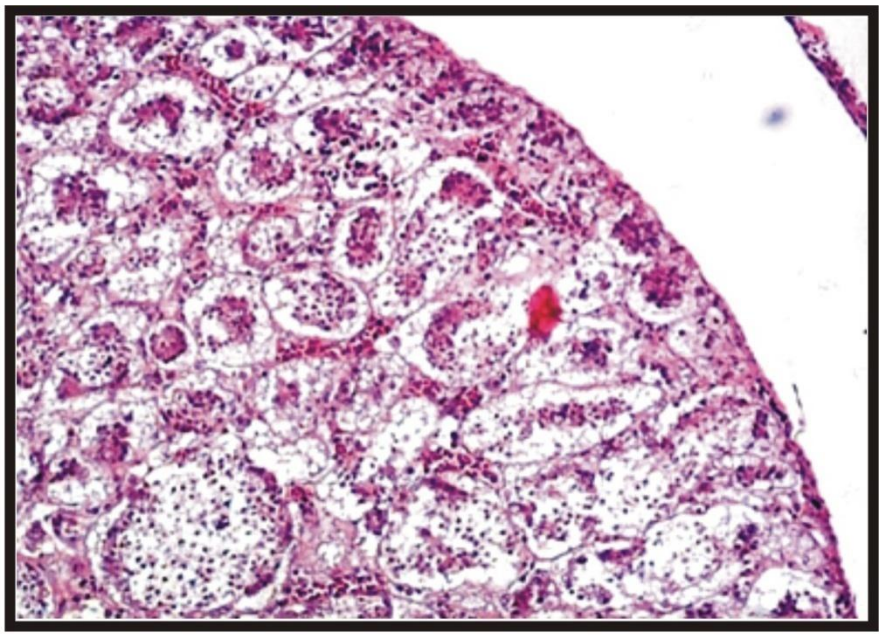


Fig. 39: T.S. of testis of *C. marulius* during April 2015. The cross section depict Tunica albuginea (Outer membrane) of single lobe of testis and spermatoza in different developmental stages with advance stages moving towards centre of the lobule. Magnification 40X.

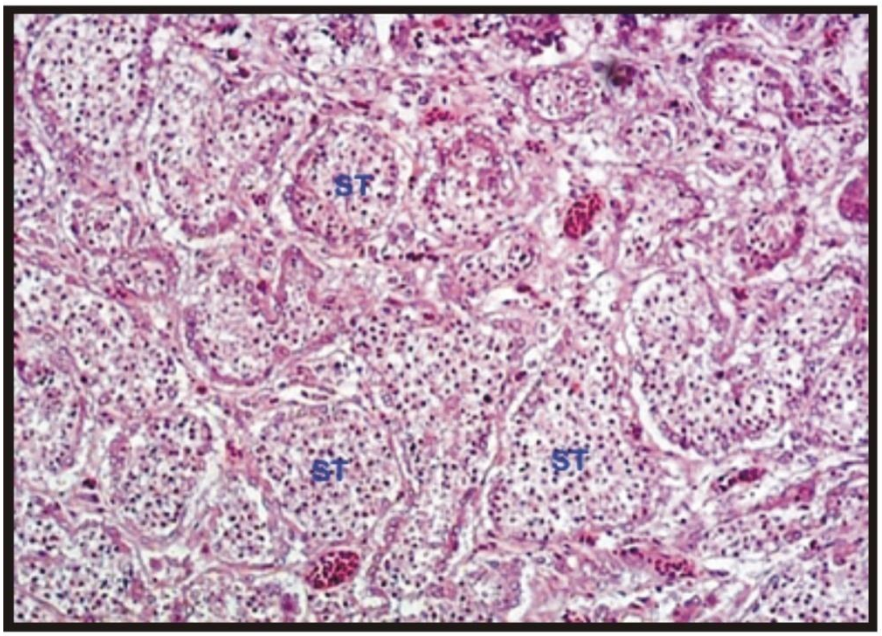


Fig. 40: T.S. of testis of *C. marulius* during April 2015 showing well organized seminiferous tubules depicting spermatocytes in different stages within the oocytes. Magnification 40X.

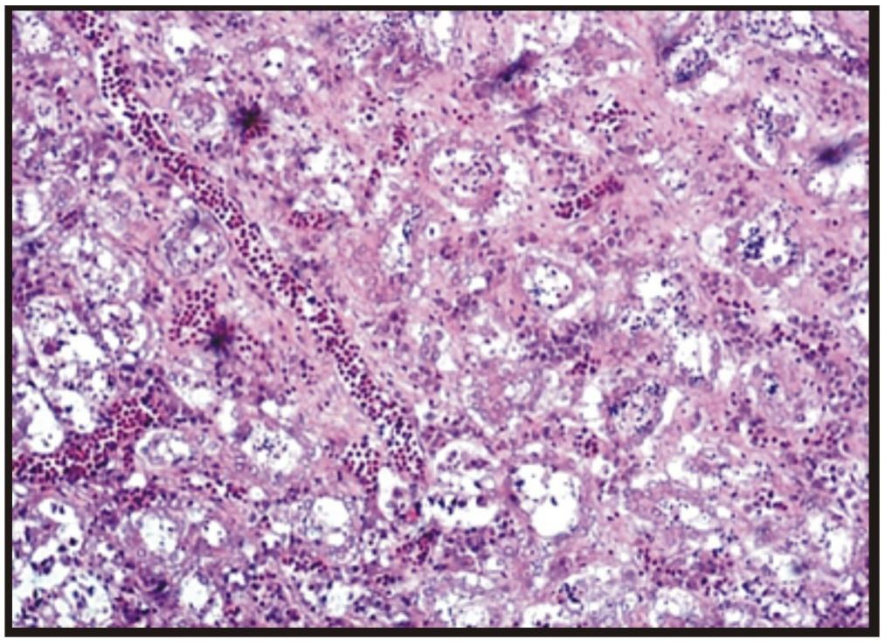


Fig. 41: T.S. of testis of *C. marulius* during April 2015 showing completion of gametogenesis and release of spermatids from seminiferous tubules to vas efference. Many seminiferous tubules are empty due to release of spermatids. Magnification 40X.

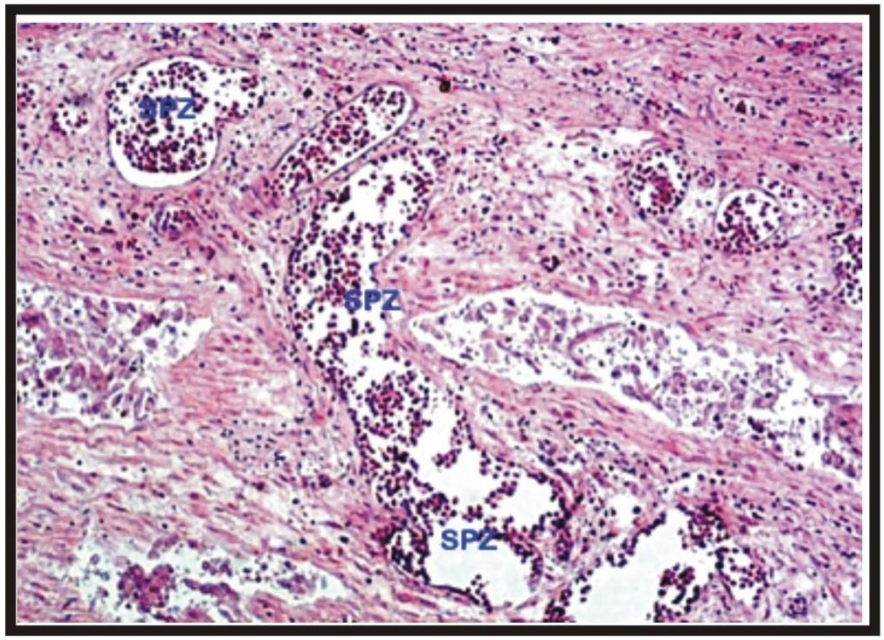


Fig. 42: T.S. of testis of *C. marulius* during April 2015 showing some seminiferous tubles with spermatozoa and some empty after release. The vas efference is full of spermatozoa. Magnification 100X.

The gonadal maturity in the snakeheads is primarily environmental dependent but prolonged and two phases of breeding in southern India may be due to the fact that the area received long summer together with higher light spells in comparison to north and eastern parts of the country.

Histological studies on gonads provide more reliable and direct indications of the reproductive status of the organism and hence are more dependable for the analysis of the reproductive cycle (West, 1990). Several studies are available that analyze seasonal variation in the level of reproductive activities in different species (Murua and Motos, 1998; Saborido-Rey and Junquera, 1998; Kjesbu *et al.*, 2003), but barring the study of Bhatti (2010) no other information is in hand on *C. marulius*. The fish reproductive physiologists, in general have placed more emphasis on the study of histological changes in the female reproductive physiology and relatively fewer studies are available on the male reproductive physiology (Bowers and Holliday, 1961; Koya *et al.*, 2002).

The shape and the size of the ovary and testes vary with the stages of the sexual maturity (Goswami and Sundararaj, 1971; Vitale *et al.*, 2005). Ovaries are generally bi-lobbed structures in most fishes with a short oviduct. Each lobe contains ovigerous lamellae, their number and dimensions verifying with growth and reproductive stage of the fish. Maturation of the egg is a long process that involves complex physiological and biochemical changes (Fishelson *et al.*, 2006). Maturation is accomplished in the fish with development of oocyte, involving mitotic division, resulting in four cells and oocyte giving rise to two polar bodies. During this process, the enlarged nucleus of the primary oocytes moves towards peripheral position, when its membrane breaks down and the first meiotic division is completed with extrusion of the polar body. The second meiotic division starts immediately, but is arrested at metaphase (Goswami and Sundararaj, 1971; Masui and Clarke, 1979). During this process, a distinct animal pole separates from vegetal pole, in some species. The yolk also undergoes some type of maturation and becomes less opaque. The oil droplets, when present, coalesce to form one or more larger droplets. Soon after, the mature (secondary) oocyte ovulates out of

the follicular envelope, and acquires a jelly coat (Goswami and Sundararaj, 1971). The ovulated eggs are then spawned in water and are almost immediately fertilized. The ovaries have been generally classified into seven stages of the reproductive activity (Saeed *et al.,* 2010) as given in the next para, which have been variously used in understanding the seasonal variation in reproductive activity of different fish species (Vitale *et al.*, 2005; McDermott and Lowe, 1996; Abuzinadah, 2001; Saeed *et al.*, 2010).

I. Virgin or immature: Oogonial nest small with dense basophilic cytoplasm with central nucleus and few large nucleoli around the edge.
II. Developing or early maturing stage: Nucleus increased in size and multiple nucleoli appearing and weakly stained area (circumnuclear ring, CNR) appearing.
III. Early maturing: Large visible eggs, CNR moving towards outer part of the cell and large number of oocytes.
IV. Late maturing stage: Large number of oocytes with some ripe ova.
V. Spawning or final maturation stage: Ripe ova dominating (chorion thicker and nucleus migrating towards the animal pole) dominant.
VI. Spent stage: Large number of empty follicles (post-ovulatory follicles; oocytes in early developing stage.
VII. Resting: All oocytes in early development stages. Some post ovulatory structure indicating previous spawning.

The testis in fishes generally appear as a pair of elongated structures, composed of a series of branched seminiferous tubules, which are embedded in the stroma in the fish. The testes mainly comprises of thin-walled tubules or lobules, which contain germ cells (spermatogonia). The spermatogonia divide in clusters and are enclosed in a cyst. The primary spermatogonia (representing the stem cell) are present throughout the year and divide mitotically to produce the secondary spematogonia, which finally produce the primary spermatocyst. The spermatocysts divide by meiosis to form the spermatids, which are finally transformed into spermatozoa (Fishelson *et al.*, 2006).

Generally four different stages i.e. immature, prespawning, spawning and post spawning (spent), of the testicular development are identified based upon the histological variations, which represent different stages of the reproductive activity of the individual fish (Muchlisin *et al.*, 2010; Koya *et al.*, 2002; Mori *et al.*, 2003; Rehman *et al.*, 2000). Using such histological classification of the testes, the cyclic change in the reproductive activity has been reported in golden rabbitfish (*Siganus guttatus*; Rehman *et al.*, 2000), *Rasbora tawarensis* (Muchlisen *et al.*, 2010) and black rockfish (*Sebastes schlegeli*; Mori *et al.*, 2003). Siddiquee *et al*. (2015) while studying histology of *C. marulius* from the samples collected during July reported that the testes of *C. marulius* observe all the maturing stages of spermatogonia in it and revealed that the fish was found in matured condition during July. According to Rehman *et al*. (2000), four maturity stages of testes in fish can be classified on the basis of the the following histological observations.

I. Immature: Seminal lobes totally occupied by clusters of spermatogoania.
II. Pre-spawning: Spematocytes constituting >50% of the germ cells, fewer spermatogonia.
III. Spawning: Having higher proportion of spermatids and spermatozoa dominating with decreased percentage of the spematocytes.
IV. Post-spawning: No spermatozoa; with sporadic appearance of spermatogonia and spermatocytes.

No study is in hand describing the histology of *C. marulius* and / or on the seasonal histological classification of the gonads into different stages of the reproductive activity excepting that of Bhatti (2010). He also observed identical pattern of cyclic changes in the histology of testis in the males of this fish species, sampled during different calendar months (Bhatti, 2010). In December sample, all the males were classified by him as immature. The testis sampled during February reflected a pre-spawning stage of development. The March samples of testes were classified as pre-spawning in 75% of cases, and as spawning in others. All the testis in the April sample were in spawning stage, while in the May sample 50% of the males were in

spawning stage and 50% in the post spawning period. In June and September samples, all testes were in the post-spawning or resting stage. He suggested that the reproductive changes in the male gonads start appearing in February and the major spawning occurs in April, with some early and late spawning in March and May respectively. No reproductive activity occurs in the other parts of the year.

The present histological study on testes also suggests that there are basically four stages of maturity in the testes as suggested by Rehman *et al*. (2000) and it also support the findings of Bhatti (2010) that this species start spawning from late March to May end which was also confirmed from the visual experience where good number of brooders in a pond have been stocked for observation of natural spawning also. However, this study differ in the respect that though major spawning was observed in this species between late March to May end, it did not stop till end of September as observed visually for 2 years in pond conditions with gradual decline in intensity of breeding.

Further, the females under the histological sections showed almost all the stages of oocyte developmental all through the season unlike carps where they are observed in more or less of similar developmental stages which indicate that in *C. marulius* the oocyte development is a regular feature during the spawning season and hence all the developmental stages are observed in the histological sections at a time. This indicates that the species is a batch spawner and hence may spawn 2-3 times in a season. Qasim and Qayyum (1961) also observed two batches of oocytes in the maturing ovary of *C. marulius* from North Indian waters indicating that the fish is a batch spawner.

The analysis of all the reproductive indicators suggests that *C. marulius* in the area under present study starts reproductive activity after December. Spawning may occurs between late March and early May, with peak spawning activity in April-May. Since the present report is based on fewer samples and also carried out on the samples collected from February to April, it needs to be carried out further on larger number of samples collected all round the year

Chapter 6

CAPTIVE BREEDING

Chapter 6
CAPTIVE BREEDING

6.1 MATERIALS AND METHODS

6.1.1 Procurement of brooders

The fingerling of *C. marulius* developd at the farm site from wild breeding were reared to brood size in two ponds of size 0.02 and 0.05 ha at NBFGR Fish Farm for three years (Figure 43) and were used for breeding in three enclosure conditions of earthen ponds, cement tanks and the indoor FRP hatchery conditions (catfish hatchery larval rearing tanks, designed and developed by CIFA, Bhubneshwar, India). The brooders were fed in earthen rearing ponds with either cut pieces of large fishes or small-size fishs depending on the availability 5% of their body weight directly in the ponds during evening time. The ponds were drained out with a portable diesel pumping set (5 H.P.), whenever brooders were required for breeding trials. Brooders which could be used within 3-days were harvested with the help of hand nets and transferred in a Fibre-reinforced-plastic (FRP) tanks of size 1.8m x 1m x 0.7m (L x W x H) for thorough washing. When all the brooders were procured, the tank water was drained out and the fishes were washed with freshwater within the tank and disinfected with potassium permanganate ($KMnO_4$) 1 $mg.L^{-1}$ for 5 minutes. The aeration was arranged inside the tank from a portable blower (HiBlow 40) and tank was covered tightely with a netting cloth to avoid fish escape. The brooders were utilized for breeding trials within 3-4 days from these tanks.

6.1.2 Breeding systems, sex-ratio and hormonal applications for induced breeding

As per the available literature, the mating in this species in nature happens in 1:1 female-male ratio and the species bred and lay eggs amongst the aquatic vegetation in the shape of a single more or less round egg mass, also called ‘nest’. Further, as the differentiation of final stage of maturity is difficult in the species, the breeding pairs

were arranged in 4 sets of sex ratios of female to male 1:1, 1:2, 2:1 and 2:3 ratios. Each set of sex ratios was tried for induced breeding in duplicate under pond, tank and hatchery conditions. Two sets of hormonal doses of Gonopro FH hormone (Manufacturer: Amrit Pharmaceuticals, Aurangabad, India; Marketed by APC Nutrients Pvt. Ltd., Secunderabad, India; containing sGnRH anologue 20 mcg.ml^{-1} and dopamine antagonist, at the rate 2.0 ml kg^{-1}) at the rate 1.0 ml and 1.5 ml per kg body weight were given to females and at the rate 1.0 ml to males in all the cases by the method of intraperotonial injection (Figure 44). These rates of hormonal doses were tried on the basis of our previous experience of successful induced breeding of striped murrel (*Channa striatus*). For inducing with hormone, the brooders were procured from the FRP tanks during evening time, where they had been stocked after procurement from the stock ponds, and transported in fish carrying bags in water near the site of induced breeding pond/tank and given hormonal injections as given in intra-peritonially at the base of pectoral fin. The brooders were then released in the pond/tanks for breeding. The brooders were observed for mating bahaviour, nest forming behavior and egg laying behavior during day time only from next day morning up to 5 days. Where the spawning did not take place up to 5 days, the brooders were discarded for further spawning and recollected from pond/tank with the help of a small drag net and released in a separate tank.

6.1.3 Breeding in open earthen ponds

The breeding experiment in earthen ponds was undertaken during April and May in two identical earthen ponds of size 4m x 4m x 1m (L x W x D) in outdoor conditions. First of all, the ponds were drained out with the diesel pumping set and cleaned from all aquatic macro-vegetation (Figure 45). The ponds were then filled with fresh water from a deep bore well. The water quality of the bore well was temperature 27.5 ^{o}C, pH 7.8, TDS 220 mg.L^{-1} and salinity <0.5 gm.L^{-1}. The ponds were planted with macrovegetation comprising of *Eicchornia crassipes* and *Hydrilla reticulata* so that approximately 25% of the water spread area was covered with it and allowed to settle for five days so that the water quality is stabilized and the aquatic

macrovegetation were set in position. Each pond was then stocked with one set of homone injected brooders as per the schedule given in Table 7. As only two ponds of this small size were available so only two sets of pair were tried on a single occasion and rest were tried subsequently after finishing work of the previous sets. The pairs in which spawning occurred were removed immediately after observing the egg mass in the pond with the help of small sciene net so that eggs could be procured and hatched under the control indoor conditions for further study.

6.1.4 Breeding in open cement tanks

Eight open cement tanks of size 5m x 3m x 1.2m (L x W x D) were used for undertaking captive breeding trials during April and May. The tanks were thoroughly cleaned and filled with bore well water up to a depth of 70 cm and provided with aquatic macrovegetation comprising of *Eicchornia crassipes* and *Hydrilla reticulata* so that approximately 25% of the water spread area was covered with it (Figure 46). The tank water was allowed to settle for five days so that the water quality is stabilized and the aquatic macrovegetation are set in position. As eight tanks of uniform dimensions were available at the site, all 8 sets were arranged on a single day. Freshly caught brooders from the NBFGR farm facilities were identified for advance maturity condition through visual observation and were stocked in these tanks in different sex ratios comprising as stated above. The brooders were given hormonal doses as given in Table 8 as prescribed in the previous paragraph and released in the cement tanks. No feeding was given to brooders during the experiment period. The brooders were observed for spawning behavior from morning of next day till 5 days daily for spawning. The spawned brooders were removed at the earliest by sciene netting and eggs were scooped out with a hapa. The water quality of the tank water was analysed for the same parameters as discussed above at the time of release of the brooders in the tank.

Figure 43: One of the rearing ponds used for raising brood stock of *C. marulius* (size 20m X 20m)

Fig 44: A brooder of *C. marulius* being given intra-peritoneal injection of hormone for induced breeding

Figure 45: One of the small earthen ponds used for induced breeding of *C. marulius*

Fig 46: Cement tanks used for induced breeding of *C. marulius*

6.1.5 Breeding in indoor hatchery conditions

Two identical rectangular FRP tanks of size 13'x3'x14'' (LxWxD) were used for spawning under the indoor conditions (Figure 47). The tanks were filled with bore well water up to 9 inch height. The entire water surface was sparsely covered with *Hydrilla verticillata*, so that around 25% of the total surface area of the tank was covered with macrophytes. The brooders were procured and selected in a same way as in the previous two trials. They were given hormonal injections as given in the Table…and released in the tanks. Four tanks were used at a time and so trial was complete in two occasions. As the tanks were shallow they were covered with wooden frames of iron netting in order to escape of the fish. The tanks were observed for spawning stimulus and laying of eggs frequently from next day morning till 5 days. The water quality was analysed using multiparameter for temperature, pH, TDS and DO immediately after stocking the injected brooders in the tanks.

6.1.6 Collection of eggs, hatching and spawn rearing

As the eggs of *C. marulius* are pelagic, laid down in the form of a mass in between the weeds, they were easily scooped out with the help of a plastic sieve. The eggs were transferred in the plastic tub with water and aeration was provided from a portable aerator. The rate of fertilization, hatching and duration of hatching were recorded. The hatchling were then pooled up and reared in a circular FRP tank (dia 6 feet dia, water depth 6") provided with aeration, till absorption of yolk-sac. The survival and duration at yolk-absorption stage was counted.

Fig 47: Breeding of *C. marulius* in FRP tanks in the indoor conditions

6.2 RESULTS AND DISCUSSION

6.2.1 Breeding in open pond conditions

Out of 8 sets arranged in pond conditions, spawning occurred in 5 sets. The spawning was observed under all the sex ratios maintained in the trial. However, only one female spawned in all the cases and females given hormonal injections of both 1.0 $ml.kg^{-1}$bw and 1.5$ml.kg^{-1}$bw equally gave more or less the same spawning response. All the females spawned within 12-24 hours of giving hormonal injection. The females laid eggs in the form of an egg mass surrounding between the aquatic macro-vegetation so that they remain in intact condition (Figure 48). Both fertilization and hatching rates were observed over 90% in all the spawned fishes (Table 7, Figure 49). The water quality analysed for both ponds were found to range temperature 28.6 ^{o}C - 32.2 ^{o}C, pH 7.9 - 8.5, TDS 310 $mg.L^{-1}$ - 340 $mg.L^{-}1$ and was in the normal range.

6.2.2 Breeding in outdoor cement tanks

A total of 4 sets spawned out of 8 arranged for induced breeding in the cement tanks. The performance with respect to sex-ratios, dose rates, incubation period, fertilization and hatching was observed to be more or less the same as in the pond conditions (Table 8). The water quality was also under the normal range with water temperature 29.2 ^{o}C - 33.2 ^{o}C, pH 8.2 – 9.2, and TDS 280 $mg.L^{-1}$ - 310 $mg.L^{-1}$.

6.2.3 Breeding in indoor FRP tanks

None of the set in FRP tank responded though a good amount of mating responce was observed in the brooders (Table 9). The water quality was observed to be under the normal range with water temperature 28.0 ^{o}C - 31.8 ^{o}C, pH 7.9 – 8.2, and TDS 230 $mg.L^{-1}$ - 260 $mg.L^{-1}$.

Table 7: Captive breeding details of *C. marulius* in open earthen ponds

Set No.	Sex ratio (F:M)	Total weight of female (kg)	Hormone dose rate to female ($ml.kg^{-1}$)	Total weight of male (kg)	Hormone dose rate to male ($ml.kg^{-1}$)	Breeding response	Incubation period (hrs)	No. of eggs procured	Fertilization (%)	Hatching (%)
1.	1:1	410	1.0	425	1.0	Bred completely	24	4200	92	90
2.	1:1	450	1.5	475	1.0	Not bred	-	-	-	-
3.	1:2	425	1.0	750	1.0	Not bred	-	-	-	-
4.	1:2	490	1.5	850	1.0	Bred completely	12	4550	95	92
5.	2:1	925	1.0	450	1.0	One female bred completely	24	3500	95	93
6.	2:1	900	1.5	525	1.0	No breeding	-	-	-	-
7.	2:3	840	1.0	1210	1.0	One female bred	12	4300	90	93
8.	2:3	880	1.5	1320	1.0	One female bred	24	4400	90	90

Figure 48: Location of nest (top), complete nest (middle), close-view of nest (bottom)

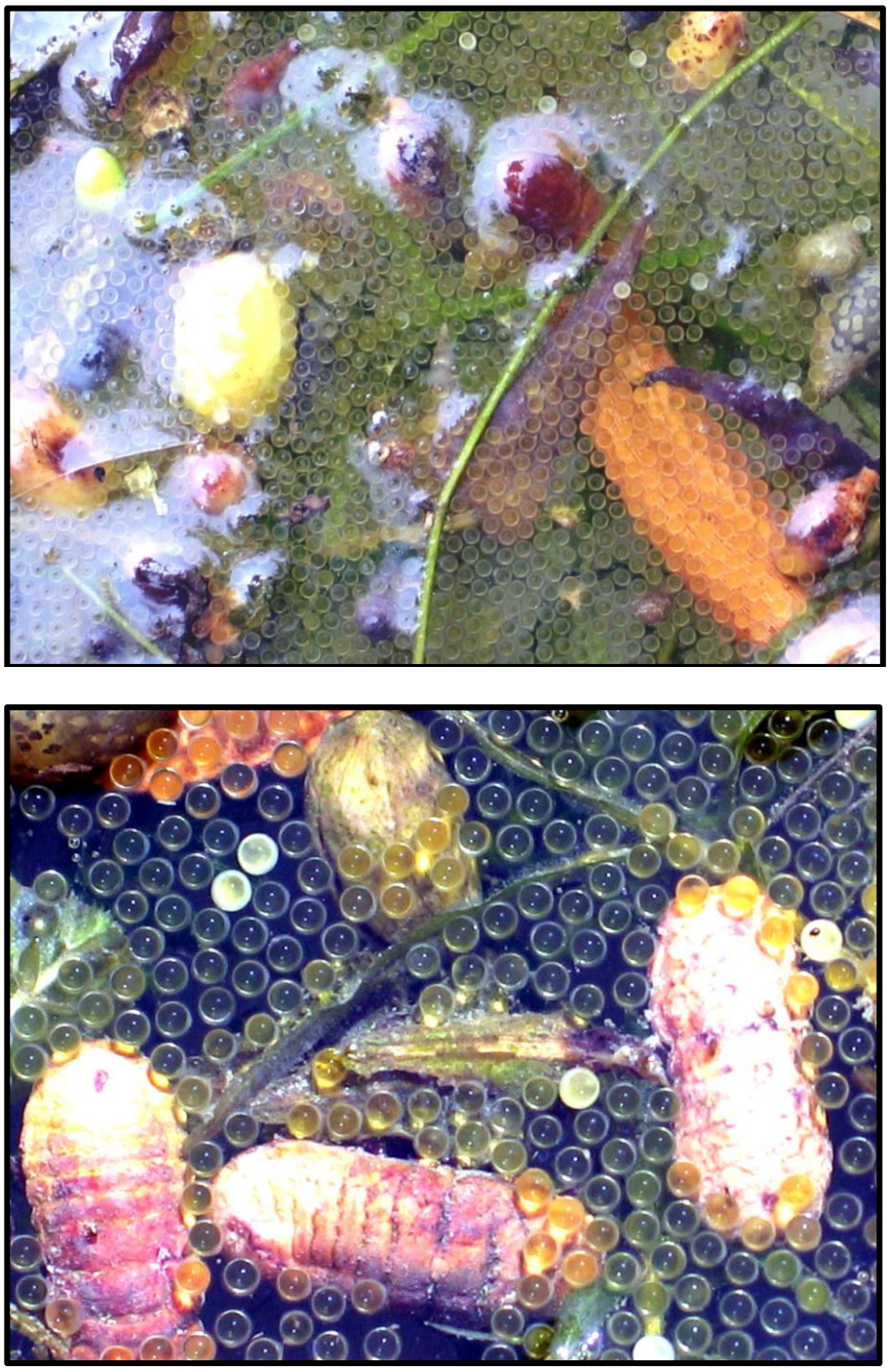


Figure 49: **Close-views of eggs, transparent eggs are fertilized and white are unfertilized. Also seen large whitish insect coccoons**

Table 8: Captive breeding details of *C. marulius* in open cement tanks

Set No.	Sex ratio (F:M)	Total weight female (kg)	Hormone dose rate female ($ml.kg^{-1}$)	Total weight male (kg)	Hormone dose rate male ($ml.kg^{-1}$)	Breeding response	Incubation period (hrs)	No. of eggs procured	Fertilization (%)	Hatching (%)
1.	1:1	375	1.0	400	1.0	Not bred	-	-	-	-
2.	1:1	410	1.5	450	1.0	Not bred	-	-	-	-
3.	1:2	400	1.0	775	1.0	Not bred	-	-	-	-
4.	1:2	525	1.5	825	1.0	Bred completely	12	4300	91	90
5.	2:1	900	1.0	525	1.0	One female bred completely	24	3700	93	90
6.	2:1	850	1.5	525	1.0	No breeding	-	-	-	-
7.	2:3	825	1.0	1250	1.0	One female bred completely	12	3900	90	92
8.	2:3	880	1.5	1420	1.0	One female bred completely	24	4500	94	96

Table 9: Captive breeding details of *C. marulius* in indoor hatchery (catfish hatchery) conditions

Set No.	Sex ratio (F:M)	Total weight of female (gm)	Hormone dose rate to female ($ml.kg^{-1}$)	Total weight of male (kg)	Hormone dose rate to male ($ml.kg^{-1}$)	Breeding response	Incubation period (hrs)
1.	1:1	350	1.0	400	1.0	No breeding	-
2.	1:1	425	1.5	425	1.0	No breeding	-
3.	1:2	375	1.5	700	1.0	No breeding	-
4.	1:2	400	2.0	750	1.0	No breeding	-
5.	2:1	750	1.5	450	1.0	No breeding	-
6.	2:1	725	2.0	425	1.0	No breeding	-
7.	2:3	725	1.5	1150	1.0	No breeding	-
8.	2:3	800	2.0	1100	1.0	No breeding	-

A very little work on captive breeding of *C. marulius* has been carried out in India and abroad in comparison to *C. striatus*. The spawning habits of both *C. marulius* and *C. striatus* have been reported more or less of similar nature in the natural conditions. In natural conditions, both the species breed in ditches, ponds and flooded paddy fields a little prior to or with the onset of monsoons (Bhattacharya 1946; Chacko 1947). According to Qasim and Qayyum (1961), the snakeheads in northern states of India breed during June to October with peak from July to August. However, this is different in southern India, where this group is reported to breed during November to January (Alikunhi, 1953). Jyotsna *et al.* (1995) reported spawning period of striped murrel to be during mid monsoon from June to August. However, according to the present study *C. marulius* attains complete maturity from April onwards in north India (Uttar Pradesh) as also evidenced in the preceding chapters and even start spawning even in late March and this has been observed many times in natural pond condition by the author at the site of this work.

While making a comparison in three sets of study using open earthern pond and cement tank and indoor FRP tank systems, it was visualized that open earthen pond and cement tank conditions are more suitable for spawning of this species in comparison to indoor FRP tank condition. Out of 8 sets arranged in three systems, 5 spawned in the earthen ponds, 4 in the cement tanks and there was no spawning in the indoor tanks. Another factor that seemed to matter in spawning of this species is the size and depth of the enclosure as the earthen pond used in the study was of size 16 m^2 with 1 m depth, the cement tank was 15 m^2 with 1 m depth whereas, the size of FRP tank was quiet small 3.9 m^2 with depth of 0.3 m (13'x3'x14'') (LxWxD).

According to Devaraj (1973) and Parameswaran (1975) the giant murrel, *C. marulius* matures sexually in two years and breeds naturally in ponds and brood size varied between 375 g - 3,000 g in different swamps recorded in Karnataka state, India. However, there are contradictory reportings of attaining maturity in the first year of life also when *C. marulius* attains length of 300-350 mm (Alikunhi 1953, Arumugam,

1966). This species has also been observed to mature and spawn in cement tank conditions in the first year of its life in August month by the author at the working site in Lucknow, Uttar Pradesh, India when reared from fingerling size in captive conditions (Personal Communication). Since the brooders used in the present study were already 3 years old, so they were supposed to have attained maturity as per the above recordings. However, unlike many fish species particularly carps where majority of them mature at more or less the same time irrespective of even the sex, the. Maturity condition is entirely different in case of *C. marulius* both within and between the sexes. Whereas, a good number of females were found in different maturity conditions in different months as also observed in the histological sections in the earlier chapter, the situation was not same in case of males, where hardly few of them were found in the matured condition at a time, in a population even during peak spawning season when collected from a stock pond. Therefore, there seems to be some social characteristic that signaled favourable conditions only for few numbers of brooders to attain maturity. As males are highly aggressive and dominant when become fully mature, their behavior may be sending negative impulse to other males in the same pond for attaining maturity as has been reported in case of *Macrobrachium rosenbergii* by many authors (Personal communication).

It has been reported that each female pair with only a single male in case of both *C. marulius* and *C. striatus* (Parmeshwaran and Murugesan, 1976; Moitra *et al.*, 1979) and other male is rejected. This behavior was also reported in all the sets where only one female cohabited with only one male. Further, in sets where more than one male and female was kept, the spawning occurred only in one female. This bahaviour also confirmed that the males are dominant at the time of spawning and they don't allow other males to mate even with other female in close vicinity. This situation has been closely watched by the scholar in pond conditions and observed that only one or two pairs used to spawn at a time in pond condition eventhough in the presence of large number of brooders even at the time of peak breeding season. It has also been visualized that in case the eggs and fry are removed from a pond, the next spawning

occurs quickly. These are some of the behavioural studies that show the species observe some sense of social behavior during spawning and that need to be studied in detail in future studies.

The courting pair just before spawning is often seen to frequently move from one place to other for selection of spawning site and construction of nest in water in a sheltered weedy margin of river (Khan, 1924). Similar observations were also made during the current study and after a long courtship the female laid eggs in small numbers at a time with regular return for releasing another batch of eggs at the same place amongst the macrovegetation which are subsequently fertilized by the male. According to Agbayani (2013), the bullseye snakehead builds floating nests of weeds and leaves where orange-yellow eggs are deposited.

The use of exogenous hormones in the induction of spawning of fishes is well documented (Lam, 1982) and different doses of hormones and sex steroids have been reported to give different results (Ayinla and Nwadukwe, 1988; Zonneveld *et al.*, 1988; Salami *et al.,* 1994). Human chorionic gonadotropin (HCG) (Mollah and Tan, 1982; Zairin *et al.,* 1992; De leeuw *et al.*, 1985; Fermin, 1992) and Ovaprim, a commercial formulation of GnRH analogue and dopamine antagonist (Alok *et al.*, 1993; Francis, 1996; Haniffa *et al.,* 1996) have been successfully used for induced breeding of air breathing fishes. LHRHa have been extensively used more effectively as an ovulating agent for the induced breeding of marine fishes (Devauvchelle *et al.*, 1988; Kestemont, 1988). Francis *et al.* (2000) described use of carp pituitary extract, human chorionic gonadotropin and ovaprim as inducing agents for the breeding of *C. striatus* successfully in earthen pond conditions.

The induced breeding of *C. striatus* in India using very high dose of pituitary gland extract have been demonstrated in the past (Alikunhi, 1953 and Paremeswaran and Murugesan 1976b). Hossain, *et al.* (2008) reported successful induced breeding in *C. striatus* by stripping using carp pituitary at very high doses in Bangladesh. Dayal, *et al.* (2013), Haniffa, *et al*. (2000, 2001), and Marimuthu, *et al.* (2007) HCG has also

been successfully reported to precipate spawning in *C. striatus* (Bilal, *et al*., 2013). Induced breeding of *C. striatus* with sGnRH anologue using Ovaprim and Ovatide hormonal formulations in pond condition by putting them directly in the open pond/cement tank or fixing large hapa inside the earthen pond have been demonstrated by Dayal *et al.* (2013). Marx and Stephen (2004) also succeeded in spawning *C. striatus* in pond condition using pituitary extract but they did not get successful response both with ovaprim and ovatide. Dayal *et al.* (2013) made an unsuccessful indoor breeding attempt of *C. striatus* in FRP tanks provided water depth of 0.60 m using sGnRH anologue, whereas Pati *et al.* (2004) succeeded in spawning this fish with Wova FH (sGnRH) in the FRP tanks though it was not clear whether this work was carried out in indoor or outdoor conditions.

In most of the breeding trials, the successful spawning rate has not been defined and different opinions have been expressed about the suitability of inducing agents and the breeding environment. Based on the present success and the views of the earlier workers, the author rate induced breeding success to be primarily based on the following four criteria.

a) Firstly, the maturity stage of both the sexes as suggested in the foreground para needs to be carefully examined before selection of the brooders.
b) Secondarily, dose rates of hormone which needs to be applied sex-wise. It is comparatively very high in comparison to carps irrespective of type of hormone
c) Thirdly, the sex-ratio and,
d) Fourthly, the type of spawning tank, its size, water depth and macrophytes it has.

The success of induced breeding in this species also depends on assessment of the maturity stage of fish particularly male fish in comparison to female as later can be identified at least with slight bulging of the abdomen together with collaborating secondary sexual characters as discussed in the preceding chapter. However, the major problem arises with identification of male brooders though up to some extent this can be identified by the secondary sexual characters as discussed earlier but it would be of

paramount importance that equipment like ultrasound may be take use for more accurate identification of maturity assessment by observing the size of testes *in situ*.

According to Selvaraj and Frances (2007), HCG implantation induced faster maturation in both female and male *C. striatus*. Therefore for maturity advancement in both the sexes, the HCG/LHRH or other similar inducing agents may be used for effecting maturity in all the brooders so that large number of brooders may be made available at a time for spawning. Further study may also require in case of males to check whether even after inducement with HCG/LHRH, in case of keeping the brooders in the same tank, the number of matured brooders are increased or it remain more or same the same as without inducement with HCG/LHRH. In such case, each male brooder needs to be reared in the separate tanks so that it remains unaffected with the presence of a dominant male for attaining maturity.

The males are highly aggressive during spawning season and only one male in a pond cohabit with single female, thereby only one or two spawning occurs in a pond at a time. As males also observe parental care, same male did not take part in subsequent spawning with other females for about 4-6 weeks. This phenomenon has some sort of social linkage which may be due to parental care observed by this species. However, this needs to be verified in future research, though in length-weight relationship (LWR) studies the male population has been reported very poor in comparison to female population in snakeheads by the scholar as also reported by Koundal (2014) and Olurin and Savage (2011).

C. marulius is low fecund as well as batch spawner fish with ova having different maturity stages at times in the ovary and hence does not release all ova in a single batch. The ovarian fecundity of this species has been evaluated to vary to much by different workers ranging from 95, 301-1112, 234 ova per kg body weight during peak season (Devraj, 1973a), 29,000-55,000 per kg body weight (Alikunhi, 1953) and 22,000-34,000 per kg body weight (Chen, 1990). Considering the above ovarian fecundity, the number of eggs procured from floating egg masses both in wild and captive spawning is generally very low, which in the instant case were 3500-4550 in

females of weight 275 and 490 gm respectively, which were more or less similar to the recording of Hossain *et al.* (2008). Overall, 20,950 eggs were collected from 5 spawnings in earthen ponds and 16,400 from 4 tank spawning, which were more or less same. This implies that the species is a batch spawner and release only small fraction of those eggs which are fully mature at the time of spawning.

The fertilization percentage was was found very high in all the cases of spawning ranging from 90-95% in case of earthen ponds and 91-94% in cement tanks (Figure 50, 51), which were comparatively higher than previous records (Hossain *et al*., 2008, Devraj, 1973b; Chen, 1990, Haniffa *et al*., 2004). A fertilization rate of 76.50% was reported in indoor conditions with *C. striatus* by Amornsakun *et al.* (2011b).

Parents lay the eggs in nests, built with weeds and leaves of aquatic plants (Pethiyagoda, 1991), and guard the spawn until the young reach the size of some 10 cm (Breder and Rosen, 1966). Males show a territorial behavior (Pethiyagoda, 1991). Similarly, hatching rate was also very high ranging from 91-94% in earthen ponds and 90-96% in cement tanks. Therefore it can be visualized that induced breeding in no way adversely affect on the physiology of the fish when sGnRH anologue is used as the quality of eggs and hatching rates were very high in all the spawning cases.

Fig 50: Hatchling of *C. marulius*

Fig 51: Rearing of spawn of *C. marulius* in a FRP tank

Chapter 7

EMBRYONIC AND LARVAL DEVELOPMENT

Chapter: 7
EMBRYONIC AND LARVAL DEVELOPMENT

7.1 MATERIALS AND METHODS

Approximately 200 immediately spawned eggs were transferred in a tray in an air conditioned room maintained with the temperature of 26±1.0 ^{O}C. The fertilized eggs and hatchlings were examined under a stereozoom microscope (Leica model S8APO) for the embryonic and larval developments up to yolk-sac absorption stage. As the eggs of *C. marulius* have a large oil globule two-third size of the yolk, the blastodisc of eggs always remain situated on the down side of the yolk as a thin layer and was not visible up to morula stage due to high buoyancy of oil globule which always keeps egg upside down. Therefore in order to observe the developments in the blastoderm, the eggs were rotated with the help of soft brush to observe cell development in the blastoderm under the microscope. Once the egg attained morula stage, the development of embryo becomes easily visible even without rotating the eggs. The digitized images were taken, whenever, changes in the internal structures were observed. The embryo development was observed till hatching of the embryo. The water of the container was changed after every 12 hours in order to optimize the water quality.

EMBRYONIC AND LARVAL DEVELOPMENT

7.2 RESULTS AND DISCUSSION

7.2.1 Water Quality

The water parameters of the spawning tanks were temperature 28±1 ^{o}C, pH 7.4±0.1, TDS 225 ± 18 mg L^{-1}, DO 6.0±0.5 mg L^{-1} which was found in the suitable range for spawning of this species.

7.2.2 Embryonic development

The details of embryonic development are given in Table 10 and Figure 52. The fertilized eggs were round, transluscent, pale yellow, pelagic, non-adhesive and had a diameter of 1.79±0.05 mm. They were unique in having single large round oil globule of dia 1.1±0.1 mm, almost two-third size of the yolk and immersed in the golden yellow yolk having a dia of 1.25±0.1 mm and both were surrounded within a space bounded by the vitelline membrane. West (1990) has also described similar structure in *C. striatus* egg. Parameswaran (1975) and Parameswaran and Murugesan (1976b) described the egg structure to be pale red-yellow with a diameter between 1.584 mm to 1.980 mm. Khan (2010) described the size of the egg as 1.79 mm which was same as reported in the present study.

The first cleavage was followed with equal division of blastoderm (2-cell stage) within 20±4 post fertilization minute (pfm). It was followed with second division resulting in the formation of 4-cell stage within 30±5 pfm (Fig. 2A). The third cleavage with formation of 16 cells was observed at 45±5 pfm (Fig. 2B). It was soon followed with fourth cleavage with the formation of 64 cells within 60±10 pfm (Fig. 2C). The blastoderm obtained morula stage in 1.45±0.15 pfh (Fig. 2D). At this stage, the embryo development becomes clear in side view when egg is moved a little bit with a brush. The embryo developed to gastrula (Fig. 2E) and early neurula stages in 5.00±0.15 pfh and 6.30±0.30 pfh respectively. At this stage, head and tail portion becomes distinct (Fig. 2F). Cephalic region becomes wider at 10 myotome stage and rudiments of heart

could be seen (Fig. 2G). The size of the embryo started increasing with increase in the number of myotomes and development of internal organs, which have been described in Table and Fig.52 The embryo hatched in 29.00±1.00 pfh at 26±1 ^{O}C. The hatching duration has been reported quiet variable in the past studies with different inducing agents ranging from 39-43h (Pituitary gland), 36-38h (HCG), 34-36h (LHRHa) and 21-23h (Ovaprim) (Haniffa *et al*., 2001) though this solely depends on the water temperature and not likely to be effected by the type of hormone used for spawning. Hatching was reported in 28 hr and 40 min with 60.26% rate at a water temperature of 26.5-29.0 ^{o}C in *C. striatus* by Amornsakun *et al.* (2011b). Marimuthu and Haniffa (2007) also described embryonic development with more or less similar recording excepting faster development 23.30-24.00 pfh at hatching, which may be due to higher water temperature. Khan (2010) is the only other worker, who has described the embryonic development of *C. marulius* in a study at Pakistan. According to him, the mouth and tail regions in the embryo developed in 13.45 pfh, heart beat started in 23.00 Pfh, twitching movement in 24.00 pfh, hatching in 32.50 pfh. However, the hatching period was found slightly different with that of Parmeswaran and Murugesan (1976b), who recorded this to be 54 hours at 16-26 ^{o}C and in 30 hours at 28-33^{o}C.

7.2.3 Larval development

The details of larval development up to yolk absorption stage are given in Table 11 and Fig.53. A just hatched larva is translucent, dull brown in colour and has well defined yolk. The head and yolk together looked like a bulb and anal portion has a distinct transparent caudal fin-fold having approximately 38 myotomes. It measured 3.14 mm in total length, 1.42 mm in anal length, 1.20 mm yolk length. The hatchling moves with jerks up and down but unable to swim (Fig. 3A). The heart was functional but flow of blood was not visible as being transparent at this stage. In subsequent developments, the hatchling increased both in head and anal lengths with development of eyes at 79.00±0.20 phh, mouth and pectoral fins at 84.00±0.30 phh (Fig. 3B-F). The yolk-sac was completely absorbed in 73.00±0.30 phh or total 102.00±0.30 pfh. At this stage, the larva reached a length of 6.56 mm and swim freely with well developed

mouth and all fins (Fig. 1H and 3G). Mouth opening and digestive tract developed fully at 62 hr phh and the larvae started feeding Moina at temperature of 28.0-30.5 ^{o}C. The mouth gap was recorded 334.87±149.78 µm (Amornsakun *et al.*, 2011b). The length of newly hatched larvae was reported 3.18±0.11 mm having yolk volume 1.279.7±196.10 µm and yolk completely absorbed in 80 hr (Amornsakun *et al.*, 2011b). These developments were found more or less same except that the total length and anal length of yolk-sac absorbed larvae were comparatively larger 6.56 mm and 3.99 mm respectively in the instant case in comparison to 5.8 mm and 2.90 mm recorded by Marimuthu *et al.* (2003). It is concluded that hatching of eggs of *C. marulius* may be undertaken in indoor conditions using plastic troughs, FRP tanks and cement tanks.

Table 10: Details of embryonic development of *C. marulius* at 26±1 ^{0}C (n=4)

Stage	Post fertilization duration	Figure No.	Developmental details
Fertilized Egg	-	-	Eggs are round, transluscent, non-adhesive, floating, and pale yellow in colour having diameter of 1.50±0.05 mm. Diameter of oil globule and yolk-sac was respectively 1.1±0.1 mm and 1.25±0.1 mm.
2-cell stage	20.00±04.00 m	-	First cleavage
4-cell stage	30.00±05.00 m	2 (A)	Second cleavage
16-cell stage	45.00±05.00 m	2 (B)	Third cleavage
64-cell stage	60.00±10.00 m	2 (C)	Fourth cleavage
Morula stage	01.45±00.15 h	2 (D)	Blastoderm cell walls become undifferentiated; embryonic shield formed and invaded around half of the yolk.
Gastrula stage	05.00±00.15 h	2 (E)	Gastrulation converts the blastoderm into a two-layered structure, with an outer epiblast and inner hypoblast. Head and tail region not differentiated
Early neurula	06.30±00.30 h	2 (F)	Cephalic region distinct with fore brain and narrow tail region
10 myotome	16.00±00.30 h	2 (G)	Cephalic region further broadened, 10 myotomes visible, tail extends up to half of the yolk periphery, heart rudimentary
15 mtotome	17.30±00.30 h	2 (H)	Length of embryo further extends, around 15 myotomes visible, heart formed, eye lens formed in the rudimentary eyes kupfer's vesicle.
22 myotome	18.00±00.30 h	2 (I)	Length of embryo further extends, around 22 myotomes visible, heart well developed and commenced blood circulation, eyes rudimentary,
25 myotome	18.30±00.15 h	2 (J)	Length of embryo further extends and cover around 80% yolk surface, heart well developed and commenced blood circulation but pulse rate very slow, around 25 myotomes visible, melanopores visible over the yolk-sac, tail narrow
27 myotome	22.30±00.15 h	2 (K)	Length of embryo further extends and cover around 85% yolk surface, cephalic and tail regions further widened, heart beat rhythmic and fast, melanophores increased in number
35 myotomes	23.00±00.15 h	2 (L)	Embryo covers entire yolk, cephalic region further enlarged, tail started detaching from yolk, melanophores increased further in number, around 35 myotomes visible,
Pre-hatched embryo	27.00±00.30 h	2 (M)	Tail detached from posterior side and moves at interval, myotomes more than 35, heart showed flow of red blood, eye precursor visible, auditory vesicle formed, twitching movement initiated, melanophores appeared on over the body.
Hatching embryo	29.00±01.00 h	2 (N)	Embryo coming out of egg shell with more vigorous twitching movement, myotomes more than 35, melanophore denser over the body, eye rudimentary

Table 11: Development of hatchling to spawn stage of *C. marulius* (n=4)

phh time	Pfh time	Figure No.	Developmental details
0.0	30.00±0.30 h	3 (A)	Hatchling dull brown in colour, yolk-sac well defined, caudal finfold transparent, myotomes around 38, average total length 3.14mm, anal length 1.42mm, yolk length 1.20mm, yolk width 0.80mm, length of eye optic 0.34mm, eye optic distinct but eyes are not developed, mouth not formed. The larvae move up and down with jerks but could not swim.
6.00±0.30 h	36.00±0.30 h	3 (B)	Average total length 3.40mm, anal length 1.58mm, yolk length 1.42mm, yolk width 1.20mm, melanophores increased in number,
22.00±0.30 h	52.00±0.30 h	3 (C)	Average total length 4.08mm, anal length 2.08mm, thorax length 2.02mm, yolk length 1.42mm, yolk width 1.08mm, eyes rudimentary
27.00±0.20 h	57.00±0.20 h	3 (D)	Rudimentary eyes prominent, average total length 4.01mm, anal length 1.95mm, yolk length 1.42mm, yolk width 1.00mm
49.00±0.20 h	79.00±0.20 h	3 (E)	Eyes formed, Eye dia 0.35mm, average total length 4.97mm, anal length 2.99mm, thorax length 1.98mm, yolk length 1.29mm, yolk width 0.89mm
54.00±0.30 h	84.00±0.30 h	3 (F)	Average total length 6.17mm, anal length 3.93mm, thorax length 2.24mm, eye dia 0.42mm, head width 1.17mm, yolk-sac reduced in size, pectoral fins developed and move vigorously for swimming, mouth formed
73.00±0.30 h	102.00±0.30 h	3 (G)	Average total length 6.56mm, anal length 3.99mm, thorax length 2.59mm; eyes increased in size with eye dia 0.55mm and eye width 0.43mm; head width 1.29mm, mouth formed, mouth cleft 0.53mm, yolk-sac consumed completely, melanophores dense, pectoral fins well developed

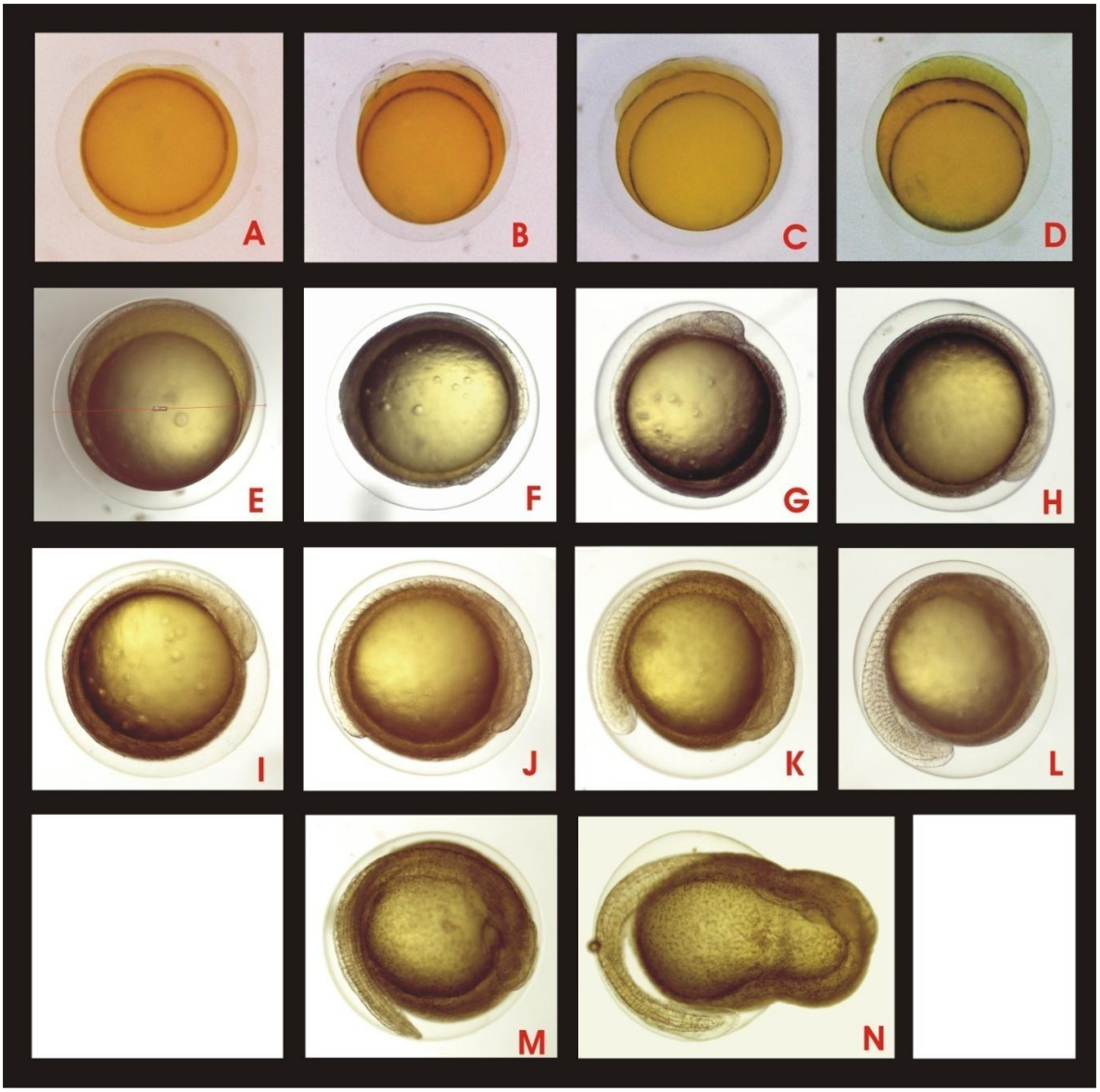


Figure 52: Development of *C. marulius* egg from up to hatching stage

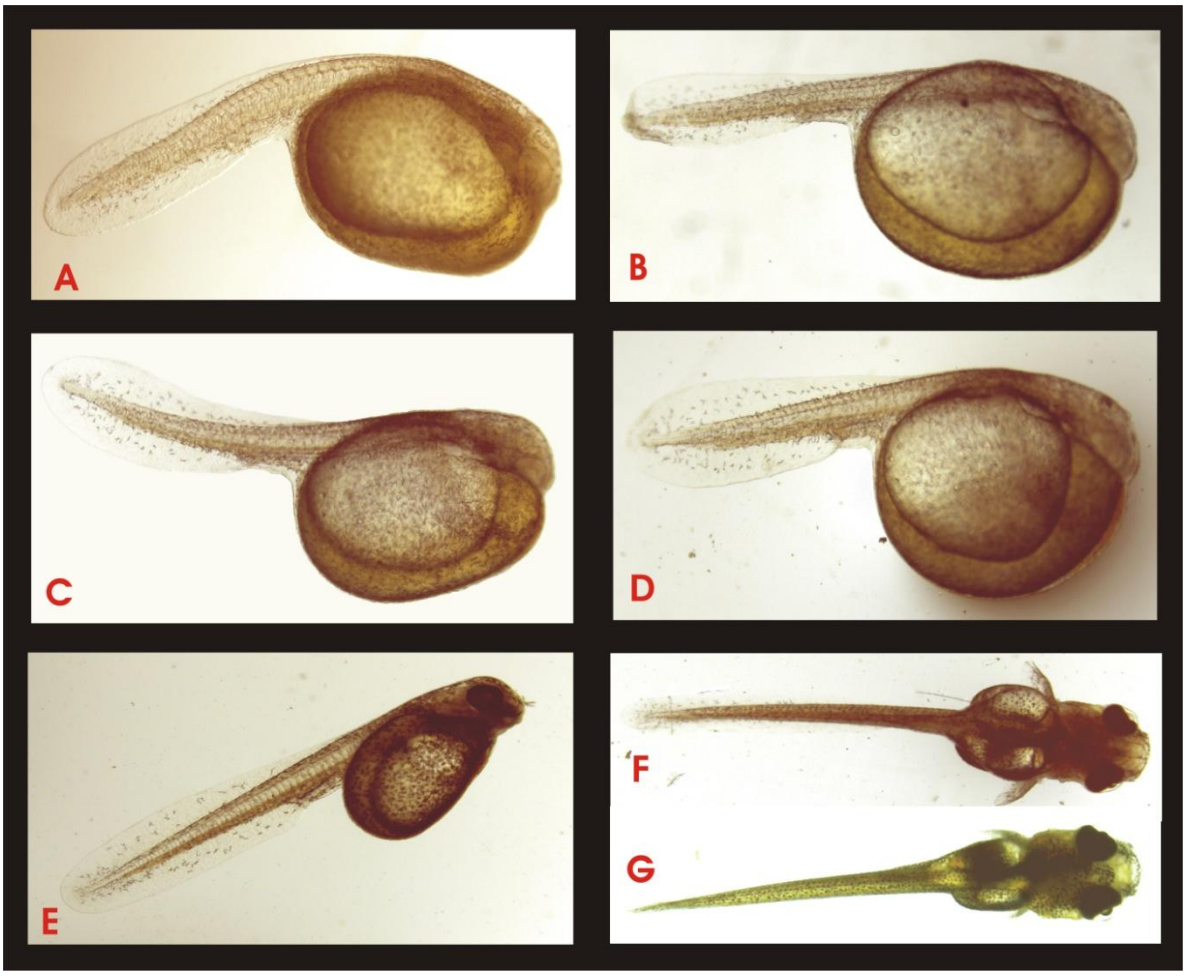


Figure 53: Development of *C. marulius* from hatching to spawn stage

Chapter 8

LARVAL REARING

Chapter 8
LARVAL REARING

8.1 MATERIAL AND METHODS

8.1.1 Procurement of test fish

The early fry of *C. marulius* were procured from the farm facilities of NBFGR, Lucknow, India where they bred naturally (Figure54). They were thoroughly washed and disinfected with formalin (15 mg L^{-1}) and reared initially in two FRP tanks of 1125 litre (size 1.5 x 1.0 x 0.75 m) for 30 days. The fry were initially given zooplankton as a main feed for 3 days followed by a combination of zooplankton and egg custard for next 7 days, subsequently zooplankton was withdrawn from their diet. The fry were then acclimatized to synthetic diet 'D' (protein 480 g kg^{-1}) for 5 days before the start of the experiment.

8.1.2 Experiment design

The larval rearing trial was conducted in triplicate set with six variable diets in 18 FRP tanks of 1125 litre (size 1.5 x 1.0 x 0.75 m) kept under an open shade at the NBFGR farm facility during July (Figure 55). The tanks were filled with 500 litre bore well water and covered with green house netting (75% light cutting). Twenty fry (mean length 4.11$\pm$ 0.59 cm) of test fish were stocked in each of the 18 designated tanks (Figure 56). The average initial biomass of stocked fry of each of the tank was recorded before placing them in the tank which did not differ significantly ($p>0.01$) among six treatments. Feed was given once in a day (11.00 A.M.) initially at the rate 8% of body weight for first 10 days and then corrected to 5% of body weight for rest of the period both by hand and tray feeding in order to ween them on artificial diet (Figure 57). The tanks were cleaned on every third day through siphoning when all the debris was removed and 50% tank water was replaced with fresh chlorine-free bore well water. Water samples for the analysis of temperature, pH, total alkalinity,

dissolved oxygen, and free carbon dioxide were tested on every third day. The temperature was measured with a centigrade thermometer, pH with digital electronic meter (Eutec), total alkalinity, dissolved oxygen and free carbon dioxide by titration methods following the standard methods (APHA 1985).

8.1.3 Preparation and application of test diets

Six semi-purified test diets A, B, C, D, E, and F containing 300, 360, 420, 480, 540 and 600 g kg^{-1} protein levels were prepared using casein as a protein supplement. The other feed ingredients mixed in the various diets are given in Table 12. The feed ingredients after proper mixing were steamed in a pressure cooker for 10 minutes and after cooling divided into 4 blocks and stored in a deep freeze (-20^{o}C) in a sealed polythene bag. One by one these feed blocks were taken out from the deep freezer, kept under normal cooling (temperature around 5-6^{o}C) condition in a refrigerator and gradually used for feeding to fish. The feed was graded to small particle size with the help of a hand held metal grater. The grated feed was provided to fish both by hand and tray feeding in order to ween them on artificial diet (Figure 57)

8.1.4 Collection of survival and growth data

Mortality was counted every day in the morning and evening and the dead fish were removed. Cannibalism rate was estimated from the difference between initial number of fish stocked and sum of survivors plus dead fish removed. All the fishes were removed on 28th day, measured for length and weight and 5 samples from each of the replicates was packed in sealed polythene sackets and kept in deep freezer (-20^{o}C) for proximate analysis of carcass (Figure 58) and left over fingerling were pooled and stocked in a pond for further rearing (Figure 59).

8.1.5 Proximate analysis

Proximate composition of diets and fish carcasses was analyzed following methods of AOAC (1990). All samples were analyzed in triplicate. Dry matter was

calculated by drying in an oven at 105^{o}C for 24 hours; crude protein (N X 6.25) by the Kjeldahl method after an acid digestion method and crude lipid by ether extraction after acid hydrolyses. Nitrogen free Extract was estimated by subtraction method.

8.1.6 Analysis of survival and growth data

All the surviving samples of fishes were measured for total length (L) and weight (W) at the end of the experiment. Survival rates (S), net biomass, specific growth rates (SGR), per day weight gain, feed conversion ratio (FCR) and length-weight relationship (W) were calculated using the following formulae:

$$S = 100 \times (n_t/n_0)$$

Where, S is survival rate (%), n_t is the number of fishes survived at time *t* and n_0 is the number of fishes at the commencement of the experiment.

$$NB = GB - IB$$

where, NB is net biomass (g) of all the surviving fishes, GB is measured gross biomass (g) of all the surviving fishes at the time of final harvesting and IB is the measured initial biomass (g) of all the stocked fishes.

$$SGR = 100(\ln Wt - \ln W_0) / (t_2 - t_{1)}$$

Where, SGR is specific growth rate; *Wt* is the total weight at time *t2*, *Wo* is initial weight at the time (t1).

$$FCR = TF/NB$$

Where, FCR is food conversion ratio, TF is total quantity of given feed (g), NB is net biomass (g).

$$W = a\ L^{b}$$

Where, W is assumed weight of fish, ‘a’ and ‘b’ are constant and ‘L’ is length of fish.

PDWG = NB/t

Where, PDWG is per day weight gain, NB net biomass and‘t’ number of days.

Analysis of variance (ANOVA) was used to determine the significance levels between different production attributes by SPSS version 16.0 software. Student’s‘t’ test was performed to analyze significance levels for diets. Regression graphs were plotted for comparing the length-weight relationship at final harvesting and ‘Y’ (simple linear regression) and ‘R^2’ (correlation) were recorded using M.S. Excel (Version 2007) and ‘W’ was calculated manually by the formula ($W = aL^b$) in M.S. Excel spread sheet. The data are expressed as mean$\pm$ standard deviation. Significant levels were considered at $p<0.05$ and $p<0.01$ and means were compared using Duncan Multiple range test.

Figure 54: Sac-fry of *C. maurlius*

Figure 55: A view of a battery of tanks used for experiment on growth studies

Figure 56: Close-up of fry of *C. marulius* in a beaker flask

Figure 57: A haul of fry of *C. marulius* being fed on palm

Figure 58: Preservation of samples of C. marulius for carcass composition

Figure 59: Pooled up fingerling of *C. marulius* at the time of final harvesting

8.2 RESULTS AND DISCUSSION

8.2.1 Mortality and survival

The highest mortality was observed in the second week of the experiment which slowed down in the subsequent weeks but continued till the completion of the experiment. The survival was significantly higher (p<0.05) with feeds E (88.3%) and F (85.0%) in comparison to feeds A (46.6%), B (46.6%), C (46.6%) and D (53.3%). The feeds A, B and C that showed poor survival were found not significantly different (p>0.05) and similarly feed E and F showing higher survival were also not significantly (p>0.05) different (Table 13).

8.2.2 Growth

No significant difference ($p > 0.05$) in average length and weight of fishes in different treatments was observed although the standard deviations showed comparatively higher fluctuations in both total lengths and weights with diets A, B, C and D with that of diets E and F confirmed that wide variations in size occurred in these treatments (Figure 60 A, B, C, D, E and F). The initial biomass did not differ significantly (p<0.01) amongst all the treatments, however, a linear relationship existed between net biomass production, SGR and per day weight gain from feed A-F containing low to high levels of protein. The net biomass, SGR and per day weight gain also did not differ significantly (p<0.05) between feeds E and F (Table 13). The FCR was found very high and variable in treatments A to D, whereas it was in the acceptable range in treatments E (3.64) and F (3.26).

8.2.3 Length-weight relationship

The values of 'R^2' and 'W' for all the treatments were found more or less the same which revealed that all the surviving fishes were in good shape of health. The fishes in all the treatments follow Fulton's condition factor where values of 'b' were observed higher than 3 (Table 13 and Figure 60 A, B, C, D, E and F).

8.2.4 Proximate analysis of diets

The details of proximate analysis of protein, nitrogen free extract (NFE), lipids, moisture and energy of different test diets is given in Table 13. No significant variations (p>0.05 and p >0.01) in the dietary protein levels in the test diets were observed in comparison to formulated diets. Similarly both NFE and lipids also did not show significant variations (p>0.05 and p>0.01) in comparison to the formulated diets.

8.2.5 Proximate analysis of carcass

Table 14 revealed that there were significant variations (p<0.05) in protein levels of carcasses of fishes fed different diets. The protein deposition in tissue was found directly proportional to the available protein in the test diets. The highest level of protein (666.3 $g.kg^{-1}$) was observed in the tissue of fishes fed diet F (CP 600 $g.kg^{-1}$) followed respectively 631.3 $g.kg^{-1}$, 627.6 $g.kg^{-1}$, 583.3 $g.kg^{-1}$, 576.9 $g.kg^{-1}$ and 560.9 $g.kg^{-1}$ with diets E (CP 540 $g.kg^{-1}$), D (CP 480 $g.kg^{-1}$), C (CP 420 $g.kg^{-1}$), B (CP 360 $g.kg^{-1}$) and A (CP 300 $g.kg^{-1}$). Similarly, NFE levels in carcass were found in increasing order with regard to its availability in the formulated diets. Diet A, C, and D respectively showed significantly higher (p<0.05) values of 114.0 $g.kg^{-1}$, 117.0 $g.kg^{-1}$ and 111.3 $g.kg^{-1}$ NFE in comparison to diets B (102.2 $g.kg^{-1}$), E (108.2 $g.kg^{-1}$) and F (108.1 $g.kg^{-1}$). In contrast to NFE, lipid levels in carcass showed highest value of 66.8 $g.kg^{-1}$ with diet F, which was found significantly higher (p<0.05) than the diets E (58.6 $g.kg^{-1}$), A (43.0 $g.kg^{-1}$), B (44.6 $g.kg^{-1}$), D (34.6 $g.kg^{-1}$) and C (34.6 $g.kg^{-1}$) respectively (table-3). Moisture values did not follow any trend and was found significantly higher (p<0.05) in diets C (842.6 $g.kg^{-1}$), D (827.8 $g.kg^{-1}$), F (819.5 $g.kg^{-1}$) in comparison to diets B (817.7 $g.kg^{-1}$), A (803.0 $g.kg^{-1}$) and F (720.4 $g.kg^{-1}$) though the later did not differ significantly (p>0.05) from the initial stocked fish.

8.2.6 Water quality

The physico-chemical parameters of water recorded were found in the normal range. The average values for temperature, pH, total alkalinity and DO were respectively recorded 28.3±1.15 ^{o}C, 7.5±0.07, 138.4±2.06 mg.L^{-1} and 6.84±0.27 mg.L^{-1}, whereas free CO_2 was found absent in all the tank water.

8.3 Discussion

A significantly (p<0.05) higher survival of 88.3% and 85.0% respectively in diets E (protein 540 g.kg^{-1}, energy 18.37 KJ.g^{-1}) and F (protein 600 g.kg^{-1}, energy 18.47 KJ.g^{-1}) in the present study in comparison to formulated diets A (protein 300 g.kg^{-1}, energy 19.95 KJ.g^{-1}), B (protein 360 g.kg^{-1}, energy 18.07 KJ.g^{-1}), C (protein 420 g.kg^{-1}, energy 18.17 KJ.g^{-1}) and D (protein 480 g.kg^{-1}, energy 18.27 KJ.g^{-1}) revealed that the protein requirement of *C. marulius* is quiet high at fry stage (size 4.11±0.59 cm). This was well corroborated with the work of Mohanty and Samantaray (1996) who obtained highest growth performances (survival was not defined by the authors) in *C. striata* fry fed formulated diet (made from natural ingredients) containing 550 g.kg^{-1} protein (4320 kcal energy.kg^{-1}) fed at the rate of 10% bw day^{-1}.

Though highly significant variations (p<0.05) in the survival rates between diets A-D with that of diets E and F were observed, the average lengths and weights were insignificant (p>0.05) with all the six diets pointing the poor survivals in diets A-D may be due to higher rate of cannibalism or cannibalism attempts that cause injury and subsequent mortality due to spread of disease. It is well known that Snakeheads observed great amount of cannibalism at all stages of life and it is one of the major reasons of low survival during snakehead culture (Ng and Lim, 1990). In the process of cannibalism although shooters are able to prey on fish measuring 2/3 in length (Diana *et al*., 1985) or 63-80% (Qin and Fast, 1996a) to predator size in case of *C. striatus*, no information as to predator-prey ratio is available for *C.*

marulius. *C. striatus* in the process of cannibalism ingested comparatively smaller numbers (more than 10%) of prey and large numbers of them die due to injury, shock and spread of diseases (Qin and Fast, 1996b). This phenomenon was observed in the present study also whereby hardly 15-20% of the populations were found missing in the tanks and rests were procured in dead condition showing signs of injury in different parts of the body more prominently on the caudal fin which was eaten up either in toto or in part.

It has been demonstrated in several of the studies that application of formulated diets had improved survival greatly in fishes that observed great amount of cannibalism (Hoelzer, 1992; Kvarnemo, *et al*., 1998; Manica, 2004; Qin and Fast, 1996b; Rohwer, 1978; Sargent, 1992). However, it is also more important that formulated feed should meet the nutritional requirement of fish in to. The poor survival with isocaloric diets A, B and C containing protein levels of 300, 360 and 420 $g.kg^{-1}$ respectively, has revealed that nutritionally deficient diets tends to aggravate cannibalism as feed applied to all the treatments was in uniform quantity and was totally consumed everyday within a short span of time. The higher size of minimum lengths and weights of fishes in all the treatments at the time of termination of experiment with that of initial lengths and weights also confirmed that the feed was accepted in all the treatments, however, it is the quality of feed which in the instant case is the levels of protein that mattered for low and higher survival in different treatments. It is better explained in case of diets E (CP 540 $g.kg^{-1}$) and F (CP 600 $g.kg^{-1}$) as the survival is better in these diets. It reveals that optimum dietary protein levels reduce the tendency of cannibalism.

The tanks showing higher rate of mortality also found to have greater size variations both in lengths and weights (Table 13 and Figure 60 A, B, C, D, E and F) that could have been occurred due to cannibalism in these tanks. Qin and Fast (1996b) observed that *C. striatus* in all treatments of feed application rates was found to cannibalize most small individuals and all treatments had a few large

individuals at the end. However this situation did not arise in the present study due to short term experiment, however greater differences in minimum and maximum size were observed in treatments showing poor survival provided with diets of low levels of protein. The wide range in initial size distribution enabled large individuals to cannibalize small ones and hence survival.

Although acceptability of feed was recognized as stated above, significant differences ($p<0.05$) existed amongst treatments in case of net biomass production, SGR, weight gain per day and FCR (Table 13). The feeds A and B, however, showed negative values for net biomass production, SGR, weight gain per day and FCR due to higher rate of mortality/ cannibalism and low protein levels in these diets. These values were, however, significantly slightly higher in diets C ($p>0.05$) and D ($p<0.05$) which were also likely to happened due to reasons stated above. However, no significant ($p<0.05$) change in net biomass, SGR and weight gain per day was observed in diets E and F provided with higher protein levels in the present study that indicated that the fish may efficiently consume protein up to 600 $g.kg^{-1}$ and protein level of 550 $g.kg^{-1}$ would be economical for its culture. This finding was partly favoured in respect that protein level of 550 $g.kg^{-1}$ in diet performed best and economical but protein level of 600 $g.kg^{-1}$ also showed more or less similar growth in fishes which was in contrast to the findings of Mohanty and Samantaray (1996), who observed depressed growth at protein levels higher than 550 $g.kg^{-1}$ in *C. striatus*. However, the observations with juvenile *C. striata* (Wee, 1986) and *C. micropeltes* (Wee and Tacon, 1982); *Chanos chanos* (Lim *et al*., 1979); *Epinephelus tauvina* (Teng *et al.*, 1978), *Cyprinus carpio* (Ogino and Saito, 1970), *Ictalurus punctatus* (Prather and Lovell, 1973) and *Sarotherodon mossambicus* (Jauncey, 1982) were reported to be in line with the present study.

The 'W' values were found to follow cube law with all the diet treatments indicating well being of the fish and was better than the adult natural stock (Johal *et al*., 1983). The 'R^2' values (0.853-0.943) were also comparable with the natural

population (Devraj, 1973a) that also confirmed satisfactory growth on all experimental artificial feeds. These facts illustrates that the cause of mortality in fish in different treatments is not because of poor feed acceptability, instead it is the quality of feed that mattered for higher survival and growth (Table 13).

Protein efficiency studies on snakehead body tissue have been performed in good number of cases both from capture and culture stocks (Aliyu-Paiko *et al*., 2010, Gam *et al*., 2005, Mohanty and Samantaray, 1996, Zuraini *et al*., 2006) however there is dearth of such literature on *C. marulius*. Barring the study of Zuraini *et al*. (2006), the level of protein in body tissues in case of *C. striatus* has been reported to be 230 $g.kg^{-1}$ (Zuraini *et al*., 2006) to 449.0 $g.kg^{-1}$ (Gam *et al*., 2005) in natural stocks, whereas in experimental culture, protein level as high up to 713 $g.kg^{-1}$ has been reported when fish fed dietary protein 450 $g.kg^{-1}$ along with a lipid level of 65 $g.kg^{-1}$ (Aliyu-Paiko *et al*., 2010). The later, therefore, support the present findings in which protein levels in body carcass of *C. marulius* was recorded from 560.9 $g.kg^{-1}$ to 666.3 $g.kg^{-1}$ when *C. marulius* was fed semi-purified diets containing dietary protein levels of 300 $g.kg^{-1}$ (energy 17.87 $KJ.g^{-1}$) to 600 $g.kg^{-1}$ (18.47 $KJ.g^{-1}$) (Table 14). The availability of protein in body carcass greatly depends on species, size, age, season, protein quality, dietary level of energy, water quality and presence of natural food and culture management (NRC, 1993, Gam *et al*., 2005).

Protein efficiency in *C. marulius* was found almost directly proportional to the dietary protein levels as all treatments had significantly ($p<0.05$) different carcass protein with highest protein in diet F (protein 600 $g.kg^{-1}$) (Table 14). These results were similar to the work of Mohanty and Samantray (1996) and Aliyu-Paiko *et al*. (2010). Higher amount of carcass protein in comparison to the dietary protein in all the treatments revealed that this fish has high sparing capacity of metabolizable NFE to protein (Aliyu-Paiko *et al*., 2010). This also fits well in case of lipid levels with diets E and diet F where carcass lipid levels were found

significantly (p<0.05) higher than the dietary lipid levels. According to Gam *et al*. (2005), carcass protein level depends on availability of natural food in water in case of *C. striatus* and found highest during rainy season when quantity of natural food is at maximum. The reason of higher deposition of carcass protein in the fish in the present study and that of findings of Aliyu-Paiko *et al*. (2010) therefore may be due to feeding higher amount of dietary protein in the present instance. The moisture content was also found significantly (p<0.05) low with diet F in comparison to other diets.

Therefore on the basis of survival, growth and protein efficiency indices recorded in the present study, the dietary protein requirement of *C. marulius* fry was assessed to be around 60 g kg^{-}1 at energy value of 18.4 KJ.g^{-1} when fed 5 percent of bw.day^{-1}. It was also evaluated that this fish has high sparing capacity of utilizing metabolizable NFE in protein and lipid replacement. However, this needs to be confirmed with natural feed ingredients in future studies.

Table12: Composition of experimental diets and proximate composition

Ingredient	Feed ingredients used in different test feeds (g kg^{-1})					
	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Casein[1]	300.0	360.0	420.0	480.0	540.0	600.0
Starch[2]	434.0	374.0	314.0	254.0	194.0	134.0
Cellulose[3]	180.5	180.5	180.5	180.5	180.5	180.5
Cod liver oil[4]	45.0	45.0	45.0	45.0	45.0	45.0
Ascorbate[5]	0.50	0.50	0.50	0.50	0.50	0.50
CMC[6]	20.0	20.0	20.0	20.0	20.0	20.0
VM+MM[7]	20.0	20.0	20.0	20.0	20.0	20.0
GE.kg^{-1}	4279	4303	4327	4351	4375	4399
KJ g^{-1}	17.95	18.07	18.17	18.27	18.37	18.47
Proximate Composition						
Protein	294.3*	349.0*	402.9*	475.7*	528.6*	586.6**
NFE	597.6**	551.6*	487.0*	426.0*	361.3*	307.6*
Lipid	44.0*	43.3*	41.6**	42.2**	44.5*	43.4*
Moisture	50.2	46.0	65.4	52.3	62.5	54.5
GE kg^{-1}	4279	4303	4327	4351	4375	4399
KJ g^{-1}	17.97	18.07	18.17	18.27	18.37	18.47

1. HiMedia, Mumbai Lot No: 0000042681; 2. HiMedia, Mumbai, Lot No: 0000028340;
3. Cellulose (HiMedia, Mumbai, Lot No: 0000040304; 4.Cod Liver Oil, Manufacturer Universal Medicare Pvt. Ltd., Mumbai Batch No. R0109J; 5. L-Ascorbate-2 triphosphate Ca salt-HiMedia, Mumbai, Lot No. 000000517; 6. HiMedia, Mumbai, Lot No. 0000042121; 7. Each kg of Vitamin and mineral mixture named (Agrimin Forte) contains Vit. A 700000 IU, Vit. D_3 70000 IU, Vit. E 250 mg, Nicotinamide 1000 mg, Co 150 mg, Cu 1200 mg, I 325 mg, Fe 1500 mg, Mg 6000 mg, Mn 1500 mg, K 100 mg, Se 10 mg, Na 5.9 mg, S 0.72%, Zn 9600 mg, Ca 25.5%, P 12.75% Manufacturer Brindavan Phosphates Pvt. Ltd, 48N, Doddaballpur Ind. Area, Doddaballapur – 561 203, India and marketed by Virbac Animal Health India Pvt. Ltd., Andheri-Kurla Road, Andheri, Mumbai-400 059, India. Batch No. BFA-611 September 2010.
* Values are significantly different at 95% confidence limit for two replicates of each diet,
** Values are significantly different at 99% confidence limit for two replicates of each diet

Table 13: Growth parameter of *C. marulius* fed different protein levels in the diets

Parameter	Feed A	Feed B	Feed C	Feed D	Feed E	Feed F
Initial No.	60	60	60	60	60	60
Survival (%)	46.6^{b}	46.6^{b}	46.6^{b}	53.3^{b}	88.3^{a}	85.0^{a}
Initial avg. length (cm)	4.11 ± 0.59^{a}	4.11 ± 0.59^{a}	4.11 ± 0.59^{a}	4.11 ± 0.59^{a}	4.11 ± 0.59^{a}	4.11 ± 0.59^{a}
Final avg. length (cm)*	6.34 ± 0.76^{a}	6.40 ± 0.79^{a}	6.50 ± 0.89^{a}	6.40 ± 0.90^{a}	6.23 ± 0.61^{a}	6.49 ± 0.6^{a}
Initial av. weight (g)	0.78^{a}	0.82^{a}	0.83^{a}	0.86^{a}	0.80^{a}	0.81^{a}
Final average weight (g)*	1.62 ± 0.57^{a}	1.68 ± 0.69^{a}	1.89 ± 0.74^{a}	1.71 ± 0.70^{a}	1.51 ± 0.46^{a}	1.72 ± 0.53^{a}
Initial biomass (g)* #	47.5^{a}	49.0^{a}	50.5^{a}	49.5^{a}	48.5^{a}	49.5^{a}
Gross biomass (g)*	46.4^{c}	47.2^{c}	53.0^{c}	62.8^{bc}	80.5^{ab}	88.6^{a}
Net biomass (g d^{-1})*	-1.1^{c}	-1.8^{c}	2.5^{c}	14.5^{bc}	33.0^{ab}	39.1^{a}
SGR % d^{-1}*	-0.0805^{c}	-0.0661^{c}	0.0748^{c}	0.3627^{bc}	0.771^{ab}	0.875^{a}
Weight gain per day (g)*	-0.01^{c}	-0.021^{c}	0.02^{c}	0.17^{bc}	0.39^{ab}	0.46^{a}
FCR*	2.37^{cd}	2.48^{d}	2.09^{c}	1.87^{b}	1.35^{a}	1.26^{a}
'y' values**	0.0709x-2.84	0.0883x-3.97	0.0795x-3.27	0.0785x-3.23	0.0745x-3.13	0.0780x-3.37
'w' values**	$0.0062*L^{3.0}$	$0.0057*L^{3.0}$	$0.0066*L^{3.0}$	$0.0064*L^{3.0}$	$0.0061*L^{3.0}$	$0.0063*L^{3.0}$
'R^2' values**	0.878	0.940	0.951	0.943	0.939	0.853

*n = Values of all the surviving fishes, ** n = 28 fishes, mean values within same rows and with different superscripts are significantly different at ($p<0.05$) and ($p<0.01$)$^{\#}$.

Table 14: Proximate composition of the carcass of *C. marulius* fed with different diets

Parameter	Initial	Diet A	Diet B	Diet C	Diet D	Diet E	Diet F
Protein (A) ($g\ kg^{-1}$)	651.5^{a}	560.9^{d}	576.9^{cd}	583.3^{c}	627.6^{b}	586.6^{b}	666.3^{a}
NFE (B) ($g\ kg^{-1}$)	123.0^{a}	114.0^{bc}	102.2^{d}	117.0^{ab}	111.3^{bc}	108.2^{cd}	$108.1d^{c}$
Lipids (C) ($g\ kg^{-1}$)	54.4^{c}	43.0^{d}	44.6^{d}	34.6^{f}	38.5^{e}	58.6^{b}	66.8^{a}
Moisture (%)	72.04^{d}	80.30^{c}	81.77^{bc}	84.26^{a}	82.78^{ab}	81.95^{bc}	72.04^{e}
Dry Matter (%)	27.95	19.70	18.23	15.74	17.22	18.045	27.96
Total (A+B+C) (DM basis) ($g\ kg^{-1}$)	828.9	718.0	723.8	734.9	777.4	798.1	841.2
GE kg^{-1}	393.19	338.36	342.15	341.94	363.10	381.82	404.98
KJ g^{-1}	16.51	14.21	14.37	14.36	15.25	16.03	17.00

*Values in a row are significantly different at 95% confidence limit

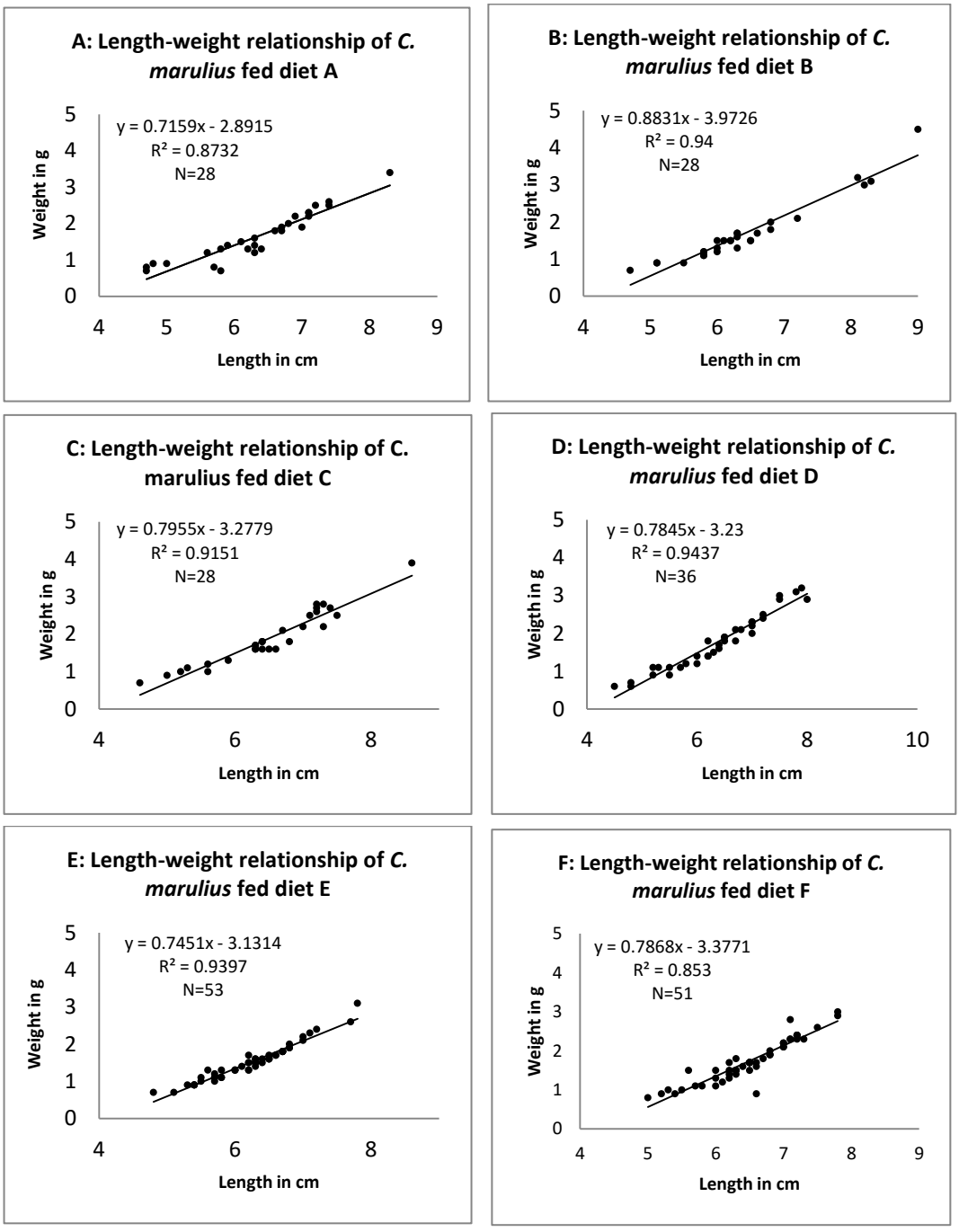


Fig 60: LWR and R^2 of *C. marulius* with diets A, B, C, D, E, F

Chapter 9

GROW-OUT

Chapter 9
GROW-OUT

9.1 MATERIAL AND METHODS

9.1.1 Procurement of test fish

The fry of *C. marulius* (length 2.96±0.16 cm, weight 0.21±0.02 g) were procured from the farm facilities of NBFGR, Lucknow from a natural spawned stock. After thorough washing and disinfection with formalin (15 $mg.L^{-1}$), reared initially in two FRP tanks of 1125 litre (size 1.5m x 1.0m x 0.75 m) for 30 days. The fry were initially fed exclusively zooplankton for 3 days followed with a combination of zooplankton and egg custard for next 7 days (7:3), egg custard and formulated diet for another 10 days (1:1) and finally only formulated diet for last 10 days.

9.1.2 Preparation of grow-out tank

A cement tank of dimension 14.2m x 10.3m x 1.2m (LxWxH) was filled up with fresh ground water to a depth of 1.0 m (146 m^3) and manured with mustard oil cake (2 kg) and diammonium phosphate (DAP) fertilizer (0.5kg), 5 days prior to stocking so that sufficient turbidity suitable for the grow-out of fish developed in the tank water (Figure 61). The test fish of length 4.11±0.042 cm and weight 4.05±0.059 g numbering 244 were stocked in the tank after proper acclimatization. Water samples for the analysis of temperature, pH, total hardness, electrical conductivity (EC), and dissolved oxygen (DO) were analyzed fortnightly using multiparameter (Orion model 5 Star).

9.1.3 Preparation and application of test diets

The feed was formulated on-farm using locally available feed ingredients (Table 15). All the ingredients were finely grounded, sieved and after mixing manually converted in to hard dough by adding bore well water. The dough was then transferred into a steel container and cooked in a pressure cooker up to 15 minutes. The prepared semi-moist feed was stored in a refrigerator at 4 ^oC and used within 4-6 days. The feed

was applied by cutting into small pieces and given once during morning (10.00-11.00 A.M.) at one side of the tank daily ad-libitum.

9.1.4 Analysis of survival and growth data

The fishes were reared for 13 months from 15 June to 14 June. The survival was estimated in the mid of every month by draining the tank entirely excepting during following December to February when netting and dewatering was avoided due to severe winter climatic conditions when chances of mortality were higher due to temperature stress. The weight-length data of 25 fishes was taken every month with the help of a measuring scale in centimeters and weight by a digital balance with a precision of 0.01 g (Figure 62, 63, 64, 65). The fishes were released back in the same tank when sufficient water was refilled in the tank. The shooters larger by 50% in total length than that of average length of others were removed after recording their weight and length and rest were restocked in the same tank.

The gross survival (GS), net survival (NS), net biomass (NB), specific growth rate (SGR), coefficient of determination (R^2), Fulton's condition factor (K) and regression analysis (WLR) were performed by the following formulae:

$$GS = 100 \times (n_t/n_0)$$

Where, 'n_t' is the number of fishes survived in a particular month and 'n_0' is the initial stocking number of fish at the commencement of the experiment.

$$NS = 100 \times (ns/np)$$

Where, '*ns* ' is the number of fishes survived in a subsequent month and '*np* ' is the number of fishes in the preceding month.

$$NB = GB - IB$$

where, 'NB' (g) is net biomass of all the surviving fishes excluding shooters in a particular month, 'GB' is the gross biomass (g) of all the fishes in a particular month and 'IB' is the measured initial biomass (g) of all the stocked fishes.

$$SGR = 100(\ln W_t - \ln Wo)/(t_2 - t_1)$$

Where, SGR is specific growth rate; '*Wt* ' is the total weight at time 't_2', 'Wo' is initial weight at the time (t_1).

$$R = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)2][n\sum y2 - (\sum y)2]}}$$

R^2 = Square of R (Correlation coefficient)

Where, 'R' is correlation coefficient and 'R^2' is coefficient of determination, x mean length (cm) and 'y' mean weight (g)

$K = 100W/L^3$

Where, W is weight in gram, L is length in centimeter

$W = a\ L^b$

The values of constant 'a' and 'b' were estimated from the equation of LeCren (1951) by transforming log values of length and weight, that is, linear regression equation given below:

$\text{Log } W = \log a + b \log L$

where, 'W' is assumed weight of fish in gram, 'a' is intercept on y-axis and 'b' an exponent between 2 and 4 and 'L' is mean length of fish in cm.

Analysis

Analysis of variance (ANOVA) was used to determine the significance levels between length, weight, survival, initial biomass, gross biomass and net biomass, SGR, per day weight gain and carcass proximate composition. Student's't' test was performed to analyze significance levels for diets. Regression graphs were plotted for comparing the length-weight relationship at final harvesting and 'Y' (simple linear regression) and 'R^2' (correlation) were recorded using M.S. Excel (Version, 2007) and 'W' was calculated manually by the formula ($W = aL^b$) in M.S. Excel spread sheet. The data are expressed as mean$\pm$ standard deviation. Significant levels were considered at $p<0.05$ and $p<0.01$ using SPSS software version 16.0.

GROW-OUT

9.2 RESULTS AND DISCUSSION

A good amount of work on culture of striped snakehead (*C. striatus*) using different models of predator-prey ratio, stocking density, and live and formulated diets have been carried out in the past (Ng and Lim, 1990; Qin and Fast, 1998; Folkvord A., 1991, Devraj M., 1973a), however, there are only few reports in case of *C. marulius*. Devraj (1973a) was the first in demonstrating experimental culture of *C. marulius* in cement tanks as well in earthen ponds at Bhavani Sagar in Tamil Nadu, India way back during 1964-65. He reported respective survivals of 19.3% in 3-months and 1.3% in 5-months with fingerling size of 5.0 to 7.9 cm in length and 10.5 to 15.5 cm when the species is reared in cement tanks and fed exclusively on fish liver. In another trial undertaken by him in earthen pond, adult specimens of 36.2 to 56.2 cm in length reared by him for 13 months gave a survival of 57.5% when fed on prey fish. Though he cultured the fish on meat and forage fish being the natural food of the species, the survival in both the earlier cases were very low with small size groups. The successful commercial farming always relies on a technology where all activities relating to culture could be easily maneuvered by a producer. Feeding a fish with artificial diet suitable in all respect to feeding bahaviour and fish nutritional requirements is one of the important aspects of successful fish farming and more important particularly for those fishes that observe high degree of predation and cannibalism. Raizada *et al*. (2012) in a 28 days study demonstrated that *C. marulius* fry could be easily weaned on semi-purified diet containing 54% CP with high survival rates (>85%). Based on the previous experience, the fingerlings of *C. marulius* were reared on artificial diets containing 55% CP for 12 months to observe the influence of artificial diet on the survival and body indices of this fish.

The survival was found low in small size fish but gradually improved and stabilized from November onwards when they attained a mean length of 15.1$\pm$0.19

cm and weight 26.96±1.30 (Table 16, Fig 66). Though gross survival of only 22.54% was observed under 12 month culture period but majority of losses was only up to October. The loss in survival could be linked mainly to the cannibalism behavior as the numbers of shooters recorded in July were the highest resulting in net lowest survival of 63.93%. As the culture progresses and fish started relishing the formulated diet, the number of shooters also subsequently reduced and no shooter was recorded from December onwards resulting in stabilizing of net surviving rate to over 90% in the subsequent months (Table 16, Fig 66). This phenomenon has also been reported in several of past studies in case of striped snakehead and Cortez damsel fish (Ng and Lim, 1990; Vivekanandan and Pandian, 1977).

The weight-length data (Table 16) indicated that both of them tend to increase gradually and reached from a mere weight and length of 4.05±0.59 g and 4.11±0.042 cm in June 2013 to 117.68±10.43 g and 23.36±0.71 cm in June 2014 although the rate of growth was very slow from November 2012 to April 2013 when winter sets in reducing the tank water temperature to low of 11.8 ^{o}C. The weight and length data also suggest that there was comparatively little difference in size amongst the fishes with progressing of culture on artificial diet.

The SGR values were found to improve gradually during July (0.3585%) to September (0.8258%) when water temperature was higher due to summer season but subsequently decreased from October 2013 (0.4964%) to April 2013 (0.06937%) due to winter climate but again improved during May-June 2014 when summer season sets in. Thus the rate of SGR was found directly proportional to the water temperature (Table 17).

A strong correlation (R^2) existed between WLR all through the culture period and no effect due to the climatic conditions was observed. Therefore, it was revealed that both weight and length increases with the same pace in different climatic conditions. The values of correlation coefficient obtained in the present

study were more or less the same as observed in natural stock of *C. marulius* in India (Vivekanandan and Pandian, 1977; Rathod *et. al*., 2011). Therefore, it could be interpreted that this fish could maintain WLR more or less same both in natural and captive conditions on artificial diet.

The 'K' values were found both size and season dependent. Small fishes (4.11±0.042 cm) in comparison to large fishes (23.36±0.71 cm) observed respective 'K' values of 5.173 and 0.9259 (Table 17). Froese (2006) noted that smaller fish grow in length in a greater ratio than they grow in other dimensions and their weight-length ratio differs from what obtains among larger individuals. Koundal (2014) also recorded similar findings in *C. punctatus*. Similarly, 'K' values were also observed to be higher (0.9259) in summer season (June 2014) and low (0.3370) in winter season (March 2014) for larger size which is also confirmed in similar study (Froese, 2006).

The analysis of regression expressed that both small (2.61 to 2.91 cm) and large groups (12.63 to 23.36 cm) observed allometric growth in 'b', though it was negative in previous case, and positive in the later. The value of 'b' was found to increase with increase in weight/age (Table 17). Similar recordings have also been made by Devraj (1973a), who suggested an exponent of 1.18 in length class <8 cm and 3.33 in length class >8 cm in natural stocks of this species in Bhavani Sagar. Excepting Rathod *et al.* (2011) who recorded low value of 'b' (1.45), others have shown values of 'b' near or above 3 from natural stocks collected in different part of the country in specimens larger than 20 cm (Khan *et al*., 2012; Froese, 2006; Koundal *et. al*., 2014). These results suggest that the species had grown in proportion to the length in captive conditions and the artificial diet given to the fish was well accepted and transformed to body indices. The present data also suggest that exponent remain unaffected with change in the season as no change in summer and winter season was noticed.

The rate of mortality in small size fish was so high that the overall biomass weight was even found lower than the stocked biomass. Therefore, smaller size fish require a different culture strategy than their larger counterpart. It may be possible that the palatability of the present diet may not be fully suitable for small-size fish. Hence, future research should focus separate strategy for developing diet for small-size group.

In 13-months culture period, the fry of *C. marulius* attained a length of 23.36±0.71 cm and weight of 117.68 g from that of 4.11±0.042 cm and 4.05±0.59 g respectively giving a net production of 5.48 kg (375.34 kg/ha/yr) at a gross survival of only 22.54%. Though the production was low but considering that the experiment was taken up in a small cement tank, where growth is always likely to suffer, there is need to take up similar study in larger earthen ponds with higher stocking densities along with the versatile method of grading the shooters.

The physico-chemical parameters of water were found in the range of temperature11.8-34.8 ^{o}C, pH 7.5-8.6, total hardness 210-230 mg L^{-1}, EC 580-630 µS $cm^{-1}$and DO 4.4-10.6 mg L^{-1} and were considered to be normal and suitable for this species in the climate of the experiment place.

It is, therefore, concluded that *C. marulius* could be successfully weaned on artificial diet for a commercial farming. The weaning on artificial diet not only gradually stopped cannibalism but also provided comparatively higher survival and better production in comparison to culture on the models of predator-prey system or extraneous feeding with fish and animals meat. The WLR indicated that the growth in terms of gain in weight and length was better than in open waters. Further research is needed to minimize the production of shooters at young stages by developing more attractive and palatable artificial feeds and modifying feeding strategy and also developing simple and cost-effective size grading methodology. Since the stocking density in the instant case was low, experiments with higher

densities in larger tanks/ponds also need to be carried out for improving production potential.

Table 15: Composition of egg custard and artificial diet

Egg Custard			
Sr. No.	**Ingredient**	**Percent composition**	**App. ratio (w/w)**
1.	Whole Hen's Egg	25.0	5
2.	Everyday milk powder	30.0	6
3.	Fish Meal	40.0	8
4.	Vitamin + mineral mixture*	5.0	1
Composition of Newtrients in feeds in (%)			
1.	Protein	50.20	
2.	Carbohydrate	20.10	
3.	Fat	9.00	
4.	Ash	5.65	
5.	Fibre	Nil	
Formulated Feed			
Sr. No.	Ingredient	Ratio (w/w)	
1.	Whole hen's egg	270	
2.	Fish meal	270	
3.	Soybean meal (extruded nuggets)	270	
4.	Rice Polish	90	
5.	Wheat flour	90	
6.	Vitamin + mineral mixture*	10	

*1. Cod Liver Oil, Manufacturer Universal Medicare Pvt. Ltd., Mumbai Batch No. R0109J; 2. Each kg of Vitamin and mineral mixture named 'Agrimin Forte' contains Vit. A 700000 IU, Vit. D_3 70000 IU, Vit. E 250mg, Nicotinamide 1000mg, Co 150mg, Cu 1200mg, I 325mg, Fe 1500mg, Mg 6000mg, Mn 1500mg, K 100mg, Se 10mg, Na 5.9mg, S 0.72%, Zn 9600mg, Ca 25.5%, P 12.75% Manufacturer: Brindavan Phosphates Pvt. Ltd, 48N, Doddaballpur Ind. Area, Doddaballapur – 561 203, India and marketed by Virbac Animal Health India Pvt. Ltd., Andheri-Kurla Road, Andheri, Mumbai-400 059, India. Batch No. BFA-611 September 2010.

Table 16: Month-wise growth and survival of *C. marulius* fed artificial diet

Months / Parameters	June 2012	July 2012	August 2012	September 2012	October 2012	November 2012	December 2012	March 2013	April 2013	May 2013	June 2013
Length (cm)	4.11± 0.042	5.38± 0.11	8.30± 0.34	12.63± 0.29	14.68± 0.24	15.1± 0.19	16.26± 0.31	16.78± 0.33	17.62± 0.32	21.13± 0.44	23.36± 0.71
Max	4.4	6.2	11.4	15.0	17.0	17.5	19.0	20.2	22.0	29.0	32.0
Min	3.8	4.7	5.4	9.5	13.0	14.0	14.5	14.5	15.00	19.0	19.0
Weight (g)	4.05± 0.0.59	5.15± 0.11	8.46± 0.38	14.96± 0.85	21.08± 1.23	26.96± 1.30	33.46± 1.95	35.04± 1.34	36.76± 1.31	73.28± 6.23	117.68± 10.43
Max	4.6	6.0	12.0	24.0	33.0	42.0	47.0	50.0	52.0	158.0	266.0
Min	3.6	4.2	4.8	6.0	14.0	20.0	20.0	23.0	27.0	45.0	68.0
Total number excluding shooters	244	156	101	79	73	65	59	59	57	56	55
Total number and weight (g) of shooter	-	26 (208)	15 (210)	2 (371)	5 (300)	1 (150)	Nil	Nil	Nil	Nil	Nil
Total biomass (g)/Net gain in biomass	988.68	803.08 (-185.60)	854.05 (-134.63)	1181.84 (193.16)	1538.84 (550.16)	1752.40 (763.72)	2001.28 (1012.60)	2060.28 (1071.60)	2079.36 (1090.68)	4103.68 (2024.32)	6472.40 (5483.77)
Gross survival (%)	100	63.93	41.39	32.38	29.91	26.63	24.18	24.18	23.36	22.95	22.54
Net survival (%)		63.93	64.74	78.22	92.41	89.04	90.77	100.00	96.61	98.25	98.21

± values are standard error of the mean, Gross survival is the percentage of fishes counting since beginning of the experiment and net survival is obtained by the number of fishes survived in the previous month.

Table 17: Growth indices of *C. marulius* fed artificial diet

Month / Parameter	June 2012	July 2012	August 2012	September 2012	October 2012	November 2012	December 2012	March 2013	April 2013	May 2013	June 2013
SGR (%)	-	0.3585	0.7184	0.8258	0.4964	0.3561	0.1042	0.0668	0.06937	0.9987	0.6857
R^2	0.888	0.6236	0.95945	0.7272	0.9459	0.8663	0.9021	0.6938	0.7670	0.7133	0.9395
(K)	5.173	3.3464	1.4861	0.7456	0.6642	0.7832	0.8080	0.3370	0.6705	0.7770	0.9259
a	0.058± 0.0010	0.034± 0.0016	0.016± 0.0013	0.007± 0.0003	0.006± 0.0012	0.007± 0.0001	0.007± 0.0001	0.007± 0.0002	0.006± 0.002	0.007± 0.0003	0.008± 0.0002
b	2.61± 0.003	2.73± 0.009	2.91± 0.017	3.10± 0.011	3.18± 0.010	3.21± 0.008	3.26± 0.013	3.28± 0.011	3.30± 0.010	3.44± 0.015	3.54± 0.022
WLR	$0.058L^{2.61}$	$0.034L^{2.73}$	$0.016L^{2.91}$	$0.007L^{3.10}$	$0.006L^{3.18}$	$0.007L^{3.21}$	$0.007L^{3.26}$	$0.007L^{3.28}$	$0.006L^{3.30}$	$0.007L^{3.44}$	$0.008L^{3.54}$

± values are standard error of mean, R^2= Coefficient of determination, K=Fulton's condition factor, a = intercept, b = slope, WLR= weight-length relationship

Fig 61: Cement tank used for grow-out of *C. marulius*

Fig 62: Sample hauls of *C. marulius* for measuring length and weight

Fig 63: Measuring length of *C. marulius* with a scale for LWR

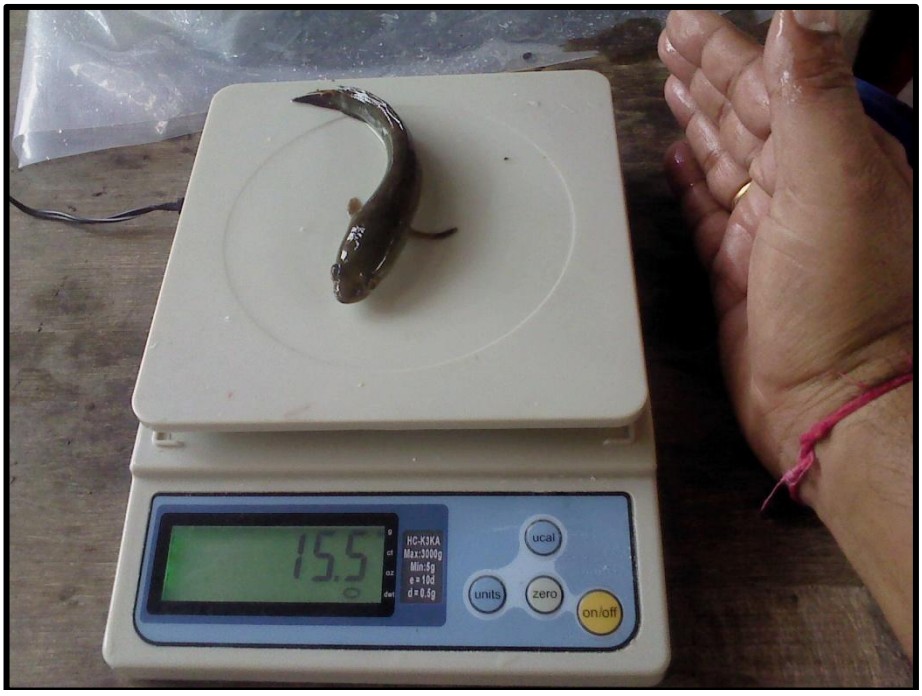


Fig 64: Measuring weight of *C. marulius* for LWR

Fig 65: Final harvesting size of *C. marulius* after 13 months rearing

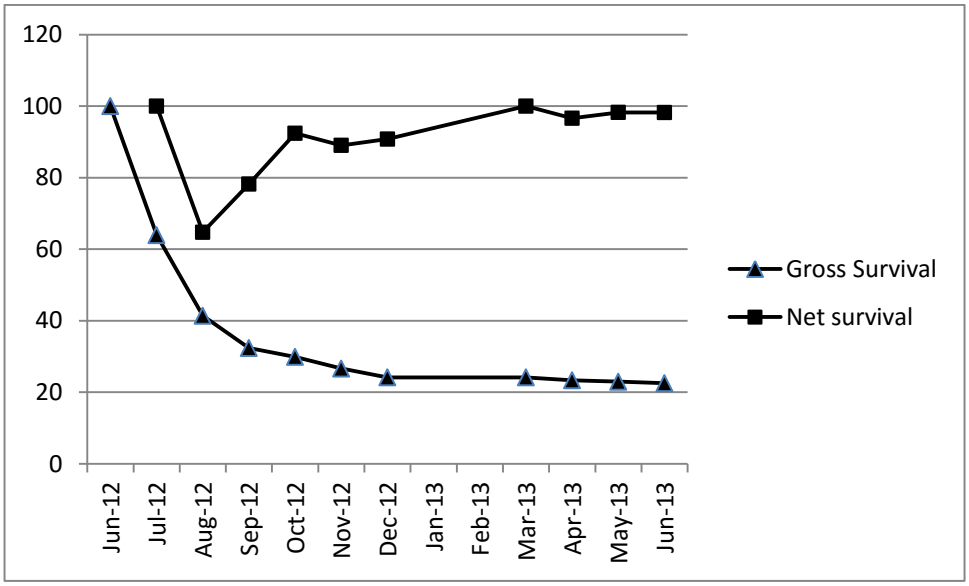


Figure 66: Gross and net survival of *C. marulius* on artificial diet during 12 months grow-out

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RESEARCH ARTICLE

Influence of Formulated Diet on Survival and Growth of Giant-snakehead, *Channa marulius* Reared in Pond Condition

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Abstract The fingerlings of giant-snakehead, *Channa marulius* were reared for 12-months in a cement tank on formulated diet to explore the possibility of their growth. The mortality was found higher during initial months but gradually stopped when the fish attained length and weight of over 15.10 ± 0.31 cm and 26.96 ± 1.30 g respectively. The cause of mortality was mainly corroborated with cannibalistic nature of the fish, leading to development of shooters which were found in higher numbers in the smaller size groups. Regular grading of shooters completely stopped their further production. The growth in terms of net biomass gain and SGR was highest during summer period, whereas it was negligible in winter. A strong correlation existed between weight and length relationship during entire culture period. Fulton's condition index was found size and season dependent. The smaller fishes observed negative exponent values; whereas larger sized were positive. The study suggests that this commercially important fish could be cultured on formulated diet when shooters are graded out at regular intervals in small size groups only.

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Introduction

Giant snakehead, *Channa marulius* (family Channidae) is the largest amongst 26 species of this genus. It represents an important food, ornamental and sport fish of India, Pakistan, China, Thailand and Cambodia [1, 2]. The fish is commonly found in rivers, canals, lakes, swamps, marshes and rice fields [3] and grows to a maximum length of 183 cm [4] and weight of 30 kg [5]. The snakeheads in general are in great demand as food fish due to their appealing flavor [6], few spines, medicinal importance and particularly their air-breathing nature [7] that facilitate their culture in oxygen-stressed ponds and easy transport in live condition. However in last few decades, the population of *C. marulius* has been drastically reduced due to several anthropogenic interventions [8]. Hence the species needs immediate conservation efforts and production demand through conservation by aquaculture practice [9].

Though commercial farming of *C. striatus* is popular in countries like Thailand, India, Philippines and Taiwan [7, 10], barring a few experimental trials, the farming of giant snakehead is not practiced due to poor understanding of their breeding, larval rearing and grow-out requirements. The major problem associated with snakehead culture is its predatory and cannibalistic behaviour that starts from the larval stage [11] and continues till adult stage consuming prey fish more than half to two–third of its length [11, 12].

Several studies have demonstrated that although cannibalistic aggression in fishes is difficult to be stopped even after feeding the fish to satiation level but it can be reduced by increasing natural food availability [12–15] or by

weaning the fish to accept formulated feed [12, 16]. Therefore, the purpose of the present study was to explore possibility of developing culture of *C. marulius* on formulated diet in pond conditions and to observe influence on survival and growth indices for developing strategies for its commercial production in the captivity.

Material and Methods

Procurement of Test Fish

The fry of *C. marulius* (length 2.96 ± 0.16 cm, weight 0.21 ± 0.02 g) were procured from the farm of NBFGR, Lucknow from a natural spawned stock. After thorough washing and disinfection with formalin (15 mg L^{-1}), they were reared for acclimatization in two FRP tanks of 1,125 litre (size 1.5 × 1.0 × 0.75 m) for 30 days. The fry were exclusively fed on zooplankton for first 3 days followed by a combination of zooplankton and egg custard for next 7 days, egg custard and formulated diet for next 10 days and finally only formulated diet for rest of 10 days.

Preparation of Grow-out Tank

A cement tank of dimension 14.2 m × 10.3 m × 1.2 m (L × W × H) was filled up with fresh ground water to a depth of 1.0 m (146 m^3) and manured with mustard oil cake (2 kg) and diammonium phosphate fertilizer (0.5 kg) 5 days before stocking for developing sufficient turbidity suitable for the grow-out of fish. The test fish of length 4.11 ± 0.042 cm and weight 4.05 ± 0.059 g numbering 244 were stocked in the tank after proper acclimatization. Around 30 % water of the tank was changed every month to control algal blooms. The tank was covered from top by a net to avoid bird predation.

Preparation and Application of Test Diets

The test feed was formulated on-farm using locally available feed ingredients (Table 1). All the ingredients were finely grounded, sieved and after hand mixing converted into hard dough by adding bore well water. The dough was then transferred into a steal container and cooked in a pressure cooker up to 15 min. The prepared semi-moist feed was stored in a refrigerator at 4 °C and used within 4–6 days. The feed was applied by cutting into small pieces and given once during morning (10.00–11.00 A.M.) at one side of the tank ad libitum.

Collection of Survival and Growth Data

The fishes were reared for 12 months from 15 June 2012 to 14 June 2013. The survival was estimated in the mid of every month by draining the tank entirely excepting during December 2012 to February 2013 when netting and dewatering was avoided due to severe cold climatic conditions when chances of mortality were higher due to temperature stress. The length data of 25 fishes was taken every month with the help of a measuring scale in centimeters and the weight by a digital balance with a precision of 0.01 g. The fishes were released back in the same tank when sufficient water was refilled in the tank. The shooters larger by 50 % in total length than that of average length of fish were removed after recording their length and weight and rest were restocked in the same tank.

Water Quality

Water samples were analyzed fortnightly for temperature, pH, total hardness, electrical conductivity (EC), and dissolved oxygen (DO) using multiparameter (model Orion 5 Star).

Data Analysis

The gross survival (GS), net survival (NS), net biomass (NB), specific growth rate (SGR), coefficient of determination (R^2), Fulton's condition factor (K) and regression analysis (WLR) were determined by the following formulae:

$$GS = 100 \times (n_t/n_0)$$

where, n_t is the number of fishes survived in a particular month and n_0 is the initial stocking number of fish at the commencement of the experiment.

$$NS = 100 \times (ns/np)$$

where, *ns* is the number of fishes survived in a subsequent month and *np* is the number of fishes in the preceding month.

$$NB = GB - IB$$

where, NB (g) is net biomass of all the surviving fishes excluding shooters in a particular month, GB is the gross biomass (g) of all the fishes in a particular month and IB is the measured initial biomass (g) of all the stocked fishes.

$$SGR = 100(\ln W_t - \ln W_0)/(t_2 - t_1)$$

where, SGR is specific growth rate; *Wt* is the total weight at time t_2, *Wo* is initial weight at the time (t_1).

$$R = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{\left[n\sum x^2 - (\sum x)^2\right]\left[n\sum y^2 - (\sum y)^2\right]}}$$

$$R^2 = \text{Square of R (Correlation coefficient)}$$

where, 'R' is correlation coefficient and 'R^2' is coefficient of determination, x mean length (cm) and y mean weight (g)

Table 1 Composition of egg custard and artificial diet

Egg custard			
Sr. no.	Ingredient	Percent composition	App. ratio (w/w)
1.	Whole hen's egg	25.0	5
2.	Everyday milk powder	30.0	6
3.	Fish meal	40.0	8
4.	Vitamin + mineral mixture*	5.0	1
Composition			
1.	Protein	50.20	
2.	Carbohydrate	20.10	
3.	Fat	9.00	
4.	Ash	5.65	
5.	Fibre	Nil	

Formulated feed		
Sr. no.	Ingredient	Ratio (w/w)
1.	Whole hen's egg	270
2.	Fish meal	270
3.	Soybean meal (extruded nuggets)	270
4.	Rice polish	90
5.	Wheat flour	90
6.	Vitamin + mineral mixture*	10

* 1. Cod Liver Oil, Manufacturer Universal Medicare Pvt. Ltd., Mumbai Batch No. R0109 J; 2. Each kg of Vitamin and mineral mixture named 'Agrimin Forte' contains Vit. A 700,000 IU, Vit. D_3 70,000 IU, Vit. E 250 mg, Nicotinamide 1,000 mg, Co 150 mg, Cu 1,200 mg, I 325 mg, Fe 1,500 mg, Mg 6,000 mg, Mn 1,500 mg, K 100 mg, Se 10 mg, Na 5.9 mg, S 0.72 %, Zn 9600 mg, Ca 25.5 %, P 12.75 % Manufacturer: Brindavan Phosphates Pvt. Ltd, 48 N, Doddaballpur Ind. Area, Doddaballapur—561 203, India and marketed by Virbac Animal Health India Pvt. Ltd., Andheri-Kurla Road, Andheri, Mumbai-400 059, India. Batch No. BFA-611 September 2010

$$K = 100W/L^3$$

where, W is weight in gram, L is length in centimeter

$$W = a\,L^b$$

The values of constant 'a' and 'b' were estimated from the equation of LeCren [17] by transforming log values of length and weight in the linear regression equation given below:

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

where, W is assumed weight of fish in gram, 'a' is intercept on y-axis and 'b' an exponent between 2 and 4 and 'L' is mean length of fish in cm.

Results and Discussion

Gross survival of 22.54 % was noticed when *C. marulius* was reared for 12 months on formulated diet in the present study. The survival was found to be low in small sized fish but gradually improved and stabilized when fish attained a mean length of 15.1 ± 0.19 cm and weight 26.96 ± 1.30 g. The net survival thereafter was almost over 90 % till completion of the experiment (Table 2, Fig. 1). The major cause of mortality in snakehead culture is attributed to its cannibalistic behaviour [7, 11], which is more prone in small sized groups in *C. striatus* [12] and the Atlantic cod (*Gadus morhua*) [18]. Though good amount of work on the culture of striped snakehead (*C. striatus*) has been carried out both in India and abroad but there is a dearth of such reporting with giant snakehead (*C. marulius*). Devraj [19] was the first to demonstrate experimental culture of *C. marulius* in cement tanks as well in earthen ponds at Bhavani Sagar in Tamil Nadu, India. He reported respective survivals of 19.3 % in 3-months and 1.3 % in 5-months with fingerling length of 5.0–7.9 cm and 10.5–15.5 cm when this species was reared in cement tanks and fed exclusively on fish liver. In another trial undertaken by him in earthen pond, adult specimens of 36.2–56.2 cm in length reared for 13 months gave a survival of 57.5 % when fed on prey fish. Though he cultured the fish on meat and forage fish being the natural food of the species, the survival in both the cases was very low with small size groups. Therefore, the present rates of survival of fish at different sizes were comparatively quiet high which could be corroborated to feeding the fish on formulated diet together with regular grading of the stock.

The formulated diets have shown significant reduction in cannibalism of siblings from that of 86 % to respective 60 and 35 % when applied @ 5 and 15 % bw in striped snakehead under experimental conditions [12]. The cannibalism in fish is generally related to genetics, larval behaviour [13] and difference in size within a cohort population in principle. On the other hand, larval behaviour is also governed by the environmental conditions, such as food availability, food type, nutritional composition of the food, population density, light intensity, refuge availability and water clarity [20]. Therefore, one of the most probable factor for reduction of cannibalism could be the supply of acceptable formulated diets of high nutritional quality to the satiation level in the system. Thus, supply of high nutritional diet of about 50 % CP fed to satiation level in the present study, could have met good amount of basic nutritional requirements of the fish.

Raizada et al. [21] in a 28 day study have demonstrated that *C. marulius* fry (length 4.11 ± 0.59 cm) could be easily weaned on semi-purified diet containing 54 % CP with high survival rates (>85 %). Based on authors'

Table 2 Month-wise growth and survival of *C. marulius* fed on artificial diet

Months/Parameters	June 2012	July 2012	August 2012	September 2012	October 2012	November 2012	December 2012	March 2013	April 2013	May 2013	June 2013
Length (cm)	4.11 ± 0.042	5.38 ± 0.11	8.30 ± 0.34	12.63 ± 0.29	14.68 ± 0.24	15.1 ± 0.19	16.26 ± 0.31	16.78 ± 0.33	17.62 ± 0.32	21.13 ± 0.44	23.36 ± 0.71
Max	4.4	6.2	11.4	15.0	17.0	17.5	19.0	20.2	22.0	29.0	32.0
Min	3.8	4.7	5.4	9.5	13.0	14.0	14.5	14.5	15.00	19.0	19.0
Weight (g)	4.05 ± 0.59	5.15 ± 0.11	8.46 ± 0.38	14.96 ± 0.85	21.08 ± 1.23	26.96 ± 1.30	33.46 ± 1.95	35.04 ± 1.34	36.76 ± 1.31	73.28 ± 6.23	117.68 ± 10.43
Max	4.6	6.0	12.0	24.0	33.0	42.0	47.0	50.0	52.0	158.0	266.0
Min	3.6	4.2	4.8	6.0	14.0	20.0	20.0	23.0	27.0	45.0	68.0
Total number excluding shooters	244	156	101	79	73	65	59	59	57	56	55
Total number and weight (g) of shooter	–	26(208)	15(210)	2 (371)	5 (300)	1 (150)	Nil	Nil	Nil	Nil	Nil
Total biomass (g)/net gain in biomass	988.68	803.08 (−185.60)	854.05 (−134.63)	1,181.84 (193.16)	1,538.84 (550.16)	1,752.40 (763.72)	2,001.28 (1,012.60)	2,060.28 (1,071.60)	2,079.36 (1,090.68)	4,103.68 (2,024.32)	6,472.40 (5,483.77)
Gross survival (%)	100	63.93	41.39	32.38	29.91	26.63	24.18	24.18	23.36	22.95	22.54
Net survival (%)		63.93	64.74	78.22	92.41	89.04	90.77	100.00	96.61	98.25	98.21

± Values are standard error of the mean, Gross survival is the percentage of fishes counted since beginning of the experiment and net survival is obtained by the number of fishes survived in the previous month

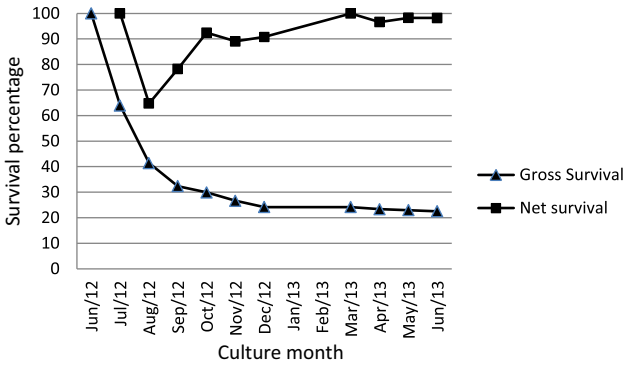

Fig. 1 Gross and net survival of *C. marulius* on formulated diet during 12 months culture period

previous experience, the fingerlings of *C. marulius* were reared on artificial diets containing approximately 50 % CP for 12 months to observe the influence of artificial diet on the survival and body indices of this fish in the present study.

As the number of shooters were highest (26) in the small size group of respective mean length and weight of 5.38 ± 0.11 cm and 5.15 ± 0.11 g, the net survival was also lowest (63.93 %), which markedly improved with the reduction of shooters subsequently with progress of culture period. This phenomenon confirmed that the survival of this fish has a direct relation with the cannibalistic nature of the fish.

The weight-length data (Table 1, 2) indicated that both weight and length tend to increase gradually and reached from a mere weight and length of 4.05 ± 0.59 g and 4.11 ± 0.042 cm in June 2012 to 117.68 ± 10.43 g and 23.36 ± 0.71 cm in June 2013, though the rate of growth was very slow from November 2012 to April 2013 when winter sets in reducing the tank water temperature to as low as 11.8 °C. The length data also suggest that there was comparatively little difference in coefficient of variation (less than 5 %) during different months in the restocked population (after removal of shooters) with a maximum of 4.09 % in August 2012 and a minimum of 1.02 % in June 2012 which indicated good method performance.

The SGR values were found to improve gradually during July (0.3585 %) to September 2012 (0.8258 %) when water temperature was higher due to summer season but subsequently decreased from October 2012 (0.4964 %) to April 2013 (0.06937 %) due to winter but improved again during May–June 2013 (Table 3). Thus the rate of SGR was found directly proportional to the water temperature. Quin and Fast [15] and Vivekanandan and Pandian [22] also reported significantly higher weight gain at high temperature (27 °C) than at low temperature (17 °C) in case of *C. striatus* though higher temperature of 27 °C did not significantly affect the survival with those reared at 21 °C.

Table 3 Growth indices of *C. marulius* fed on artificial diet

Month/ Parameter	June 2012	July 2012	August 2012	September 2012	October 2012	November 2012	December 2012	March 2013	April 2013	May 2013	June 2013
SGR (%)	–	0.3585	0.7184	0.8258	0.4964	0.3561	0.1042	0.0668	0.06937	0.9987	0.6857
R^2	0.888	0.6236	0.95945	0.7272	0.9459	0.8663	0.9021	0.6938	0.7670	0.7133	0.9395
(K)	5.173	3.3464	1.4861	0.7456	0.6642	0.7832	0.8080	0.3370	0.6705	0.7770	0.9259
a	0.058 ±0.0010	0.034 ±0.0016	0.016 ±0.0013	0.007 ±0.0003	0.006 ±0.0012	0.007 ±0.0001	0.007 ±0.0001	0.007 ±0.0002	0.006 ±0.002	0.007 ±0.0003	0.008 ±0.0002
b	2.61 ±0.003	2.73 ±0.009	2.91 ±0.017	3.10 ±0.011	3.18 ±0.010	3.21 ±0.008	3.26 ±0.013	3.28 ±0.011	3.30 ±0.010	3.44 ±0.015	3.54 ±0.022
WLR	$0.058L^{2.61}$	$0.034L^{2.73}$	$0.016L^{2.91}$	$0.007L^{3.10}$	$0.006L^{3.18}$	$0.007L^{3.21}$	$0.007L^{3.26}$	$0.007L^{3.28}$	$0.006L^{3.30}$	$0.007L^{3.44}$	$0.008L^{3.54}$

R^2 coefficient of determination, *K* Fulton's condition factor, *a* intercept, *b* slope, *WLR* weight-length relationship

± Values are standard error of mean

A strong correlation (R^2) existed between WLR all through the culture period and no effect due to the climatic conditions was observed (Table 3). Therefore, it was revealed that both weight and length increases with the same pace in different climatic conditions. The values of correlation coefficient obtained in the present study were more or less the same as observed in natural stock of *C. marulius* in India [23–28]. Therefore, it could be interpreted that this fish maintains more or less same WLR both in natural and captive conditions when fed on formulated diet.

The 'K' values were both size and season dependent. Small fishes (4.11 ± 0.042 cm) in comparison to large fishes (23.36 ± 0.71 cm) observed respective 'K' values of 5.173 and 0.9259 (Table 3). Froese [29] noted that smaller fish grow in length in a greater ratio than they grow in other dimensions and their weight-length ratio differs from what is seen among larger individuals. Koundal [30] also recorded similar findings in *C. punctatus*. Similarly, 'K' values were also observed to be higher (0.9259) in summer season (June 2013) and low (0.3370) in winter (March 2013) for larger size which is also confirmed in similar study [29].

The analysis of regression expressed that both small (4.11–8.30 cm) and large groups (12.63–23.36 cm) observed allometric growth in 'b', though it was negative (<3) in previous case, and positive (>3) in the later. The value of 'b' was found to increase with increase in weight/age (Table 3). Similar recordings have also been made by Devraj [24], who suggested an exponent of 1.18 in length class <8 cm and 3.33 in length class >8 cm in natural stocks of this species in Bhavani Sagar. Excepting Rathod et al. [27] who recorded low value of 'b' (1.45), others have shown values of 'b' near or above 3 from natural stocks collected in different part of the country in specimens larger than 20 cm [26, 28]. These results suggest that the species had grown in proportion to the length in captive conditions and the artificial diet given to the fish was well accepted and transformed to body indices. The present data also suggest that exponent remain unaffected with change in the season as no change in summer and winter season was noticed.

The rate of mortality in small size fish (up to August 2012) was so high that the overall biomass was even found less than the initial biomass which improved thereafter. Therefore, smaller size fish may require a different culture strategy than their larger counterparts. It may be possible that the palatability of the present diet may not be fully meeting the acceptable nutritional requirements for small-size fish. Hence, future research should focus separate strategy for developing diet for small-size group.

In 12-months culture period, the fry of *C. marulius* attained a length of 23.36 ± 0.71 cm and weight of

117.68 g from that of 4.11 ± 0.042 cm and 4.05 ± 0.59 g respectively giving a net production of 5.48 kg (375.34 kg/ha/yr) at a gross survival of only 22.54 %. Though the production was low but considering that the experiment was taken up in a small cement tank, where growth is always likely to be low, there is a need to take up similar study in larger earthen ponds with higher stocking densities along with the development of versatile method of grading the shooters.

Water Quality

The physico-chemical parameters of water were registered in the range of temperature 11.8–34.8 °C, pH 7.5–8.6, total hardness 210-230 mg L^{-1}, EC 580–630 μS cm^{-1} and DO 4.4–10.6 mg L^{-1} and were considered to be normal and suitable for this species in the present climatic conditions.

Conclusion

It is, therefore, concluded that *C. marulius* could be weaned on artificial diet for commercial farming. The weaning on formulated diet together with grading of shooters not only gradually stopped cannibalism but also provided comparatively higher survival and better production in comparison to culture on the models of predator–prey system or extraneous feeding with fish and animal meat. The WLR indicated that the growth in terms of gain in weight and length was better than in open waters. Further research is needed to minimize the production of shooters at young stages by developing more attractive and palatable artificial feeds, modifying feeding strategy and also developing simple and cost-effective size grading methodology. Since the stocking density in the instant case was low, experiments with higher densities in larger tanks/ponds also need to be carried out for improving production potential.

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STUDY OF GONADO-SOMATIC INDEX OF MALE AND FEMALE GIANT SNAKEHEAD FISH, *CHANNA MARULIUS* (HAMILTON, 1822)

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ABSTRACT : Conservation and aquaculture of *Channa marulius* have much importance for present scenario because population of this fish species is declining by several anthropogenic activities. GSI is the important part of reproductive biology as it has direct bearing on breeding mechanism and seed production. This study expresses the relative change in gonad weight in term of gonad somatic index to the percentage of body weight. Present study reports the changes in GSI of *Channa marulius* in and around Lucknow (Uttar Pradesh, India) during January to April. GSI increased with gonadal development and maturation of this fish. In females, the minimum GSI value (0.184 ±0.010) was observed in January (during pre-spawning period) while maximum (2.556±0.233) value during April at peak maturation period while in male minimum value (0.038±0.029) in January and (0.080 ± 0.009) maximum in April.

Key words : Gonado-somatic index, maturity, *Channa maruliu*, Lucknow.

INTRODUCTION

Channa marulius is a largest fish with 183 cm length (Shrestha, 1990) and 32 kg weight (Talwar and Jhingran, 1991) of the family Channidae so it is called giant snakehead. They are found in river, canals swamp marshes, pond, lake and reservoir *etc.* (Kilambi, 1986). *Channa marulius* have high market prize with good nutritive value, delicious taste and less muscular spins with air-breathing nature (Wee, 1982). Due to environmental changes, anthropogenic activities and high exploration rate, its population is declining tremendously from natural water bodies. This fish need conservation immediately by induced reproduction and aquaculture. Reproductive capacity depends upon gonado-somatic index (GSI) (Gogoi and Biswas, 2015). Scientific study of GSI helps in understanding breeding mechanism for aquaculture production of fish and their conservation. This study of gonado-somatic index expresses the relative change in gonad weight to the percentage of body weight.

MATERIALS AND METHODS

Specimen fishes of the research work were collected from local ponds connected to Gomti river near Lucknow. Specimens of different in size and weight were collected during different time interval from January to April 2015 and were in various maturity stages. Total 53 fish specimens were collected during the study and sex identification done by examination of morphology and anatomy of gonads. The collected fishes were transported to the Fish Reproductive Laboratory, ICAR-NBFGR, Lucknow. Fish weights were taken by a digital balance with minimum capacity 5 gm. After the weight, fishes were dissected out for obtaining gonads. The weight of gonad were taken by another digital balance with 0.05 gm minimum capacity and fixed in 5% formalin solution for further study. Weight of sample fish and their gonad were taken at monthly intervals. Later (GSI) % of gonads weight in relation to the total body weight was calculated by using the following formula:

$$\text{Gonado-somatic index (GSI)} = \frac{\text{Weight of gonad}}{\text{Weight of fish}} \times 100$$

GSI of *Channa marulius* was calculated in relation of fish weight with gonads weight. Gonado-somatic index of the fish increased with maturation and maximum values were recorded during peak period of maturity (April) and declined after spawning.

RESULTS AND DISCUSSION

The GSI of *Channa marulius* in Lucknow area estimated monthly during study period from January to April 2015 in male and female. The maturity and peak breeding season were determined by the GSI study. The value of female GSI value expressed in Table 1 and male and value expressed in Table 2. The GSI values increased from January to April. March and April, the peak period for spawning. February-April was the vittelogenic period or active cum quiescent period. GSI value was minimum at January during prespawning period (0.184±0.010) and maximum at peak maturation period during April

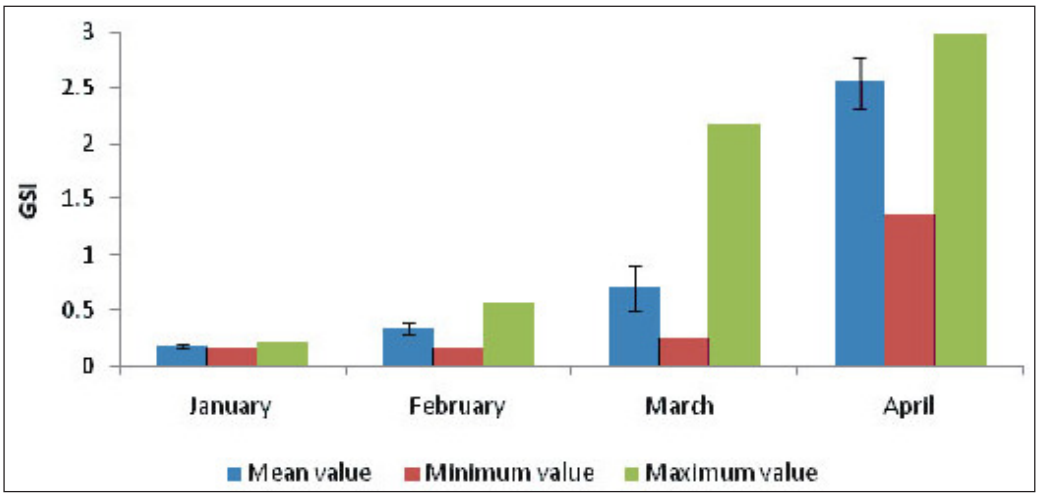

Fig. 1 : GSI values of female *C. marulius* during different months.

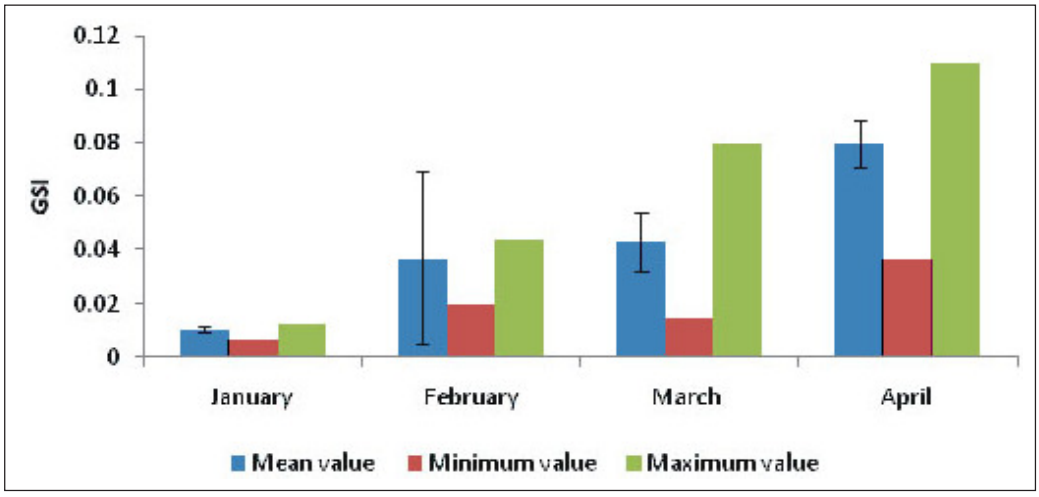

Fig. 2 : GSI values of male *C. marulius* during different months.

Table 1 : Gonado-somatic index of female *C. marulius* during January-April.

Month	Number of specimens	Mean±SEM	Minimum	Maximum	95% Confidence interval for mean	
					Lower bound	Upper bound
January	4	0.184±0.010	0.17	0.21	0.151	0.217
February	9	0.340±0.051	0.16	0.58	0.221	0.458
March	9	0.705±0.199	0.25	2.17	0.246	1.163
April	9	2.556±0.233	1.37	3.69	2.019	3.092

Table 2 : Gonadosomatic index of male *C. marulius* during January-April.

Month	Number of specimens	Mean±SEM	Minimum	Maximum	95% Confidence interval for mean	
					Lower bound	Upper bound
January	4	0.038±0.029	0.006	0.127	-0.05449	0.132441
February	4	0.037±0.032	0.002	0.044	0.024065	0.047135
March	6	0.043±0.011	0.015	0.080	0.013292	0.073375
April	8	0.080±0.009	0.037	0.110	0.058497	0.102253

(2.556±0.233) in female (Fig.1) and (0.038±0.029) minimum and (0.080±0.009) maximum in male (Fig. 2). *Channa marulius* spawn during April-May spawning season. Similar results were shown by Gaikwad *et al* (2009) in *Channa punctatus*. Narejo *et al* (2015) obtained about similar results in *Channa striatus*. They observed GSI 0.20-2.20 in male and 4.50-8.00 in female. According to Ghanbahadur *et al* (2013) fecundity increases from February to May in *Channa gachua*. Nandikeswari and Anandan (2013) reported that during March high fecundity indicate the maturation period of the fish. Kapil *et al* (2011) reported that the highest GSI of ovaries was 0.775 and minimum was 0.285 while highest GSI of testes in male was 0.74 and minimum is 0.42 average GSI about (0.62. This study had given same result as the present study. Tiwari *et al* (2014) reported that the highest value of GSI in *Channa marulius* was found during April-May.

CONCLUSION

The findings of this study related to gonado-somatic index of *Channa marulius* have shown positive response with the gonadal maturation. Highest GSI was recorded during March-April (2.556±0.233) in female and (0.080±0.009) in male. Further study in this direction will help to find out more detail information about reproductive biology of *Channa marulius*. Findings of the preset study will help in hormonal manipulation for seed production of the candiadate species for diversification of aquaculture and better conservation in natural water bodies.

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