

**A STUDY ON SEXUAL DIMORPHISM AND
REPRODUCTIVE CYCLE IN SOME
ORNAMENTAL BARBS OF CENTRAL KERALA**

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DECLARATION

I hereby declare that the thesis entitled “A study on sexual dimorphism and the reproductive cycle in some ornamental barbs of central Kerala ” is the result of investigations carried out by me in the Research and Post graduate Department of Zoology, Christ college, Irinjalakuda, Calicut University under the supervision and guidance of Dr. N.D. Inasu , Prof.& Head, Department of Zoology, Christ College, Irinjalakuda, and has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles or recognition.

Irinjalakuda,

April, 2009.

JEEJA THARAKAN

CERTIFICATE

This is to certify that the thesis entitled “A study on sexual dimorphism and the reproductive cycle in some ornamental barbs of central Kerala” is the bonafide record of the work carried out by **JEEJA THARAKAN** under my guidance and supervision in the Zoology Department, Christ College, Irinjalakuda, University of Calicut and that no part thereof has been presented for any other degree.

Irinjalakuda,
April, 2009.

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CHAPTER - 1

INTRODUCTION

CHAPTER – 1

INTRODUCTION

Water is the essential and basic resource of fish life on earth .The state of Kerala, situated on the south west coast of India, abounds with extensive inland water spreads which are highly rich in fishery potentialities suitable for fish culture. In spite of having immense scope and potential for the development of culture as well as capture fisheries in the state, the yield from these water bodies are far below optimum level. The principal constraint facing the inland fisheries is the under utilization of the existing water bodies. It has therefore become imperative to evolve a master plan for development of the inland water fisheries and careful management of the available inland water resources. The scientific management of the aquatic resources involves various inter related aspects such as the physical, chemical and biological characteristics of the environment, manipulation of the living stock and proper management techniques for exploitation. A thorough knowledge of these fundamental aspects is a pre requisite before tackling the problems of a virgin aquatic environment. An ecological approach to the management of the aquatic resources is a pre requisite for the effective utilization of the available waters suited for fish culture.

However, with the increasing demand for fish as a source to cater the ever-increasing protein requirements of the human being and also to meet the emergency demand for the indigenous fish in the ornamental fish trade industry in recent years, studies on fresh water fish are gaining momentum in Kerala and other states of India. According to Kurup (2002) of the 175 species of fish collected from the rivers and streams of Kerala, 67 species are food fish and 106 species are having all the desirable qualities for propagating as ornamental fish. The contribution of Kerala to the inter national fish trade is almost negligible at present when the turn out from the world ornamental fish trade is estimated to be approximately U.S. \$ 4.5 billion .With judicious tapping of the freshwater fishery resources, Kerala could become one of the leading states in India in ornamental fish trade, thereby generating employment opportunities and increasing export earning considerably.

Sustainable utilization of fishery resources calls for rational exploitation of the resources together with the implementation of appropriate conservation and rehabilitation programs. A scientific database on resource characteristics and bionomics of fish is indispensable for any program designed for the management and conservation of fish species of commercial importance. The primary need for selecting a fish for culture is the availability of knowledge on different aspects of life cycle of the species that are of major significance for successful culture operations. A study on reproductive characters is essential in the determination of population stock, size from egg numbers, periodicity of strength of broods (year class recruitment), spawning time and place and sex composition of exploited stock. Reproductive parameters are of great value in fishery predictions and formations of management resources

The important freshwater fishes of Kerala belongs to the following families ., Cyprinidae, Claridae, Siluridae, Bagridae, Anguillidae, Belonidae, Ambassidae, Cichlidae, Gobidae, Channidae, Anabantidae and Mastacembelidae. In India, the Cyprinidae including the carps and carp minnows, form 35.2 % of the fresh water catches. Among the major carps the well known forms such as Catla (*Catla catla*) Rohu (*Labeo rohita*) Calbasu (*Labeo calbasu*) Mrigal (*Cirrhina cirrnosus*) are used extensively for fresh water culture operations in almost all the states in India. In addition to such major carps the cyprinids include a number of species under the genus *Puntius* (Barbus) which occupy an important place among the fresh water fishes and are encountered in all kinds of fresh water habitats. Most of the members belonging to the genus *Puntius* are attractive, colourful and have a tremendous potential for development as candidates for the international ornamental fish market, which has yet to be exploited. The large scaled barb such as *Puntius curmuca*, grow to a very large size and form excellent table fish. The still smaller species like *Puntius stigma* , *Puntius ticto*, and *Puntius vittatus* which have high fecundity are cultured in many parts of India as forage fish and used as food in the culture of predatory fishes like Murrels (*Channa* species) and catfishes (Clarids and Silurids). Majority of *Puntius* species are smaller in size but they compensate for this by their abundance. Some species are considered as effective larvicidal fishes and also widely used as experimental laboratory animals.

A thorough knowledge on maturation cycle and depletion of gonads is essential for effective fishery management (Biswas *et al.*, 1984).Determination of maturity stages finds primary application in providing basic knowledge on the reproductive biology of a

stock. Information derived from these analysis is useful in ascertaining the age and size at which a fish attains sexual maturity, the time and place of spawning and duration of the cycle from the beginning of development of the ovary to the actual release of the eggs. In addition, to enable an estimation of fecundity, this information can be used to calculate the size of a stock and its reproductive potential. All these studies are essentially meant for elucidating both the short and long-term variations in the production of fish broods, which are finally recruited in the population as exploitable stocks. . Gonadal development in fish is governed by a number of biotic and abiotic factors of the environment (De Vlaming *et al*, 1972). Any alteration in the environmental conditions may affect the gonadal development process. Information relating to population stability and year class fluctuations can be obtained by undertaking studies on reproductive biology. Variations in the production from year to year may be due to these fluctuations.

SCOPE OF THE PRESENT WORK

The shining live fish is an attraction for people all over the world. It has been proved that maintaining a well-set aquarium gives peace of mind and act as a stress reliever. However, the scope of this sector and the impact on human and aquatic communities are often inaccurately known and unappreciated. Statistics reported to FAO from member States indicate that the world export value in 2006 of ornamental fish was U.S. \$ 698 million. Such a vast and important industry has the potential to contribute to the economic growth of states concerned and the sustainable development of aquatic resources.

With the decline in production from many capture fisheries., people are looking for other ways of utilizing aquatic biodiversity. One useful option is the sustainable harvest and culture of ornamental fishes. In many developing countries the harvest of fresh and marine ornamental fish provides income in areas where little other options exist for employment. Popularity of tropical ornamental fishes is now days attain a higher degree. Most tropical fish are smaller, daintier and more colourful. They are accustomed to warmish water. The warmth speeds up metabolism, making them more active.

The Indian fresh water ornamental fish have great demand in U.S.A. and other European countries because they have great ability to adapt and acclimatize to an entirely new environment. And also these are found to respond very well to the artificial feeds. It is rather unfortunate that these valuable fish are at present used as food fishes for the local population fetching a low price. However when the same is exported one fish could get many times. If we organize the ornamental fish farming and exporting properly, besides generating foreign exchange, it would create job opportunities and self employment to the rural population.

The three freshwater species undertaken for the present study were *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.) which have all the desirable qualities of ornamental fishes. They are smaller, daintier and more colourful. All these three species need less of oxygen, and more active inside the aquarium and they react very well to the artificial feeds. For technologically and economically viable culture practice, selection of the candidates species is equally or more important than any of the

environmental factors and culture system management. In order to achieve the maximum production from a well managed culture system it is highly imperative to have a deep knowledge on the reproductive biology of the species cultured. In this thesis an attempt is made to bring out all the important biological aspects of sexual cycle of *Puntius amphibius* (Val), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.).

Geographical Position and Physical Features of the Study Area

The geographical position and effects of environmental parameters greatly influence the nature and pattern of population of a locality. The importance of such information is immense in studying the biological aspects of any fishery species. The present study was conducted at Chalakudy River, a part of which is flowing through Thrissur and Ernakulam, the central districts of Kerala.

Kerala is a monsoonal area. This area experiences a northeast monsoon from October to December (Thulavarsham) and the strong and steady southwest monsoon from June to September (Edavapathi) and have an inter-monsoon during April- May. The present study area of Chalakudy River basin experiences more than 3000 mm rainfall. The steady and seasonal availability of the two monsoons is a critical factor for the water availability.

Chalakudy River is the fifth longest river in Kerala (100 05' to 100 35' North latitude and 76 15' to 76 55' East longitude) having a length of 145 km occupies a central position among the rivers of Kerala. The total drainage area is 1704 sq km out of which 1404 sq

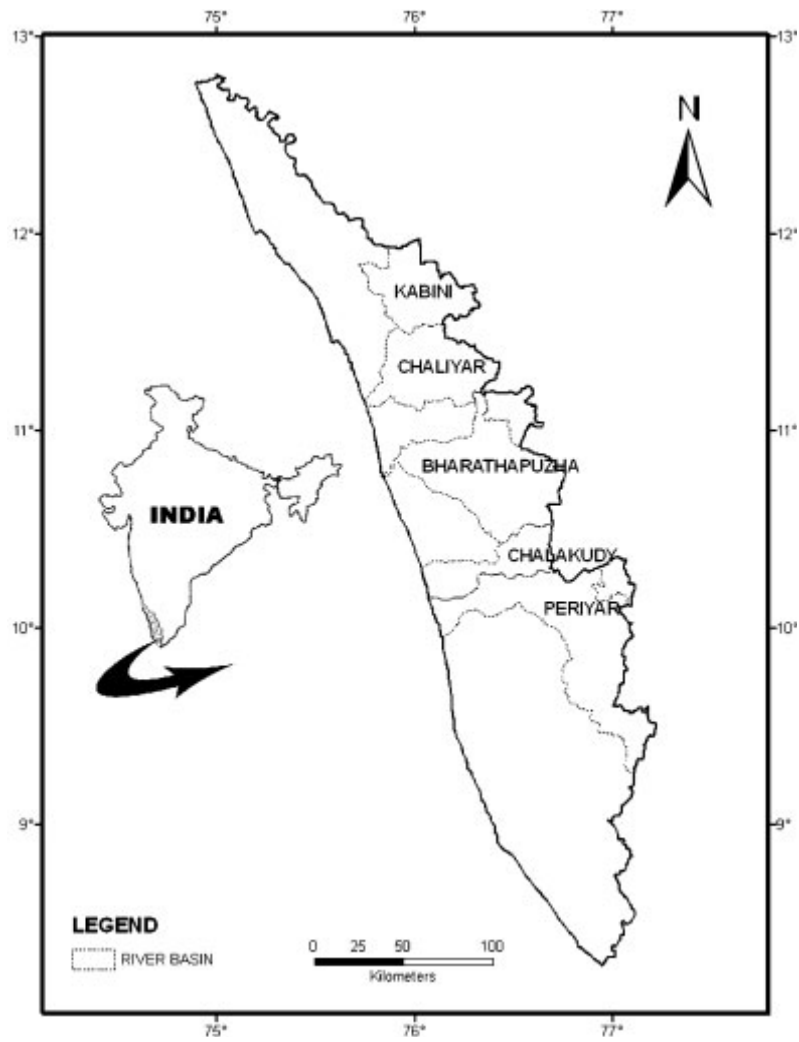
km is in Kerala and the rest 300 sq km in Coimbatore district of Tamil Nadu state. It originates from the Anamala and Nelliampathy ranges of the Western Ghats. It flows westward through the heart of the Kerala state, i.e., through the central districts Palakkad, Thrissur and Eranakulam. A major portion lies in the Thrissur district. When coming to the plains it joins with the northern distributaries of Periyar at Elanthikkara just 9 km before they together end in the Arabian Sea at Kodungallur estuary. (Amitha bachan, 2003).

Streams and rivers in the Kerala, part of the Western Ghats are an exceptional hotspot of freshwater fish diversity, having an immensely rich and diverse ichthyofauna of around 207 species, including food, ornamental and sport fishes. Among these, the native ornamental fishes form the most important component of the regional biodiversity from an eco-biological and socio-economic perspective. Annual report (2005) of the National Bureau of Fish Genetic Resources, Lucknow, mentioned that the Chalakudy River is the richest river in fish diversity, perhaps in India. A detailed analysis by Ajith Kumar *et al* (1999) reports 98 species of fishes of which 5 species are new to science. Sixtyone species were found in low elevation areas of < 75 m , 68 species in the midlands, 75- 500 m, 36 species in the highland (500 - 750m) and 14 species from the high range areas >750m. Out of the 98 species 36 are endemic to the Western Ghats, 10 are endemic to Kerala part of the Western Ghats. Among the 98 species listed, 27 were identified as good edible fishes and most of the fishes have great ornamental value.

The present study was carried out at the lowest zone of Chalakkudy River. The fish specimens were collected from Mambra, Annamanada, Kundoor, and Kanakkankadavu

regions of the river. In this area the river is flowing as a single channel and do not have any major tributaries and obstructions. This zone has a length of 43 km along the main river and the altitude difference is 50m. Slope is very less when compared to other zones of the river. The riverbed is sandy in the down stream area. Large deposits of sand were noticed in this zone.

Cast net (Veesuvala) is used to collect the specimens from the river. It is a circular net webbing attached to a centrally located hauling line. There are a number of lead sinkers along the circumference of the net. During operation the circumference forms inwardly directed purse like structure or pockets which prevent the fish from escaping. On the basis of mesh size two types of cast nets were used. They were (a) net with smaller mesh size locally called as 'Pattuvala' and (b) net with larger mesh size locally called as 'Thalivala'. Depending upon the mesh size almost all type of fishes can be caught by cast nets.



Chalakudy river

Review of the important previous works on Different species of Cyprinidae

Notable studies on the freshwater fish fauna of Kerala were those of Day (1878, 1889), John (1936) Hora and Law (1941), Silas (1951), Remadevi and Indra (1986), Pethiyagoda and Kottelat (1994), Kurup (1994), Inasu, 1991, Easa and Shaji (1995), Menon and Jacob (1996), Manimekalan and Das (1998), Ajithkumar *et al.* (1999) and Raju *et al.* (1999). The breeding biology of the major carps, among the cyprinids has received great attention, where as the minor carps remain comparatively neglected. Reproductive biology of a number of species belong to **Cyprinidae** were worked out as in *Labeo calbasu*, (Khan, 1954., Rao and Rao ,1972., Pathak and Jhingran, 1977., Vinci and Sugunan ,1981), *Labeo gonius*, (Joshi and Khanna,1980) *Labeo rohita* (Khan and Jhingran, 1975., Varghese, 1973), *Labeo bata* (Alikunhi, 1956., Siddiqui *et al.* ,1976), *Labeo fimbriatus* (Bhatnagar ,1972), *Labeo dusmieri* (Kurup, 1994). *Cirrhinus mrigala* (Khan, 1954., Chakrabarty and Singh, 1967) *Cyprinus carpio* (Parameswaran *et al.* ,1972), *Garra mullya* (Somavanshi, 1985), *Crossocheilus latiusdiplocheilus* (Kaul,1994), *Osteobrama cotio* (Parameswaran *et al.*,1971), *Tor tor* (Chaturvedi, 1976) ,*Noemacheilus triangularis* (Ritakumari and Nair, 1979), *Coilia dussumieri*, (Bal and Joshi 1956) and *Schizothorax longipinnis* (Sunder, 1986).

The important works on reproductive biology of fishes belong to the genus **Puntius** were done in *Puntius stigma* (Ibrahim, 1957), *Puntius dorsalis* (Sivakami, 1982),

Puntius vittatus (Ibrahim, 1957), *Puntius titteya* (Sundarabarathy *et al.* 2004), *Puntius lateristriga* (Sutin *et al.* 2008), *Puntius bimaculatus* (De Siiva and Kortmulder K. 1977), *Puntius sarana* (Sinha, 1975., Ganapathy and Dutt 1989., Murthy V.S, 1975., Kumar and Siddiqui, 1991., Mustafa *et al.*, 1982., Kumari N.M and Dutt N. H, 1990), *Puntius ticto* (Manissery J. K *et al.* 1979., Kapoor, 1976), *Puntius stigma* (Islam and Hossain, 1990) *Puntius sophore* (Bhuiyan and Parween, 1998) and in *Puntius cumingii* (J. Chandrasomav *et al.*, 1994). There were very few works done on the biology of *Puntius amphibius* (Val), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.). The mentionable works were those of, Observation on the maturation and spawning of *Puntius sarana subnasutus* in Veli lake done by Sobhana and Nair (1976), A dietary effect of protein on growth, feed utilization and body composition in *Puntius parrah* by Bindhu *et al.* (2002), Breeding biology of the scarlet banded barb, *Puntius amphibius* from Chackai Canal by Premkumar and Balasubramanian (1984), Length weight relation ship in *Puntius amphibius* by Premkumar *et al.* (1984), and Observations on the comparative efficiency of the allochthonous and autochthonous food of fishes based on their biochemical analysis were by Premkumar and John (1986). Nevertheless, detailed studies on the breeding habits and reproductive cycle of species such *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.) have not been attempted except for some general observations.

This paucity of knowledge on this species having immense ornamental potential thus has prompted to under take the present investigation on *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.). In order to unravel the reproductive

characteristics of the species during the periods of study from April 2003 to March 2005, the following aspects were dealt with:-

1. Length-weight relationships and condition factor
2. Biochemical variations during reproductive cycle..
3. Sexual dimorphism
4. Sex ratio
5. Length at first maturity
6. Gonado somatic index
7. Fecundity
8. Spawning season

CHAPTER - 2
SYSTEMATICS

CHAPTER - 2

SYSTEMATICS

Fish can be defined as aquatic poikilotherm vertebrate that has gills throughout life and limbs, if any, in the shape of fins. Fishes constitute the major half of the whole vertebrates, with 21723 living species (Jayaram, 1999). Of these 8411 are fresh water inhabitants. Fishes are present in every aquatic habitat on earth from lakes, rivers, caves, and hot fresh water pools (44°C) to polar sea (-2°C), tropical reefs and the deepest ocean bottoms. The inland waters of Indian sub continent holds about 930 fish species.

Classification of fishes for scientific study is done through taxonomy or systematics. Taxonomy of fishes is a tool for the identification of species, there by providing the fishery workers in country with an aid, which will allow continued improvement of precision in future data gathering and analysis .Not only that, for the harvest of these aquatic resources, a scientific understanding of the fish species with respect to their morphological, biological and adaptive characters along with their natural distribution is imperative to back up their optimum exploitation. In investigations of fishery biology and distribution of concerned species, the correct identification of fish is an essential pre-requisite. Systematics is the study of organisms, their diversity and interrelationship with the aim of arranging them in an orderly manner. A detailed analysis of the morphometry is very useful in eliminating taxonomic ambiguity.

A detailed account of the fishes of the Indian region was published by Francis Day in 1889. The other major contributors in the field of taxonomy are Munro(1955), Misra (1976), Jayaram (1981), Datta Munshi and Srivastava (1988), and Talwar and Jhingran (1991).

The group of barbs belongs to the family “**Cyprinidae**”. This is the largest family of fishes and comprising about 194 genera and 2070 species (Nelson, 1994). Barbs belong to the subfamily “Cyprininae”. This subfamily consists of fishes which are economically important as both food fishes as well as ornamental fishes. The present study gives an accounts of the taxonomy of *Puntius amphibius*, *Puntius parrah* and *Puntius sarana subnasutus*.

MATERIALS AND METHODS

The fishes of the present study belonging to the family “Cyprinidae” were collected from different spots like Mambra, Annamanada, Kundoor and Kanakkankadavu along the lower zone of Chalakudy river during the period from April 2003 to March 2005. Immediately after collection they were put in ice box and brought to the laboratory. After careful washing, the specimens were kept on a plywood board to spread out the dorsal, anal and caudal fin by using small pins. With the help of a camel brush a little concentrated formalin was applied on the fins and allowed to remain for a few minutes to harden these structures. Then these fishes were preserved in 5 % formalin for the detailed

study of morphometric and meristic characters. Details of colouration in the fresh specimens were noted. In the laboratory the specimens were carefully washed and the following measurements were taken. Then the collected fishes were identified up to the species level with the help of best and recently available identification keys. The following measurements of all the collected fishes were taken.

Total length: The greatest distance between the most anterior projected parts of head to the posterior most tip of caudal fin including filamentous prolongation.

Standard Length: Distance from the tip of the snout to the end of the vertebral column.

Maximum Body Depth: Vertical measurement from a point in the body of the fish on its back where its height is greatest to a straight line to the ventral surface or profile.

Head Length: Taken from the tip of the snout to the most point reached by the fleshy margin of the opercle.

Pre-dorsal Length: Measurement from the tip of the snout or upper lip or anterior most part of the head to the structural base of the first dorsal fin ray.

Caudal Peduncle Length: Distance from the last point of anal fin posterior to the end of vertebral column.

Snout to Anal fin length: Length from the tip of the snout to the basal part of the beginning of the anal fin.

Length of the basal part of Dorsal fin: The length of the basal part between the two ends of dorsal fin.

Length of the basal part of anal fin: The length of the basal part of the anal fin between the two ends of it.

Eye diameter: Distance between margins of the cartilaginous eyeball across the cornea.

Inter orbital space: The least distance between the upper rims of each orbit wherever the eyes are separate.

Snout length: The distance between, the tip of the snout to the anterior rim of fixed eye.

Of these, all body measurements were expressed in percentage of total length except snout length, orbit diameters and inter orbital width, which are expressed in percentage of head length. The accurate enumeration of eristic data or counts of fin rays is of diagnostic importance. The number of simple and branched rays of fins was taken with great care to get the fin formula.

RESULTS AND DISCUSSION

CHARACTERS AND KEY OF THE IDENTIFIED FISHES

The out line classification of Barbs is as follows:

Phylum - *Chordata*

Subphylum - *Vertebrata*

Super class - *Gnathostomata*

Grade - *Pisces*

Class - *Osteichthyes*

Subclass - *Actinopterygii*

Subdivision - *Teleostei*

Order - *Cypriniformes*

Sub order - *Cyprinodei*

Family - *Cyprinidae*

Sub family - *cyprinae*

Genus- *Puntius*

Genus - *Puntius* (Hamilton-Buchanan)

Puntius Hamilton – Buchanan, 1822, *Fishes of Ganges*: (type species: *Cyprinus sophore* Hamilton – Buchanan), Yazdani and Rao, 1975, *J. Bombay Nat. His. Soc.*, 73(1):171-174 (Synopsis), Taki *et al.*, 1978, *Jap. J. Ichthyol.* , 25(1): 1-8 (Inter specific relationships).

Puntius is a complex genus, which exhibit high degree of variability in colour pattern, size, and habitats such as lake, ditches, ponds, rivers and hill streams. The status of *Puntius* is obscure., the delimitation and nomenclatural validity of the genus have remained unsettled (Hora and Mukerji, 1935., Smith 1945., Selvaraj and Abraham, 1987 Myers, 1960). Kottelat (1999) described *Puntius* as a catchall genus in which a large number of unrelated small barbs have been placed, however, restricted to a very limited geographical area and without information on the limits of the genera. The obscure status of the group is owing to scantiness in the knowledge of its inter and infra generic relationship. They also reported the *Conchoniis* group of the genus is to be widely distributed in India. The genus *Puntius* with 53 species having tremendous diversity is distributed throughout Indian subcontinent. (Jayaram, 1991).

Fishes of the genus *Puntius* are prolific and occupy all possible niches. Almost all the fresh water bodies are occupied by some species of *Puntius*. Consequently they exhibit a high degree of variability in their characters. The members found in the Western Ghat water bodies show different colour patterns which vary inter specifically also.

Distinguishing characters

Body short to moderately elongate, often deep, and slightly compressed. Head short., snout often overhanging mouth. Mouth terminal to inferior, not protrusible., lips thin, devoid of a horny covering. Barbels present in one or two pairs, or entirely absent. Pharyngeal teeth in three rows. Dorsal fin short, inserted nearly opposite to pelvic fins, with 9-13 rays, its last unbranched ray often osseous. Anal fin with 7-9 rays. Caudal fin forked. Scales are small to large, with few and strongly radiating striae. Lateral line complete or incomplete, with 20-47 scales in longitudinal series.

***Puntius amphibius* (Plate-1)**

Synonyms:

Capoeta amphibius : Valenciennes, 1842. *His. nat. Poiss.* 16: 282 pl. 478

Barbus amphibius: Day, 1878., *Fishes of India*: p. 574, pl. 142, fig. 8

Specimens Examined

A total of 1478 specimens were collected during the period.

Mambra - 425, Annamanada - 521, Kundoor - 320, Kankankadavu - 212

Total length – 106 mm, Standard length - 83 mm, Maximum Body depth - 24%, Head length -20% , Pre - dorsal length -33%, Caudal peduncle length -15% , Length of basal part of dorsal fin –16 % Length of basal part of anal fin – 8 % , Eye diameter-28 % , Inter orbital space -38 % Snout length – 28 %.

DESCRIPTION

D. ii 8., A iii 5., P i 14., V i 8 – 9., C-18

Body spindle shaped, narrowing towards both ends. Length 3 - 4 times the width, body covered with cycloid scales, eyes lateral, snout blunt, mouth terminal, and corners of the mouth with a pair of maxillary barbels. Lateral line system complete on either side. Dorsal fin just opposite the base of the ventral fin with scaly basal sheath., 3 un branched rays and 8 branched rays., 3rd dorsal ray spinous. Pectoral fin short,situated just behind the opercular openings. Ventral fins placed close together with scaly basal sheath. Anal fin with 3 unbranched and 5 branched rays. Caudal fin symmetrically forked with 19 branched rays.

Pharyngeal teeth well developed and arranged in three rows of 5, 3, 2 / 2, 3, 5 (five, three, two/ two, three, five) pattern. First one of the first row small with blunt tip, last one of first and second rows spatulate and the remaining ones slightly bicuspid.Inner part of, roof of the buccal cavity, toughened and thrown into ridges.

Scales are cycloid. Scales in the lateral line 24-25, in transverse series $4\frac{1}{2} + 2\frac{1}{2}$

Colour: Fresh specimens were steel blue above the lateral line and silvery white beneath.

A black spot on either side of the caudal peduncle, extending from 22-24 scale along the lateral line system.Caudal and anal fin were black tipped.

.Fishery information: This barb is a food fish, also used in mosquito control and can be utilized as an ornamental fish.

***Puntius parrah* (Plate - 2)**

Synonyms:

Barbus parrah: Day, 1878, Fishes of India: 572, pl. 142, fig. 3

Specimens Examined

A total of 1053 specimens were collected during the period.

Mambra - 225, Annamanada - 428 , Kundoor - 220, Kanakankadavu – 180

Total length – 107 mm, Standard length - 85 mm, Maximum Body depth - 21%, Head length -19 % Pre - dorsal length – 38 %, Caudal peduncle length – 20 % , Length of basal part of dorsal fin- 17 % Length of basal part of anal fin -9 %, Eye diameter – 29 %, Inter orbital space – 42 % Snout length – 33 %.

DESCRIPTION

D. ii 8., A iii 5., P i 14., V i 8., C 18

Body fairly deep, mouth moderate: barbels single maxillary pair, shorter than orbit.

Dorsal fin inserted equidistant between tip of snout and base of caudal fin., its last unbranched ray osseous and smooth. Scales medium., lateral line complete ,with 25or 26 scales., pre-dorsal scales 8.

Colour: Fresh specimens were dark greenish above the lateral line and silvery white beneath. Cheeks golden red. A diffused black spot present on the lateral line after 12th scale. Eyes are golden. Paired and anal fins tinged with yellow., dorsal and caudal fins

dusky. Either side of the caudal peduncle extending from 22-24 scale along the lateral line system. Caudal and anal fin black tipped.

Fishery information: This barb is used as a food fish can be used as an ornamental fish.

***Puntius saranasubnasutus* (Plate 3)**

Synonyms:

Barbus subnasutus Valenciennes, 1842, *Hist. Nat. Poiss.* 16:154

Cyclocheilichthys pinnauratus Day, 1865, *Proc. Zool. Soc. Lond.*: 300., Menon, 1963, *Spolia Zeyl.*, 30 (1):69

Barbus pinnauratus: Day, 1878, *Fishes of India*: 561, pl. 139., Hora, 1942, *Rec. Indian Mus.*, 44:195

Puntius sarana subnasutus : Menon, 1963, *Spolia Zeyl.*, 30 (1) : 69., Jayaram *et al.*, 1982, *Rec. zool. Surv. India Occ. Paper*, (36): 62

Specimens Examined

A total of 568 specimens were collected during the period.

Mambra - 102, Annamanada - 248, Kundoor - 105, Kanakankadavu -113

Total length - 180mm, Standard length -145 mm, Maximum Body depth – 27 % , Head length -16 % Pre-dorsal length – 41 % , Caudal peduncle length – 22 % , Length of basal part of dorsal fin -11 % Length of basal part of anal fin – 9% Eye diameter = 19 % , Inter orbital space - 44 % Snout length -34%

DESCRIPTION

D. iii 8., A ii 5., P i 16., V i 7., C 18

Body oblong and fairly deep and head fairly small. Eyes moderate Barbels two pairs, maxillary pair much longer than orbit, rostral pair slightly shorter. Dorsal fin inserted equidistant between tip of snout and base of caudal fin, its last un-branched ray osseous, fairly strong and posterior part serrated. Scales moderate., lateral line complete, with 28 to 31 scales, pre dorsal scales -10.

Colour: The fresh specimens were greenish on back and upper half of the body, fading to white., most scales with black bases., a dark band behind operculum and a black blotch on lateral line on about 24th scale. Fins were orange and caudal fin with a black superior and inferior edge.

Fishery information: This barb is very common in Kerala and used as a food fish and can be utilized as an ornamental fish.



Plate 2.1 *Puntius amphibius*



Plate 2.2 *Puntius parrah*



Plate 2.3 *Puntius sarana subnasutus*

CHAPTER -3

LENGTH WEIGHT

RELATIONSHIP

AND

CONDITION FACTOR

CHAPTER -3

LENGTH WEIGHT RELATIONSHIP

AND

CONDITION FACTOR

INTRODUCTION

Growth of an organism means a change in length or weight or both with the increasing age. The growth in fish is considered in terms of increase in volume. Increment in size is due to conversion of the food matter into building matter of the body by the process of nutrition. Measurement of growth as length quantifies axial growth, measurement as weight quantifies growth in bulk. These two categories of growth are highly correlated. A fish can change its weight without changing in length or vice versa. Weight of a fish expressed as a function of length. The length-weight relationship is an important tool in fish biology, physiology, and ecology and fisheries assessment. The general length weight relation equation provides a mathematical relationship between the two variables, length and weight so that the unknown variable can be easily calculated from the known variable (Le Cren, 1951).The collection of data on length in the field can be done rather more easily and quickly than the collection of weight data. If a relationship is established between these two variables, the length data can be transformed into weight data.

This expression had been extensively used in the study of fish population dynamics for estimating the unknown weight from the known lengths in yield assessments (Pauly ,1987) , in setting up of yield equation for estimating population strength (Beverton and Holt, 1957.,Ricker, 1958), in estimating the number of fish landed and in comparing the population over space and time (Sekharan, 1968.,Chanchal *et al.* 1978). The length-weight relationship also helps in predicting the condition, reproductive history, and life history of fish species (Nikolsky, 1963., Wootton, 1992.,Munro and Pauly, 1983), and in morphological comparison of species and populations (King, 1996).Length-weight relationship is also important for comparative growth studies (Moutopoulos and Stergiou, 2002).The length-weight relationship is a very useful tool in fisheries assessment. Furthermore, standing crop biomass can be estimated (Morey *et al.*, 2003) and seasonal variations in fish growth can be tracked in this way (Ritcher *et al.*, 2000).

Antony Raja (1967) noted that in the case of fishes of commercial importance, this study has been found essential to convert the catch statistics of that species from weight to number in order to obtain the abundance of stock in space and time. The weight of the fish normally increases with increase in length showing that weight of a fish is a function of length. The weight is a measure of volume and the length is linear, it has been shown that their functional relationship can be described by the hypothetical cube law $W = c L^3$, where W = weight of fish 'L' is the length and 'c' is a constant. This equation can be applied to serve as the basis for the calculation of the weight of fish of known length or vice versa, only if the form and specific gravity of the fish remains constant throughout

life, obeying isometric growth pattern, in which exponent of length 'L' is found to be equal to 3 (Allen, 1938). The length-weight relationship also helps in predicting the condition, reproductive history, and life history of fish species (Nikolsky, 1963., Wootton, 1992., Pauly, 1993., Avsar, 1998), and in morphological comparison of species and populations (King, 1996; Gonçalves *et al.*, 1997). Understandably, however, the same fit is not always necessarily being the same.

According to the general cube law governing length weight relationship, the weight of fish would vary as the cube of length. However all fish species don't strictly obey the cube law and deviation from the law are measured by condition factor (Ponderal index – K factor). Le Cren (1951) proposed relative condition factor (K_n) in preference to K as the former considers all the variations like those associated with food and feeding, sexual maturity, etc., while the latter does so only if the exponent value is equal to 3. Thus K factor measures the variations from an ideal fish which holds the cube law while K_n measures the individual deviations from the expected weight deviated from length weight relationship.

The length weight relationship of cyprinids from India has been subjected to detail studies, notably by Jhingran (1952), Bhatnagar (1963), Natarajan and Jhingran (1963), Sinha (1972), Pathak (1975), Chatterji *et al* (1977), Vinci and Sugunan (1981), Sivakami (1982), Choudhury *et al.*, (1982) Malhotra (1982,1985), Mohan and Sankaran (1988), Reddy and Rao (1992), Biswas (1993), , Sarkar *et al.* (1999), Sunil (2000), Mercy *et al.* (2002) and Mohan and Singh (2003). However no detailed information is available on the length weight relationship and condition factor of *Puntius amphibius*, *Puntius parrah* and

Puntius sarana subnasutus and there fore the present study was undertaken to elucidate the pattern of growth and general well being of these three fish species.

MATERIALS AND METHODS

Random samples of the three species *Puntius amphibius*, *Puntius parrah* and *Puntius sarana subnasutus* were collected. After blotting the specimens to remove excess water, the total length to the nearest millimeter and weight to the nearest 0.01g were recorded. The data so generated was subjected to statistical analysis by fitting length-weight relationship following Le Cren (1951).Length weight relationship can be expressed as

$$W = a L^b$$

The logarithmic transformation of gives the well known linear equation

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

Where W = Weight in gm, L = length in mm, a = a constant being the initial growth index and b = growth coefficient constant. 'a' represents the point at which the regression line intercepts the Y axis and 'b' the slope of regression line.

The relationship between length and weight was determined for males and females separately by transforming the values of both variables to logarithmic values and fitting a straight line by the method of least squares. The data was processed in EXCEL software. Bailey's t test (Snedecor and Cochran,1967)was employed to find out whether 'b' value significantly deviated from the expected cube value of 3.

$$t = (b - 3) / sb$$

where b = regression coefficient , sb = standard error of 'b'

The t-test (Snedecor and Cochran, 1967) on 'r' values reveals whether significant correlation exist between length and weight.

Relative condition factor (Kn) as per Le Cren (1951) and Ponderal index (condition factor, K), as per Hile (1936) are expressed as follows.

Relative condition factor (Kn) = w / W

Where w = observed weight , W = calculated weight derived from length-weight relation.

Condition factor (K) = $W * 10 / L^3$

Where W = Observed weight L = body length, the number 10 is a factor to bring the Ponderal index to near unity. Fluctuations in condition were examined both seasonally and size wise.

RESULT

Length weight relationship of *Puntius amphibius* can be expressed as follows,

For males,

$$\text{Log } W = \log 0.000365 + 2.20013 \log L \quad (r = 0.7987)$$

For females,

$$\text{Log } W = \log 0.000003121 + 3.15598 \log L \quad (r = 0.912215)$$

The relationship between length and weight of males and females of *Puntius amphibius* together with correlation coefficients is depicted in Table 3.1 and 3.2. The correlation coefficient 'r' between log length and log weight was found to be 0.7987 in males 0.912215 in females .The 't' test on 'r' values showed the existence of very good

relationship between length – weight ($p < 0.01$). The length weight regression was found to be highly significant in both sexes. ($P < 0.001$). The results of the analysis of covariance revealed significant difference in the regression coefficient of males and females there by indicating heterogeneity of the samples. Hence comparison between males and females as carried out using students ‘t’ test (Zar, 1974). The results show that ‘b’ values are significantly different. The comparison of elevations disclosed significant difference among two sexes ($P < 0.01$) hence pooling of data to provide single equation expressing the length-weight relationships of *Puntius amphibius* will not be justifiable, thus necessitating fitting up of separate equation for males and females.

The value of regression coefficients in males was 2.20013 while in females it was 3.15598. The ‘t’ values arrived at 12.07 (df: 516) in males and 1.3567 (df: 960) in females manifested significant departure of b value from 3 in males ($p < 0.01$). The fluctuations noticed in Kn values of males and females during the study period were represented in Fig 3.7 and 3.8. The Kn values showed two peaks (oct-June) and two troughs (July August and January February). The values of Ponderal index (k) were found in conformity to the Kn values during this period. A scrutiny of the lengthwise variation in relative condition factor showed low kn values in smaller length groups.

Length weight relationship of *Puntius parrah* can be expressed as follows,

For males,

$$\text{Log } W = \log 0.0002988 + 2.2307 \log L \quad (r = 0.7907)$$

For females,

$$\text{Log } W = \log 0.000003234 + 3.08569 \log L \quad (r = 0.9712)$$

The correlation coefficient 'r' between log length and log weight was found to be 0.7907 in males 0.9712 in females. The logarithmic relationship between length and weight of males and females of *Puntius parrah* together with correlation coefficients is presented in Table 3.3 and 3.4. The 't' test on 'r' values showed the existence of very good relationship between length –weight ($p < 0.01$). The length weight regression was found to be highly significant in both sexes. ($P < 0.00$). The results of the analysis of covariance revealed significant difference in the regression coefficient of males and females there by indicating heterogeneity of the samples. Hence comparison between males and females as carried out using students 't' test. The results show that 'b' values are significantly different. The comparison of elevations disclosed significant difference among two sexes ($P < 0.01$) hence pooling of data to provide single equation expressing the length-weight relationships of *Puntius parrah* will not be justifiable, thus necessitating fitting up of separate equation for males and females. The value of regression coefficients in males was 2.2307 while in females it was 3.08569. The 't' values arrived at 12.1254 in males and 1.3567 in females manifested significant departure of b value from 3 in males. ($p < 0.01$)

The fluctuations noticed in Kn values of males and females during the study period were represented in fig 3.9 and 3.10. The Kn values showed two peaks (Oct-June) and two troughs (July August and December and January). The values of Ponderal index (k) were found in conformity to the Kn values during this period.

Length weight relationship of *Puntius sarana subnasutus* can be expressed as follows,

For males ,

$$\text{Log W} = \log 0.000022322 + 2.861298 \log L \quad (r = 0.983157)$$

For females,

$$\text{Log W} = \log 0.000005016 + 3.119967 \log L \quad (r = 0.9596)$$

The logarithmic relationship between length and weight of males and females of *Puntius sarana subnasutus* together with correlation coefficients is depicted in Table 3.5 and 3.6. The correlation coefficient 'r' between log length and log weight was found to be 0.9831 in males and 0.9596 in females. The 't' test on 'r' values showed the existence of very good relationship between length –weight ($p < 0.01$). The length weight regression was found to be highly significant in both sexes. ($P < 0.00$). The results of the analysis of covariance revealed significant difference in the regression coefficient of males and females there by indicating heterogeneity of the samples. Hence comparison between males and females as carried out using students 't' test. The results show that 'b' values are significantly different. The comparison of elevations disclosed significant difference among two sexes ($P < 0.01$) hence pooling of data to provide single equation expressing the length-weight relationships of *Puntius sarana subnasutus* will not be justifiable, thus necessitating fitting up of separate equation for males and females.

The value of regression coefficients in males was 2.861298 while in females it was 3.119967. The 't' values arrived at 2.618 (df: 195) in males and 1.356764 (df: 371) in females manifested slight departure of b value from 3 in males ($p < 0.01$). The fluctuations

noticed in Kn values of males and females during the study period were represented in Fig 3.11 and 3.12. The Kn values showed two peaks (November, July) and two troughs (August and January February). The values of Ponderal index (k) were found in conformity to the Kn values during this period. A scrutiny of the lengthwise variation in relative condition factor showed low Kn values in smaller length groups.

DISCUSSION

Length-weight relationship was expressed by the cube formula $W=a L^3$ by the earlier workers (Brody, 1945., Brown, 1957). The cube law confers a constancy form and specific gravity to an ideal fish .However, adverting to the inadequacy of the cube law in explaining the length weight relationship in fish, many researchers adopted the general formula in the form $W= a L^b$.Le Cren (1951) suggested that the deviations from the cube law might be attributed to the condition of the fish reproductive activities, taxonomic differences or environmental factors. Ricker (1958) explained that due to changes in body proportions during the life of fish, their body form and specific gravity can vary and hence cube law does not hold true for them. According to Raounefell and Everhart (1953), generally the values of 'b' is 3 in fish but the cube law needn't always hold good. In the present study the highest 'b' value was arrived in females of *Puntius amphibius* followed by *Puntius sarana subnasutus* and *Puntius parrah*. The exponential value of 3.15598 in females of *Puntius amphibius* implies that the females gain weight at a faster rate in relation to its length .This is also true in the case of *Puntius sarana subnasutus* and *Puntius parrah*.

The very low exponential value of 2.20013 observed in males of *Puntius amphibius* and 2.2307 observed in males of *Puntius parrah* reveals the reverse condition. The low 'b' value of males indicates that negative allometry which means the fish get relatively thinner as they grow (Wootton, 1992). The females manifest positive allometric pattern of growth. Reports on the length-weight relationship of cyprinids fish divulge that many of them strictly follow cube law, while there are many in which the weights of fish either tends to increase or decrease in proportion to the cube of length. Isometric growth pattern has been reported in *Cirrhinus mrigala* and *Labeo rohita* (Jhingran, 1952) *Labeo calbasu* (Pathak, 1975), *Puntius dorsalis* (Sivakami, 1982) *Catla catla* (Kantha and Rao, 1990) *Schizothorax plagiostomus* (Bhagat and Sunder, 1983) and *Puntius denisonii* (Mercy *et al.*, 2002). Deviation from cube law has been observed in Indian major carps by many authors (Jhingran, 1952., Natrajan, and Jhingran, 1963., Shrivastava and Pandey, 1981., Choudhary *et al.*, 1982., Mohan and Sankaran, 1988., Sarkar *et al.*, 1999). The slope value less than 3 has been reported in *Barilius bendelesis* (Gairola *et al.*, 1990) and *Ctenopharyngodon idella* (Dhanze and Dhanze, 1997). The value of the slope was found to be higher than 3 in *Puntius sarana sarana* (sinha, 1975), *Labeo bata*, (Chatterji *et al.* 1977) and *Puntius sophore* (Reddy and Rao, 1992). All these earlier reports corroborate the present findings on the length weight relationship in *Puntius amphibius*, *Puntius sarana sarana subnasutus* and *Puntius parrah* in which departure of 'b' value from the isometric value of 3 was noticed in respect of both males and females. Females of these three species were found to be surpass males in weight in relation to length as evidence from the disparity in 'b' values. Similar trend has been observed in other cyprinids too viz, *Puntius kolus* (Bhatnagar, 1963), *Labeo fimbriatus* (Bhatnagar, 1972), *Labeo dero*

(Malhotra and Chauhan, 1984), *Rasbora daniconius* (Thakre and Bapat, 1984), and *Labeo dussumieri* (Kurup and kuriakose, 1991). Biswas (1993) while studying *Labeo pangusia* from Meghalaya found that the females have higher slope values (3.197) when compared to males (2.785). According to Thakur and Das (1974) the males of *Heteropneustes fossilis* become lighter for their length as they grew larger. In *Ompok bimaculatus* (Rao, 1990) the regression coefficients of males (2.3148) was found to be lower than that of females (2.7223). Similar sequels were also recorded in *Salmotrutta fario* (Kumar *et al.* ,1979), *Tilapia mossambicus* (Nair, 1988) *Horabagrus brachysoma* (Kumar *et al.* 1999) and *Momnopterus cuchia* (Narejo *et al.* 2002). The results of the present study are in conformity to the above findings.

The difference in growth performances between the two sexes may be due to intrinsic changes in their physiological systems. Females are heavier than the males of the same length in these three species probably because of the difference in fatness and gonadal development (Le cren, 1951). While discussing the seasonal effects on length weight relationship of *Clarias batrachus*, Mitra and Naser (1987) found that higher metabolic activity with spawning season lowered the 'b' value while less metabolic activities, accumulation of fat, weight of gonad etc, during the pre-spawning period, increased the values. The higher regression coefficients in the females may be attributed to their more robust appearance and deep body as against the more slender males. The value of regression coefficients of specimens under the present study were in the range between 2 to 4 as reported by Tesch (1968). Martin (1949) stated that the values of 'b' usually

fluctuate between 2.5 and 4, the exponential value in females with the range proposed by the above authors.

Beverton and Holt (1957) opined that since 'a' and 'b' of allometric formula, might vary within a wide range of very similar data and are very sensitive to even the unimportant variations in various factors. Allometric formula worked better than cubic formula. Any indications in biological events could be recorded by allometric law. The significant departure of regression coefficients from the isometric growth value in male and female of the three species of *Puntius amphibius*, *Puntius sarana subnasutus* and *Puntius parrah* indicates that the general parabolic equation $W=a L^b$ express the length weight relationship in adults better than cubic law.

Fluctuations in the condition of the fish is related to the reproductive cycle (Le Cren, 1951., Sarojini, 1957., Pantalu, 1963., Qasim, 1973., Neelakandan and Pai, 1985., Gairola *et al.*, 1990., Narejo *et al.*, 2002), feeding rhythms (Hile, 1948., Qasim, 1957., Bal and Jones, 1960., Blackburn, 1960., Bhatt, 1977., Shrivastava and Pandey, 1981., Das and Misra 1989) or physio-chemical factors of environment, age physiological state of fish or some other factors (Brown, 1957., Kumar *et al.* 1979., Kalita and Jayabalan, 1997). In *Puntius amphibius* and *Puntius parrah*, the higher K_m value recorded in May, June and October almost coincided with the occurrence of high gonado somatic index (G. S. I.) in both males and females where in *Puntius sarana subnasutus* it was in June, July and November, linked to the peak breeding season. Thus it appears that reproductive cycle

in *Puntius amphibius*, *Puntius sarana subnasutus* and *Puntius parrah* was related to the variations in the condition factor.

Sex wise analysis of Kn values revealed that the mean Kn value in females of *Puntius amphibius* (1.033) was higher than that of males (0.93124). In *Puntius parrah* 1.053 and 0.8998, where in *Puntius sarana subnasutus* the Kn values for males and females were 1.0115 and 0.9986 respectively. According to Le Cren (1951) Kn values greater than 1 indicated good general condition of the fish whereas values less than 1 denotes reverse condition. Vinci and Sugunan (1981) and Biswas (1993) reported higher Kn values in females of *Labeo calbasu* and *Labeo pangusia* respectively. High Kn values observed in female specimens of present study suggest that the females are in better condition when compared to males.

A comparison of the Ponderal index (K) and Kn values revealed that in the case of females, both the values were closely synchronized whereas male showed different trends. The results of the present findings strongly corroborated with the earlier findings that the Ponderal index is applicable only if the fish obeys the cube law, in its length-weight relationship (LeCren, 1951). Females having 'b' value slightly higher than 3 showed almost the same nature of variation in the values. On the other hand, males with low regression coefficients rendered different patterns for the K and Kn values.

Based on the results of the length-weight relationship and condition factor, of *Puntius amphibius*, *Puntius sarana subnasutus* and *Puntius parrah* it can be concluded that the

growth of females is quite satisfactory and the overall growth performance of females is significantly higher than that of males.

Table 3.1: Statistical analysis to test deviation from the cube law of *Puntius amphibius*

Group	b	DF	Sb	$T=(b-3)/Sb$	P%
Male	2.20013	516	0.0684	-12.0789	8.21 E - 28
Female	3.15598	960	0.1188123	1.356764	2.5319 E -55

Table 3.2: Statistical analysis to test deviation from the cube law of *Puntius parrah*

Group	b	DF	Sb	$T=(b-3)/Sb$	P %
Male	2.2307	376	.05892	-12.1254	7.9867 E -24
Female	3.085698	675	0.0630220	1.356764	2.5319 E -55

Table 3.3: Statistical analysis to test deviation from the cube law of *P. sarana subnasutus*

Group	b	DF	Sb	$T=(b-3)/Sb$	P%
Male	2.861298	195	0.048930	-2.618	2.36 E-53
Female	3.119967	371	0.0536940	1.356764	2.5319 E -55

Fig 3.1 Relation between Length and Weight in *Puntius amphibius* - Female

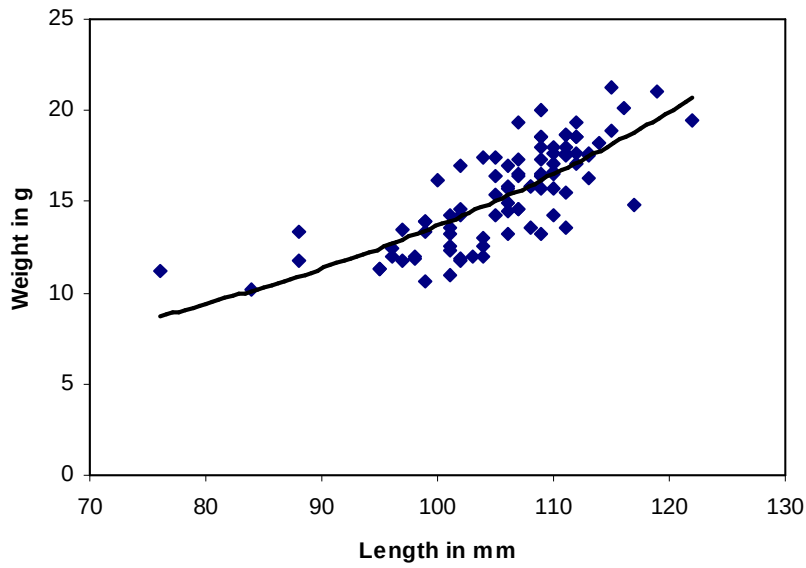


Fig 3.2 Relation between Length and Weight in *Puntius amphibius* - Male

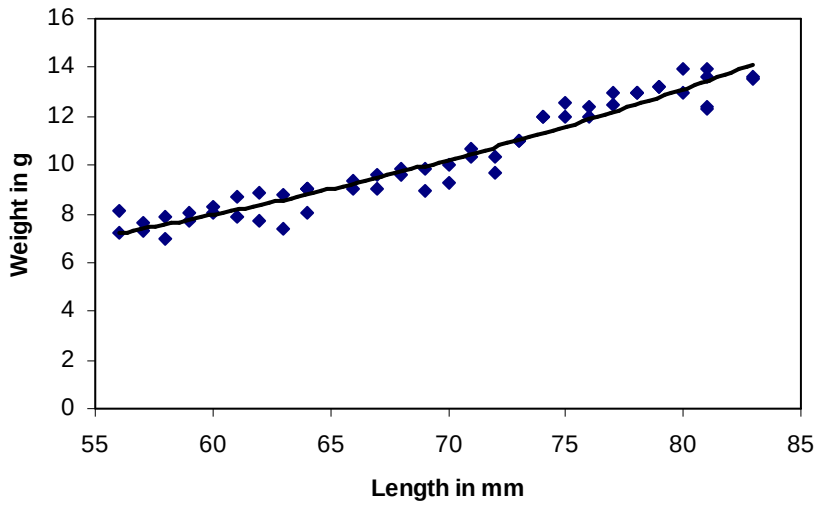


Fig 3.3 Relation between Length and Weight in *Puntius parrah* - Female

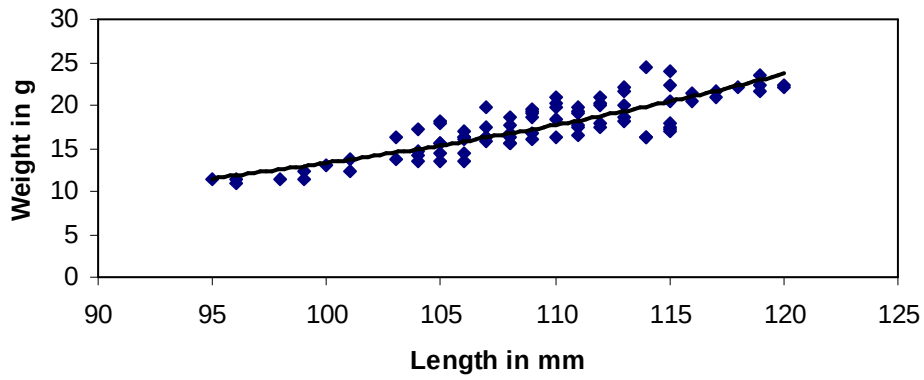


Fig.3.4 Relationship between Length and Weight in *Puntius parrah* - Male

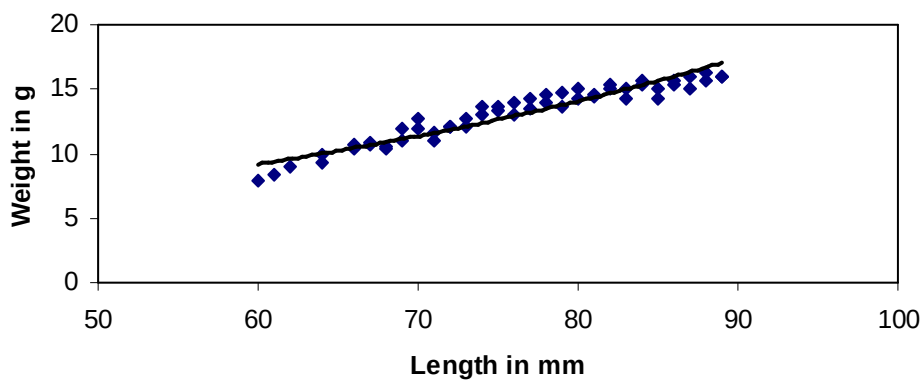


Fig.3.5 Relation between Length and Weight in *Puntius sarana subnasutus* - Female

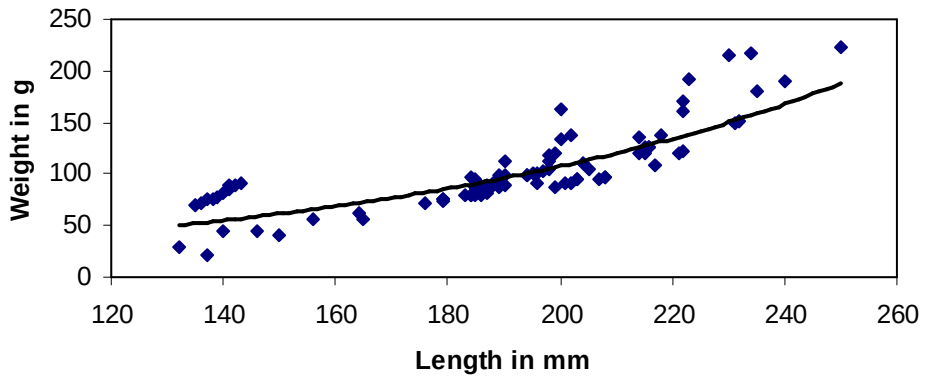


Fig 3.6 Relation between Length and Weight in *Puntius sarana subnasutus*- Male

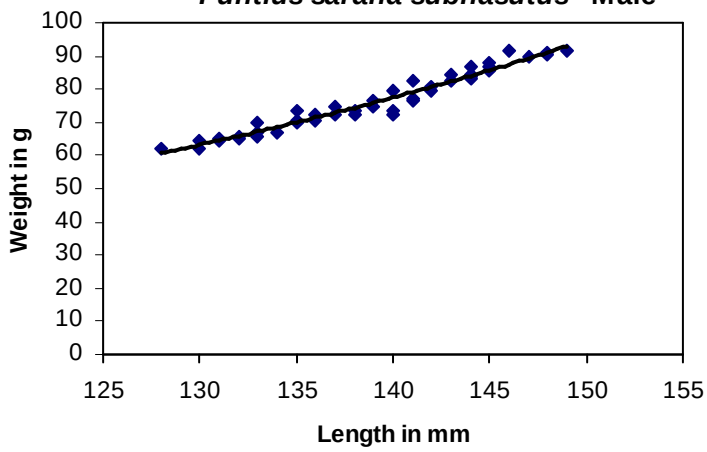


Fig 3.7 Seasonal variation in Relative condition factor of *Puntius amphibius* - Female

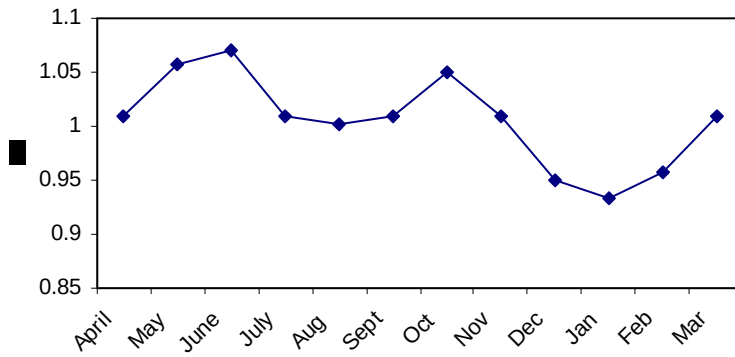


Fig 3.8 Seasonal variation in Relative condition factor of *Puntius amphibius* - Male

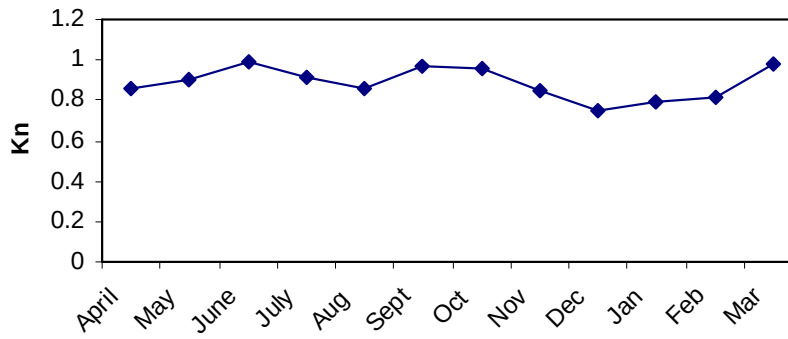


Fig 3.9 Seasonal variation in Relative condition factor of *Puntius parrah* - Female

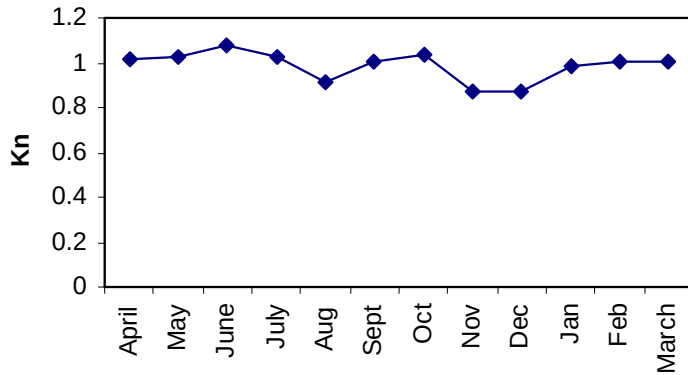


Fig 3.10 Seasonal variation in Relative condition factor of *Puntius parrah* - Male

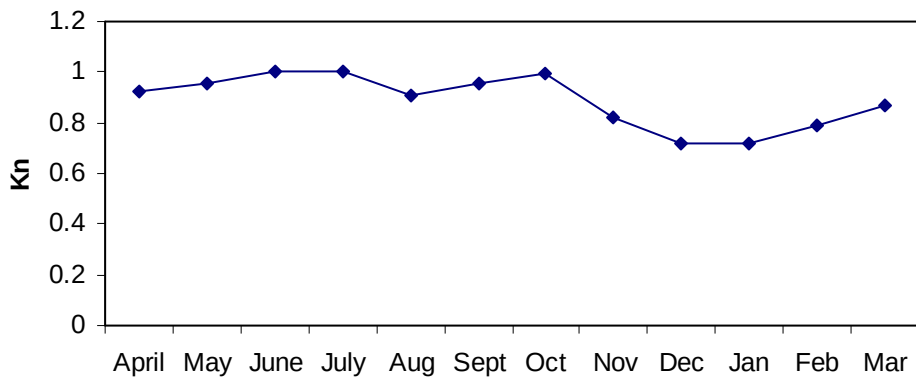


Fig 3.11 Seasonal variation in Relative condition factor of *Puntius sarana subnasutus* -Female

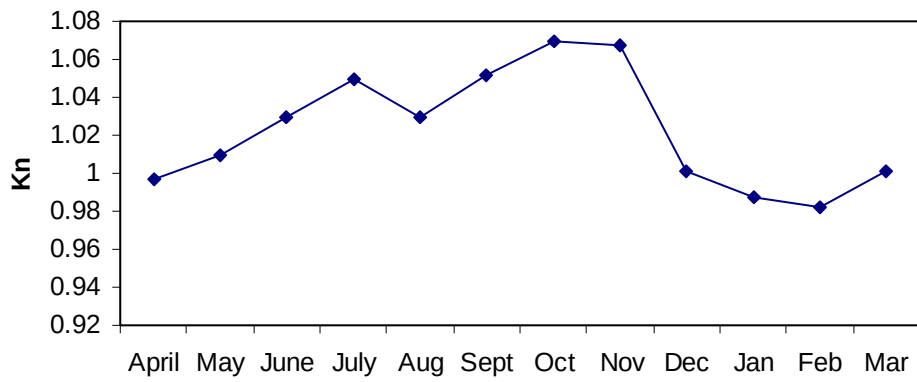
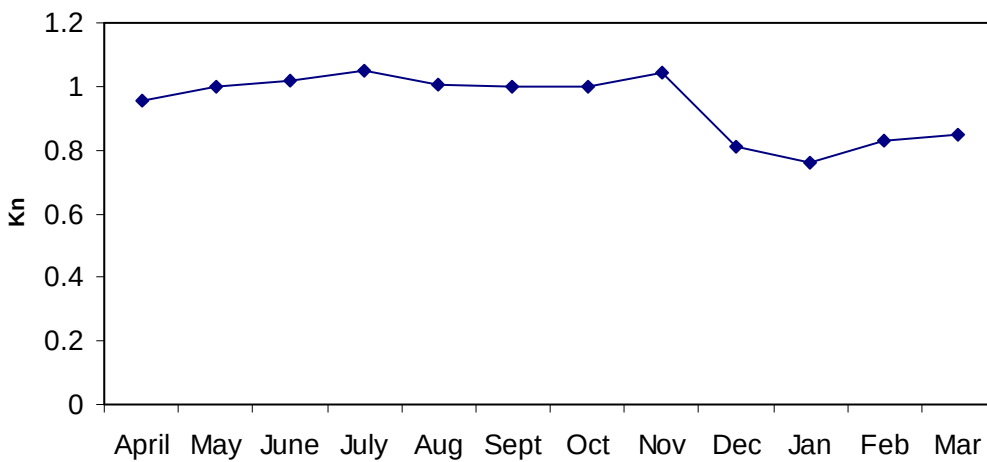


Fig 3.12 Seasonal variation in Relative condition factor of *Puntius sarana sibnssutus* - Male



CHAPTER - 4
BIOCHEMICAL VARIATIONS
DURING REPRODUCTIVE
CYCLE

CHAPTER – 4

BIOCHEMICAL VARIATIONS DURING REPRODUCTIVE CYCLE

INTRODUCTION

The biochemical composition of fish varies widely from species to species and influenced by changes in physiological status such as growth, feeding and maturation. . Further there will be a difference in the biochemical composition of various body parts such as muscle, liver, and gonads etc, since some body parts or organs are storage in their function and some others are the site for metabolism.

Maturation refers to cyclic morphological changes which a female and male undergo to attain sexual maturity. It has long been recognized that marked changes occur in many species of fish during the normal sexual cycle, but in comparison with mere observation, actual chemical studies have not been plentiful .Attainment of full maturity almost always marks a change in the growth pattern resulting from the reproductive drain due to diversion of materials meant for somatic growth to the gonads. Germinal tissue can require a significant proportion of the total annual production in sexually mature fishes. In females, mature ovaries that are rich in

protein and lipid can reach 15 % total body weight. In many fishes, gonads grow during the time when food supplies are limited and feeding is reduced. Therefore, nutrients for germinal tissue growth may need to be drawn from somatic tissues (Medford and Mackay 1978). There is a marked reduction in growth rate at the onset of maturity reflecting the utilization of the growth material for gonad maturation. Thus gonadal maturation is mainly dependent on the nutritional store accumulated during the major feeding and growing period. It is well established that most of the biochemical constituents of fishes are subject to marked seasonal changes which have been attributed to factors such as maturation, spawning, age, growth and feeding (John and Hameed, 1995).

Biochemical studies on fish tissues are of considerable importance as they help in evaluating the nutritive value as well as the physiological needs of fish at different periods of life. To a commercial aqua culturist, knowledge of the physiological conditions and consequently the health status of fish at different stages can be helpful in reckoning the quality and quantity of feed to be given (Shearer, *et al* 1994). According to Basimi and Groves (1985), harvest strategies could be planned in such a way that the harvesting is done at a time when the protein is at its highest levels and yield is substantial without being detrimental to the stock. Besides these, the data on the composition of fish is necessary in order to standardize the technology of processing fish and fishery products. So it will be quite informative and useful to understand the distribution of the major biochemical constituents such as protein and lipid in various size

groups, in different age groups and maturity stages for their rational and effective utilization

There is a considerable body of literature on the variations in body composition in relation to the eco-physiological changes like attainment of sexual maturity and spawning migration of fish. Milroy (1968) investigated the function of fat during the reproductive period of herrings. Adhikari and Noor (1986), Rubbi *et al.* (1987) did mentionable works on variation of lipid content during different maturity stages. Shahnaz fida (2006) explained the attainment of sexual maturity in fish by analyzing the differences of chemical composition of body muscles, liver and gonads. Observations on the biochemical changes of maturation and spawning were made by Bechanlal (1963) on *Cirrhina mrigala*, Appa Rao (1967) on *Pseudosciana aneus*, Idler and Bitners (1960) on sockeye salmon, Krivobock, (1964) on *Clupea*, Mackinnon, Medford and Mackay (1978) on *Esox lucius*, Nassour and Leger (1989) on *Salmo gairdneri*. Singh A.K. and Singh T. P. (1979) worked on *Heteropneustes fossilis*, Thaibault *et al.* (1997) on *Oncorhynchus mykiss*, Yagano Bano (1976) on *Clarias batrachus*, Sivakami *et al.* (1986) on *Cyprinus carpio*, Rao.K.S (1967) on *Leiognathus splendens*, Chaturvedi *et al.* (1976) on *Heteropneustes fossilis*, Delahaunty and de Vlaming (1980) on *Carassius auratus*, John and Hameed (1995) on *Nemipterus japonicus mesoprion*, Kingston and Venkataramani (1994) on *Selaroides leptolepis*, Pathak *et al.* (1989) on *Channa punctatus*. Mustafa Doruco (2000) on *Coregonus lavaretus*, Dawson and Grimm (1980) on *Pleuronectes platessa*, Shahnaz Fida *et al.* (2006) on *Cyprinus carpio*, Gersande *et al.* (1997) on *Perca fluviatilis*, Chellappa *et al.* (1989) on *Gasterosteus aculeatm*, Somavanshi (1983) on *Garra mullya*, and Basade *et al.* (2000) on *Tor putitora*.

Despite the voluminous literature that have accumulated on the proximate composition of various groups of fish, contribution on similar aspects in fish of the genus *Puntius* are very few. The important works did by Natarajanand Sreenivasan (1961) on *Puntius dubius*, Vijayakumar (1987) on *Puntius filamentosus*, Salam *et al.* (1995) on *Puntius gonionotus* require special mention. In spite of the fact that high quality seed production depends on the understanding of proximal composition of the breeders, contributions relating body composition with that of spawning habits of the three species *Puntius amphibius* (Val.), *Puntius parrah* (Day) *Puntius sarana subnasutus* (Val.) are not much accomplished.

Because of the physiological and biological hazards in the environment, fish like other vertebrates have a high reproductive potential where they produce sufficient quantities of eggs so as to enable successful recruitment. The most of genital products constitute up to 30 % of the body and even much more – a process calling for intensive energy. The first source of energy is suggested to be body fat (Giesse and Hart 1966) with their mobilization becoming prominent at the time of maturation, even in non migratory fish. Lipids such as the fats and oils represent the chief form in which excess nutrients are stored in the animal body. They serve as a concentrated source of energy and heat insulators. One gram of lipid supplies 9 calories, which is more than twice the amount of energy supplied by each gram of carbohydrate. Milroy (1968) investigated the function of fat during reproductive period of herrings. McCartney(1967) tried to explain the attainment of sexual maturity in fish by analyzing the difference of chemical composition

of body muscles, blood and gonads. Adhikari and Noor, Rubbi *et al.* and Soldevilla (1989) determined the differences of fat elements in different fishes in different season.

Eggs are found to use protein steadily for energy purpose during development, since newly spawned eggs can't absorb much of the nutrients directly from the water, it stores adequate quantities of essential substances for the growth. It was noted that as the concentration of free amino acids rise in the developing gonads there is a fall in corresponding amino acids in the liver. The translocation of muscle proteins for gonad development has been reported by Masurekar and Pai (1979) in *Cyprinus carpio*.

Investigations on the biochemical composition of fish enable us to unveil the potential store house of various nutrients and to trace the pathways through which they are mobilized for the biological needs of the fish. Further the levels of these substances could indicate the quality of the eggs and exact stage of maturation of the fish (Love, 1970) and so there appears necessity of continuous effort in this field. These factors and the lack of proper information on the biochemical composition of concerned species in relation with maturity have been prompted to take up the present work. The biochemical composition of three tissues namely muscle, liver, and gonad were studied. Two parameters viz. protein, fat are estimated in relation to the different maturation stages of the gonads.

MATERIALS AND METHODS

Collection of specimens

The specimens were cut open to identify the sex. Males were discarded from the collection. And females alone were used for further analysis. Maturity stages of females were determined based on the macroscopic appearance of the ovary like, shape and size in relation to body cavity, colour, extent of yolk formation and microscopic structures, such as ova diameter measurements. The gonads were assigned to five representative stages. Tissues of muscles, liver and gonads were removed from the samples. Muscles tissue was taken from the base of the first dorsal fin, carefully taken not to include any skeletal parts.

Estimation of total protein

Kjeldahl method is used to found out total protein content. For this the amount of nitrogen present was experimentally found out and multiplied by an appropriate conversion factor. The sample was oxidized in presence of H_2SO_4 and nitrogenous compounds were converted into ammonium sulphate. Mercury is added to the digestion mixture as a catalyst and alkali sulphate as a boiling point elevator. Ammonia is liberated by adding excess of alkali and is quantitatively distilled into a measured volume of standard H_2SO_4 . The acid not neutralized by ammonia is back titrating with standard alkali. The digestion was conducted over a heating device adjusted to bring 250 ml water

at 25°C to rolling boil in approximately 5 minutes. Add 3 to 4 boiling chips to prevent superheating. Accurately weighed 0.2 - 0.3 (0.7 - 2.0) g of the sample was digested in a digestion flask. 0.7 g Hg O, 15 g powdered potassium sulphate and 25 ml of H₂SO₄ were added. Each g of protein consumes 10 ml H₂SO₄ during digestion. The flask was placed in an inclined position on a heater and heat gently until forming ceases. A small amount of silicon antifoam added to reduce foaming. The solution was boiled vigorously until it became clear and then continued boiling for 1-2 hrs.

Distillation: Cooled the solution and add about 200 ml distilled water and in order to avoid complex formation add 25 ml of the thiosulphate solution. Mix to precipitate the mercury. A few Zn granules were added to prevent bumping, incline flask and added without agitation 25 g of sodium hydroxide solid to make solution strongly alkaline. The flask was immediately connected to distillation bulb or trap on the condenser and with tip of the condenser immersed in measured quantity of standard acid (50 ml 0.5 N or an approximate quantity of 0.1 N) in the receiver, rotates the flask to mix the contents thoroughly. Then heat immediately until all the ammonia has distilled over (150 ml distillate). Lower the receiver before stopping distillation and wash the tip of condenser with distilled water. Excess acid back titrated with standard 0.1N Na OH using methyl red as indicator.

Calculation:

Nitrogen content in g = $(a - 0.2 b) - (c - 0.2 d) \times 0.007$

a. = Volume in ml 0.5 N acid measured for main distillation

b. = Volume in ml 0.1 N alkali used for back titration.

c. = Volume in ml 0.5 N acid measured for blank distillation

d. = Volume in ml 0.1 N acid measured for main distillation.

$$\text{Total protein} = N * 100 * \text{Conversion factor} / W$$

Where N = Mass of nitrogen content in gram of the original sample.

W = Mass of sample in gram.

Estimation of total lipid

Accurately weighed 2 grams of well mixed sample was put in a 50 ml beaker and added 2 ml HCl. Using stirring rod break up coagulated lumps until the mixture was homogenized. Added additional 6 ml HCl and heat on a steam bath for 90 minutes. The mixture was stirred occasionally with a rod. Cooled the solution and transferred it to a Mojonnier fat extraction flask. Rinse the beaker and rod with 7 ml alcohol and added it to the extraction flask, and mixed the solution very well. Rinse the beaker with 25 ml ether, added it to the extraction flask. Tight the flask with a stopper with cork, and shake vigorously for 1 minute. Added 25 ml petroleum ether to the extraction flask and repeated vigorous shaking. After that the Mojonnier flask was centrifuged for 20 minutes with a speed of 600rpm. Draw off as much as possible of ether fat solution through filter consisting of cotton packed just firmly enough in funnel stem to let ether pass freely into weighed 125 ml beaker containing boiling chips. Before weighing, dry it and similar

flask as counterpoise in oven 100°C: then keep it to stand in desiccator to constant weight.

Calculation

$$\text{Fat \%} = A * 100 / B$$

Where

A = Weight difference in the beaker

B = Sample weight

RESULT

Proximate composition of muscle, liver and ovary of *Puntius amphibius*

Protein content

Variation of protein content of muscle, liver and ovary of *Puntius amphibius* during different stages of maturity was shown in Table 4.1. The muscle protein content varied between 15.85 % and 18.26 percent. The protein content of muscle was maximum at the maturing stage (18.26 %) and gradually descending through the ripening, ripe and spent stages and encounters a minimum of 15.85 % during the spent stage. A more or less descending trend in the amount of protein content was noticed from maturing stage (17.13 %) to ripe stage (14.13 %) in the case of liver protein. An increase was shown from immature stage (16.23 %) to the maturing stage and there after a sharp decrease was

noticed .The liver protein showed a maximum of 17.13 % during maturing stage and a minimum of 14.13 % during ripe stage. The spent stage showed a recovery phase (14.67 %). As ovary attained maturity, the protein content gradually increased from immature (21.82 %) to ripe stage and reached its maximum value, (26.42 %) at the ripe stage and descended to a value of 22.55 % during the spent stage. The variations of protein percentage in muscle, liver and ovary through different months are given in Fig. 4.1.

Lipid content

The variation of lipid content of muscle, liver and ovary during different stages of maturity was present in Table 4.4. Lipid content of muscle gradually decreased while maturation proceeds onwards. The lipid content was maximum (2.12 %) during the immature stage and showed a minimum value (1.63 %) during ripe stage. The spent stage showed a slight recovery (1.75 %). From immature(9.35 %) to Maturing stage (9.84 %) the liver lipid content ascended gradually and onwards a descending trend is shown, reached its minimum at ripe stage (7.24 %). The spent stage showed an increasing phase (8.91 %). The ovary showed a tremendous increase in its lipid content during the maturation process. The maximum lipid content was found in the ripe stage (14.22 %) and minimum in the spent stage (12.2 %). The variations of lipid percentage in muscle, liver and ovary through different months are given in Fig. 4. 2.

Proximate composition of muscle, liver and ovary of *Puntius parrah*

Protein content

The protein content of muscle, liver and ovary of *Puntius parrah* during different stages of maturity was shown in table 4.2 .The muscle protein content varied between 16.18 % and 18.82 %.The protein content of muscle was maximum at the immature stage (18.82 %) and gradually descending through the maturing (18.6 %) ripening (17.91 %), ripe (17.35 %) , and encountered a minimum of 16.1% during the spent stage. A more or less descending trend in the amount of protein was noticed from maturing (18.46 %) to ripe (15.76 %) stage in liver protein content. An increase was seen from immature (17.14 %) stage to the maturing stage and there after a decrease was noticed .During the spent stage (16.63 %) the liver lipid content shows a recovery trend. As ovary attained maturity, the protein content gradually increased from immature (22.57 %) to ripe stage (26.9%) and reached its maximum value at the ripe stage and descended to a value of 22.51 % during the spent stage. The variations of protein percentage in muscle, liver and ovary through different months are given in Fig .4.3.

Lipid content

The variation of lipid content of muscle, liver and ovary during different stages of maturity was present in the table 4.4. Lipid content of muscle decreased from 2.14 % to 1.92 % during the first phase of maturation. There was a slight decrease (.1 %) in the next stage. The lipid content was minimum (1.59 %) during the ripe stage. The lipid content percentage showed a recovery tendency during the spent stage (1.91 %). From immature to ripe stage the liver lipid content descended gradually and reached its minimum at ripe stage (8.07 %).The peak value of 10.32 % was recorded at the maturing phase. During

the spent stage liver again recovered the lipid content to a certain extent (9.13 %). The ovary showed a tremendous increase in its lipid content during the maturation process (10.4 % -13.64 %) The maximum lipid content was found in the ripe stage (13.6%) and minimum in the spent stage(10.4%).During the spent stage the lipid content percentage showed a decreasing trend (11.6 %). The variations of lipid percentage in muscle, liver and ovary through different months are given in Fig. 4.4.

Proximate composition of muscle, liver and ovary of *Puntius sarana subnasutus*

Protein content

The protein content of muscle, liver and ovary of *Puntius sarana subnasutus* during different stages of maturity was shown in Table 4.3 .The muscle protein content varied between 20.16 % and 18.33 %. The protein content of muscle was maximum at the beginning of maturation (20.16 %) and gradually descending through the maturing stage (19.52 %) ripening (18.84 %), ripe (18.33 %). Spent stage encountered a rising (19.45 %) value. At the early stages of maturation the liver protein content shows an increase (16.14 % -17.37 %) but on the progression of maturation the protein content shows a gradual decrease (17.37%- 14.8%). At the ripe stage the liver protein contents reduced to a minimum of (14.83 %).A recovery phase was occurred during the spent stage (15.94 %). As ovary attained maturity, the protein content gradually increased from immature to ripe stage (21.4 %-25.42 %) and reached its maximum value (25.42 %) at the ripe stage and

descended to a value of 22.4 % during the spent stage. The variations of protein percentage in muscle, liver and ovary through different months are given in Fig .4.5.

Lipid content

The variation of lipid content of muscle, liver and ovary during different stages of maturity was present in the table 4.6. Lipid content of muscle decreased from 2.3 % to 1.63 % during the different phases of maturation. Ripening stage (1.82 %) marked the thinning of lipid content of muscle and this pattern is followed till ripe stage (1.63 %). The spent stage showed a recovery phase with an increased value of (2.31 %).The lipid content pattern of liver showed a more or less gradual decrease during the reproductive cycle except a slight increase during first stage (Fig. 4.). The minimum liver lipid was present during the ripe stage (1.63 %).At the spent stage liver showed a recovery pattern attaining a value of (10.15 %).The ovary showed a tremendous increase in its lipid content accumulation during the maturation process (11.45 %-14.8%). The maximum lipid content was found in the ripe stage (14.8 %) and showed a steep decrease during the spent stage. The variations of lipid percentage in muscle, liver and ovary through different months are given in Fig. 4.6

From a close scrutiny of the results obtained in the investigations on the biochemical composition the following relationship were obvious. With the advent of maturation, in all the three species the lipid content and protein content increased in the gonad with a .corresponding decrease in lipid and protein content of muscle and liver. So a possible mobilization of lipid and protein from the liver and muscle can be suggested.

DISCUSSION

Maturation of gonads was carried out with significant changes in the chemistry of fish. The gradual accumulation of the different energy yielding substances in the somatic cells by anabolism and their subsequent catabolic processes for productive activities were the two distinct phases of development culminating in maturation and subsequent spawning. Major body components like protein and lipid undergo variations during the process of reproduction with an overall purpose of making the developing egg self sufficient. During the sexual resting period, females accumulated energy as perivisceral fat and , to a lesser extent ,into the liver (Gersande *et al.*1997). Fat stored in the body during the period of active growth and feeding is the ultimate source of energy expended by the fish during spawning. Masurekar and Pai (1979) and Dawson and Grimm (2006) have noted that much of the gonadal tissue were built from protein drawn from the musculature .

In the present study the variations in fat and protein content of muscle, liver and ovary of the three species *Puntius amphibius* (Val.), *Puntius parrah*, (Day) *Puntius sarana subnasutus* (Val.) and their inter relations were considered. In *Puntius amphibius* (Val.) The muscle protein content was comparatively high at the initial stages of maturation .Later protein content showed a decreasing tendency when maturation progressed. These differences signify the accumulation of protein in fish tissues which

could be transferred to gonads, prior to the spawning of fish. Similar results were obtained by Dawson and Grimm (1980) on the maturation process of female plaice *Pleuronectes platessa*. The liver protein content had a steady increase from stage I to stage II which was followed by sharp decrease as in *Clarius batrachus* (Yagana bano, 1976). In the ovary the protein content was gradually increasing and attained a peak value at the ripe stage, but displayed a significant decrease just after spawning as reported in *Perca fluviatilis* by Gersande *et al.* (1997). The increases in protein content of the ovary at different stages of maturation were related with the corresponded decrease in the muscle and liver protein content. This was in accordance with the findings of Love (1970), Medford and Mackay (1978), Sivakami *et al.* (1986) who thought that nutrients for germinal tissue growth may be drawn from somatic tissue.

The monthly variations of protein in muscles, liver and ovary of *Puntius amphibius* significantly correspond with the maturity stage of that month. The protein level in muscle was high during January, February, March, August which denotes the presence of immature ovaries or in other words during these months there was minimum translocation of protein towards ovary. While ovary begins to mature, the muscle protein content decreases gradually. This denotes the movement of protein to ovary. The minimum protein level was during the month of June which was corresponded to the full ripe ovaries. The liver protein content shows a little different pathway. The liver protein content shows an increase during the months of April and August which denotes a conduction and storing effect of protein from the somatic tissue for maturation of ovary where the following months call for heavy need of protein for the ovaries. The months of

May, June, September and October show significant thinning of liver protein corresponds with the proceeding of ovary maturation. The liver protein content showed minimum values during the months of June and October which denote the full ripening of the ovary and thus the spawning period. The ovary protein content showed a gradual increase and attained a peak value during the breeding months.

In *Puntius parrah* (Day) the values of protein contents were little higher and the mobilization pattern was resembles that of *Puntius amphibius* (Val.) .The month wise analysis of protein content of muscle, liver and ovary of *Puntius parrah* (Day) more or less follows that of *Puntius amphibius* since the pattern of ovary maturation and breeding seasons were coincides . In *Puntius sarana subnasutus* (Val.) along with the ovary maturation proceeds there was gradual thinning of muscle protein occurred. The liver protein utilization followed the same pattern of the other two species. The month wise variations of protein content in muscle, liver and ovary of *Puntius sarana subnasutus* (Val.) showed significant difference from the other two species *Puntius amphibius* (Val.) and *Puntius parrah* (Day) since the two peak spawning months are different .The muscle protein content was maximum during the months of January, February and March .The diminution of muscle protein from the months of April to July and October to November corresponds with the ovary maturation of two breeding seasons. During the months of August and December there was an increase in muscle corresponds with the two spent seasons. The liver protein content showed diminishing from May to July and September to November corresponded with the two breeding seasons .The ovary protein content showed a maximum value at July and November when the peak breeding season appeared.

The lipid content analysis of various parts like muscle, liver and ovary showed much variation in their lipid content corresponded with the progression of ovary maturation as reported by Mustafa dorucu (2000), Chellappa *et al.* (1989), Dawson and Grimm (1980). The muscle of *Puntius amphibius* showed depletion of lipid content when the maturation progresses as in *Leiognathus splendens* (Rao, 1967). The liver fat may mobilized to the ovary because it showed a tendency of reduction when the reproduction cycle was going on (Plack *et al.* 1971). These differences signify the accumulation of lipids in fish tissues which could be transferred to gonads, prior to the spawning. The ovary gather tremendous amount of lipids during its maturation process and attained a maximum value at the ripe stage. The monthly variations of lipid content percentage in muscle, liver and ovary exactly follows the maturation pattern of ovary .The muscle lipid content was maximum during the months of January, February, March, August and December, which corresponds with the sexually rest phase. The draining of muscle lipids starts from April and September corresponds with the two breeding seasons. The muscle lipid show maximum decline during the months of June and October when the ovaries maximum maturation , the ripe stage .The liver lipid content showed a inclination during the months of April and August .This rise may be corresponded with the storing phase of lipid for ovary maturation procedures. During the months of April to June and September to October the liver lipid content showed a declining pattern since the there was translocation of lipid to ovary. The minimum lipid content was seen in the months of June and October when the ovary attain ripe stage and spawning began.The showed a continuous increase in its lipid content from the month of March to June and August to

October which corresponded with the maturation phase and attained maximum value during June and October, the breeding seasons.

In *Puntius parrah* (Day) the lipid content was slightly higher in muscle and liver but ovary showed a slight depression at all the maturing stages when compared to *Puntius amphibius*. The month wise analysis of lipid percentage follows the same pattern as found in *Puntius amphibius* (Val.) since the breeding months coincides (June and October). *Puntius sarana subnasutus* topped in lipid content among three. The translocation of lipids into ovary was in greater percentage when compared to *Puntius amphibius* and *Puntius parrah*.) Rifaat (1984) suggested that lipid deposition and subsequent mobilization are associated with autosomal reproductive cycle. The monthly analysis of lipid content of muscle, liver and ovary showed slight variation since the peak breeding occurred at the months of July and November.

Fishes are grouped as fat fish and lean fish depending on the fat storing mechanism. Fat fish are described to store fat in the muscle while lean fish, in the liver (Love, 1970). In the present study *Puntius amphibius* was with a hepatic lipid at a range of 7.2 %- 9.8% which is much higher than the muscle fat ranged between 1.55 % and 2.1% can be included under lean fish. The *Puntius parrah* was with a hepatic fat content range 8.5 %- 10.3 % where the muscle fat ranged between 1.6 % and 2.3 % so it is included under lean fish. *Puntius sarana subnasutus* also belongs to lean fish category since the muscle protein content ranged between 2.1 % to 2.4 % and liver fat between 8.9 % and 11.2 %.

Table 4.1 Variations of protein percentage in muscle, liver and ovary of *Puntius amphibius* during different maturity stages

Tissue	Maturity stages				
	Immature	Maturing	Ripening	Ripe	Spent
Muscle	17.61 ± .2	18.26± .4	17.14 ± .2	16.6 ± .2	15.85 ± .3
Liver	16.23 ± .3	17.13 ± .2	14.51 ± .2	14.13 ± .3	14.67 ± .2
Ovary	21.82 ± .1	24.23 ± .1	25.47 ± .2	26.42 ± .1	22.55 ± .1

Table 4.2 Variations of lipid percentage in muscle, liver and ovary of *Puntius amphibius* during different maturity stages

Tissue	Maturity stages				
	Immature	Maturing	Ripening	Ripe	Spent
Muscle	2.12 ± .2	1.92 ± .4	1.74 ± .2	1.63 ± .2	1.75 ± .3
Liver	9.35 ± .3	9.84 ± .2	7.91 ± .2	7.24 ± .3	8.91 ± .2
Ovary	12.86 ± .1	13.13 ± .1	13.62 ± .2	14.22± .1	12.2 ± .1

Table 4.3 Variations of protein percentage in muscle, liver and ovary of *Puntius parrah* during different maturity stages

Tissue	Maturity stages
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	Immature	Maturing	Ripening	Ripe	Spent
Muscle	18.82 ± .3	18.6 ± .4	17.91 ± .2	17.35 ± .3	16.18 ± .1
Liver	17.14 ± .2	18.46 ± .2	15.72 ± .2	15.76 ± .3	16.63 ± .2
Ovary	22.57 ± .1	24.65 ± .4	25.52 ± .2	26.9 ± .1	22.51 ± .2

Table 4.4 Variations of lipid percentage in muscle, liver and ovary of *Puntius parrah* during different maturity stages

Tissue	Maturity stages				
	Immature	Maturing	Ripening	Ripe	Spent
Muscle	2.14 ± .2	1.92 ± .4	1.8 ± .2	1.59 ± .2	1.91 ± .1
Liver	9.2 ± .1	10.32 ± .3	8.3 ± .1	8.07 ± .3	9.13 ± .3
Ovary	10.4 ± .1	12.4 ± .1	12.9 ± .2	13.64 ± .4	11.6 ± .1

Table 4.5 Variations of protein percentage in muscle, liver and ovary of *Puntius sarana subnasutus* during different maturity stages

Tissue	Maturity stages				
	Immature	Maturing	Ripening	Ripe	Spent
Muscle	20.16 ± .1	19.52 ± .4	18.84 ± .2	18.33 ± .4	19.45 ± .2
Liver	16.14 ± .2	17.37 ± .2	15.9 ± .1	14.83 ± .3	15.94 ± .1
Ovary	21.4 ± .1	23.16 ± .1	23.91 ± .3	25.42 ± .1	22.43 ± .2

Table 4.6 Variations of lipid percentage in muscle, liver and ovary of *Puntius sarana subnasutus* during different maturity stage

Tissue	Maturity stages				
	Immature	Maturing	Ripening	Ripe	Spent
Muscle	2.34 ± .1	2.25 ± .4	1.82 ± .2	1.63 ± .2	2.31 ± .1
Liver	10.62 ± .1	11.24 ± .3	9.24 ± .1	8.92 ± .3	10.15 ± .3
Ovary	11.45 ± .3	13.7 ± .1	14.1 ± .2	14.8 ± .4	12.1 ± .1

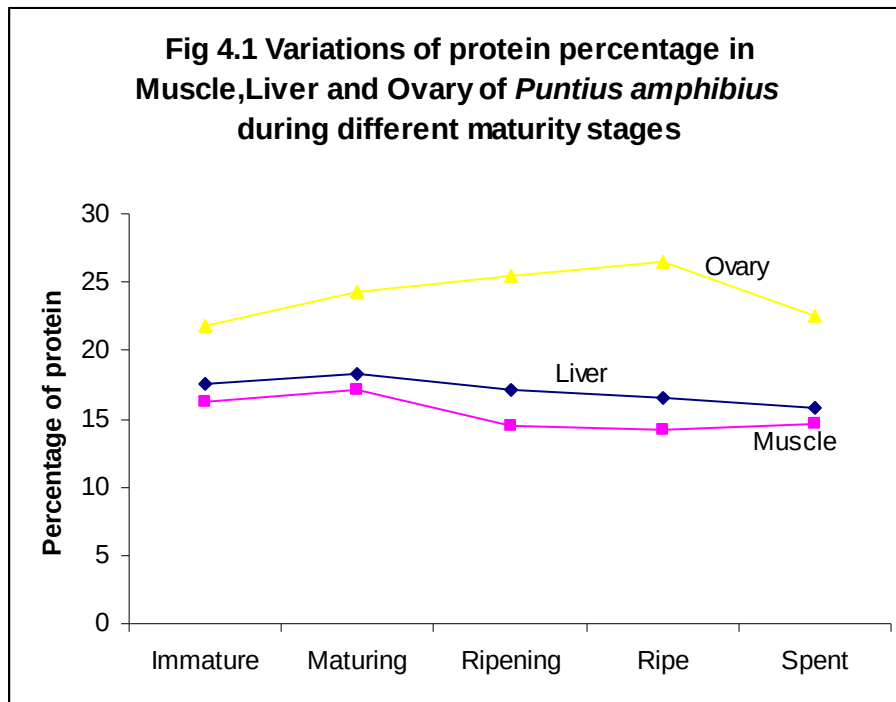


Fig 4.2 Variations of Lipid percentage in Muscle, Liver and Ovary of *Puntius amphibius* during different maturity stages

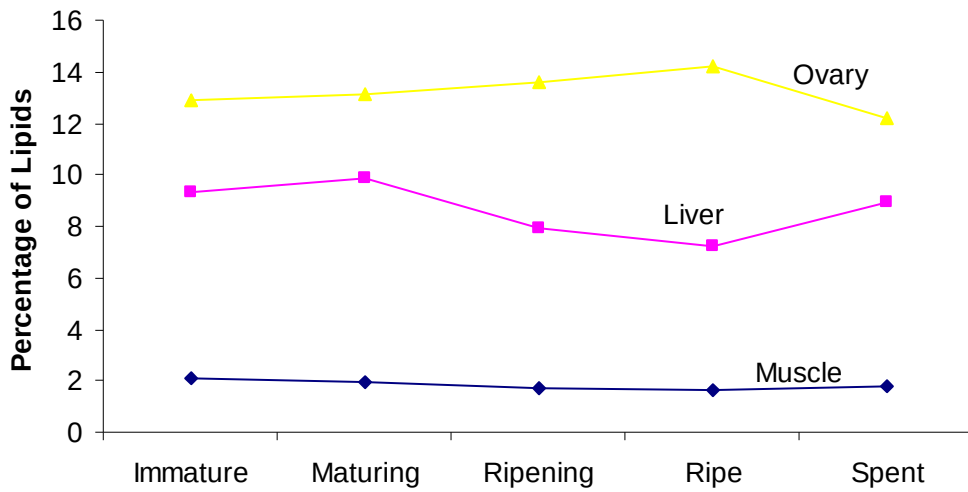


Fig 4.3 Variations of Protein percentage in Muscle, Liver and Ovary of *Puntius parrah* during different maturity stages

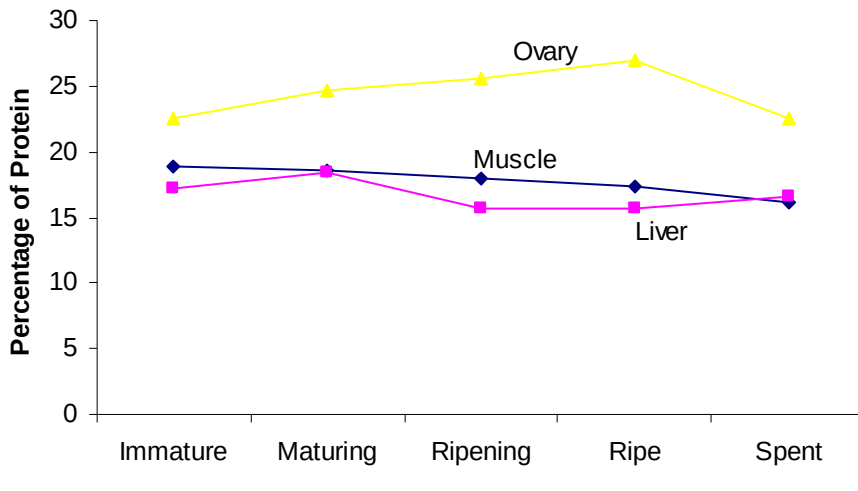


Fig 4.4 Variations of Lipid percentage in Muscle ,Liver and Ovary of *Puntius parrah* during different maturity stages

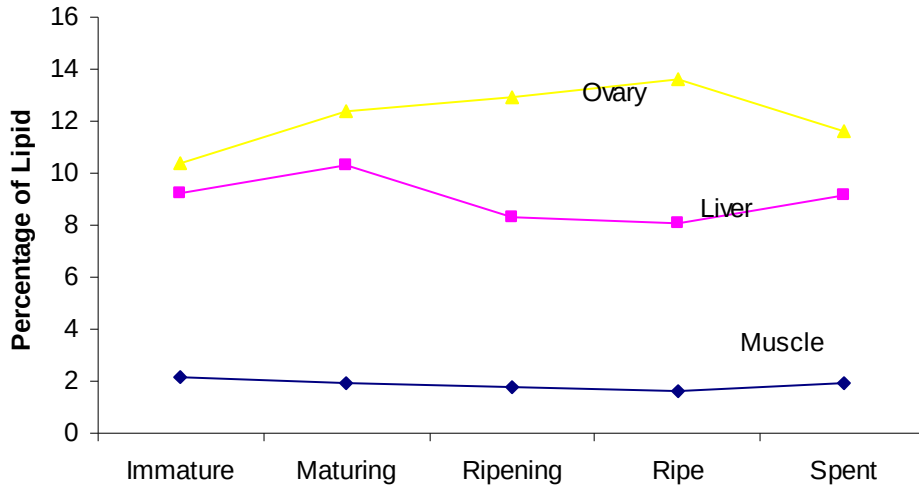


Fig 4.5 Variations of Protein percentage in Muscle,Liver and Ovary of *Puntius sarana subnasutus*

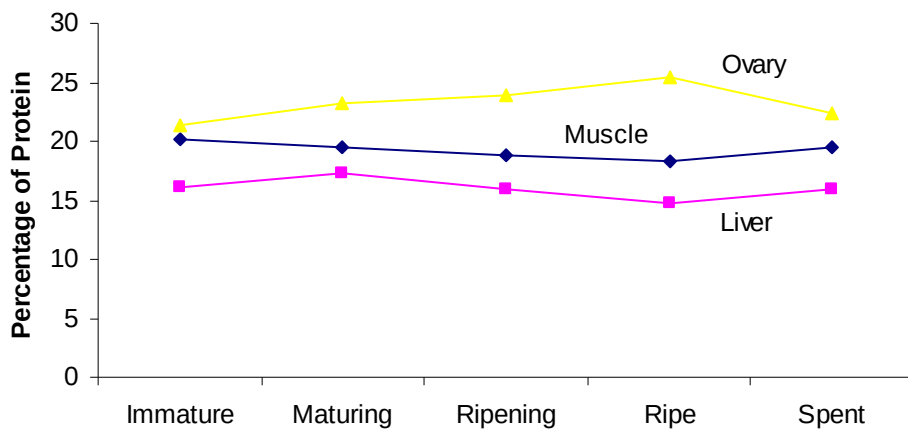
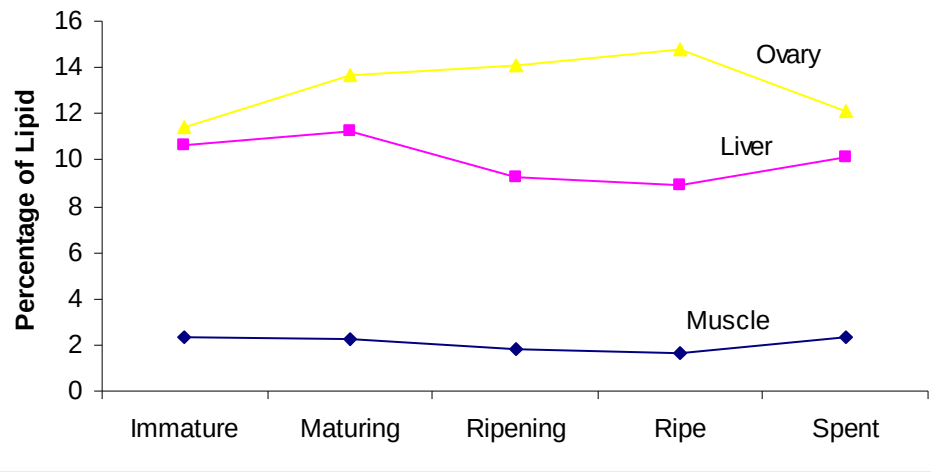


Fig 4.6 Variations of Lipid percentage in Muscle, Liver and Ovary of *Puntius sarana subnasutus*



CHAPTER - 5

SEXUAL

DIMORPHISM

CHAPTER - 5

SEXUAL DIMORPHISM

INTRODUCTION

Generally, the influence of gender is not considered in the studies of fish biology. It has long been known that sexual dimorphism is a common characteristic of many fishes worldwide. As such, further investigations into sexual dimorphisms could improve understanding of the autecology of fishes and dynamics of fish populations. The fine morphological difference between the two sex groups of the same age composition within a species is called as sexual dimorphism. Secondary sexual characters are very important as far as sex determination in fishes is considered. Identifying sexually dimorphic traits has led to appropriate species descriptions, as well as studies investigating mate choice, male antagonism, intra sexual selection or other sex characteristics (Hopkins *et al.*,1990 ., Anderson, 1994).

The knowledge about the morphological difference between the sexes of fish species is mainly need for applied works like induced breeding, fish behavior study, fish seedling production, fish genetics and fish physiology. The male and female partners should place in pairs during induced breeding and fish seedling procedures. Therefore, scientists and

farmers should know how to segregate the male and female of a particular species. A study on sexual dimorphism is also helpful in fish taxonomy.

Differences in the selective pressures experienced by the sexes can result in the evolution of sexual dimorphism of morphological traits (Casselman and Schulte, 2004). There are three classes of sexual morphism., monomorphic fish show no differences in form or colour between the sexes, fish that are temporarily dimorphic or dichromic show differences in colour and or form during the breeding season or differences in colour just during courtship and or spawning, permanently dimorphic, or dichromic fish always different in colour and or form.

The patterns of sexual dimorphism in fish are often a reversal of that are seen in other taxa, where males being larger than females with female fecundity in fish often co-varying with body size, selecting for larger females, which often out grow males (Magurran and Garcia, 2005). In species that are sexually dimorphic in body size, the extent of sexual dimorphism may be closely linked to the mating system, as in polygamous species males have a much larger reproductive potential than females, leading to sexual selection. This can take the form of intra sexual selection, predominantly through male-male competition for access to females or inter sexual selection, predominantly through female choice for high quality males. Sexual selection may result in sexual dimorphism in body size (Anderson, 1994) as seen in a number of fish families including Cyprinidae, Gobidae, Labridae, Salmonidae and Crotalidae. However patterns of sexual dimorphism are complex with some of the most extreme

examples coming from monogamous species like deep sea fishes of the genus *Photocurrents* (Mugurran and Garcia, 2000). Female fish can often be larger than males because of a tendency to be more motile (Jonsson and Jonsson, 2006). Comparative analyses of sexual size dimorphism across species have led to the discovery of Rensch's rule. This rule states that sexual size dimorphism increases with body size when males are the largest sex, but decreases with increasing size when females are larger. Body-size dimorphisms were frequently related to gonadal size differences (Downhower et al. 1983., Parker 1992). A divergence in body size between sexes could be related to different reproductive investments, which promotes a geometric relationship between female body size and fecundity where in larger females produce exponentially more ova (Benton 1987). Consequently, natural selection is most likely to favor females that maximize fitness by growing to the largest sizes. Yet, sperm count of males is less dependent on body size and growth., thus, males maximize reproductive fitness by alternative measures.

Sexual dimorphism in nuptial colouration has been reported in a number of fish families including, Cyprinidae, Cyprinodontidae, Percidae and Poeciliidae. The colour dimorphism in fishes can split into three groups colour difference, small difference and no difference. The extent of sexual dimorphism in nuptial colouration may depend on the seasonality of breeding. Poecillides have more or less continuous colouration, which expressed through out the year as in guppies (Houde, 1997) .

Length and structure of the fins vary significantly between males and females in many species. Thus, in *Labeo dero*, dorsal fin in the mature male is highly elongated (Biswas

et al. 1982). In some other forms, the tips of the dorsal spine of males become club shaped whereas female possess normal dorsal spine. Bright, horny tubercles (*Labeo pangusia*), hook shaped jaws (humped back salmon) are some of the features by which males can be distinguished from the female specimens. Courtenay (1971) described in detail sexual dimorphism of the sound producing mechanism of the shallow water striped cusk eel, *Ophidian marginata* and described that it exhibits external sexual dimorphism in the form of an occipital hump in males. The present study analyses the sexual dimorphism present in *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.).

Materials and Methods

Fresh specimens of *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.) collected from different spots along the Chalakudy River were brought to the laboratory. Gender was determined by dissection and visual examination of the gonads. The gender of very small individuals could often not be discerned and thus were not used for this study. The males and females of same age group were selected for the comparison. The weights (g) of all fish were measured, and the total lengths (TL, mm) recorded. The eye diameter, maximum width and inter orbital space were measured.

Then the fine morphological differences between male and female groups were studied and recorded.

RESULTS

Sexual dimorphism in

(a). *Puntius amphibius* (Val.)

Clear sexual dimorphism was noted in *Puntius amphibius*. Considerable difference in total length is noted among males and females of same age group (Plate 5.3). A sharp difference in body weight exists between two sex groups. Males are slender when compared to females. Females exceed males in all body parameters like total length, body weight, maximum body width (Table 5.1.). The opercular bone in the male has a dark red colouration, but opercular bone in female has no prominent red colour. In males, the eyes are brightly orange-red and about eight out of ten part of orbital margin (Plate 5.1). Caudal black spot is brighter in males than females. The scarlet coloured band is more sharp and bright in males than females (Plate 5.2). The central part of caudal fin is more coloured in males than females. So male can be easily distinguishable from female of same length age group in the population.

(b). *Puntius parrah* (Day)

Sexual dimorphism was prominent in *Puntius parrah*. Considerable difference in total length was noted among males and females of same age group (Plate 5.6). Total body weight was more in female than males when both sexes of same age group were

considered. Males were slender when compared to females. Females exceeds male in all body parameters like total length, bodyweight, maximum body width (Table 5.2.) The lateral line area is decorated with an orange red colouration in males (Plate 5.4). The pelvic fins of male are more yellowish than females. The tip of pelvic fins are bright yellow in males where they are little pale in females(Plate 5.5). The anal fins are a yellowish shade in males where they are pale in females. In females the orange red colour is extended up to the black spot at the caudal region where in males the colour spread more around the black spot. Caudal black spot is brighter in males than females. The central part of caudal fin is more coloured in males than females. So male can be easily distinguishable from female of same length age group in the population.

(c). *Puntius sarana subnasutus* (Val.)

Sexual dimorphism in *Puntius sarana subnasutus* is moderate. In the same age group females overtakes males in all morphological measurements slightly (Table 5.3 & Plate 5.9).In male there was a golden orange mark present on the gill opening of both sides which was absent In female (Plate 5.7).The pectoral fins of males are with orange colour in their throughout length where in female the pectoral fins are in very pale yellow. The ventral and anal fins are deep orange in colour throughout the length in males. The female ventral and the anals are orange at the tip only (Plate 5.8). In female the inner marginal caudal fin is tinged with Orange where in male the caudal fin is dull coloured .

Discussion

Many fish species show sexual dimorphism, a condition where males and females are different in form and or colouration, thus the sexes can distinguished externally. Sexual dimorphism in Indian major carps is prominent during the breeding season. The male is generally having rough pectoral fins and comparatively slender body while the females are easily recognizable by their bulging abdomen and protruding vent. In fact, most of the carp exhibits similar features during spawning (Mohanta *et al.*, 2008)

Sexual dimorphism, with females attaining larger body sizes than males was studied in different fish species by many workers (Winemiller and Rose 1992 .,Love 2002_).Female size depends on fecundity and male size on male-male competition. If sperm competition is very weak, small males are produced (Joseph Love, 2000). Increase in sperm competition increases male size, but only with high numbers of competing males male size approach that of females. In *Puntius amphibius* the male are noticeably shorter and leaner than females which are comparatively stout. Small sized males were produced in the population either they attain maturity earlier and also have shorter life span or the sperm competition is minimum. Since within population, the female male ratio is near 1:1.85, so naturally minimum competition may exist between the males within the population. In *Puntius parrah* the males are comparatively shorter and linear than females.The length at first maturity in male lies within the range of 56 mm to 67 mm where female attains sexual maturity at about 82 mm onwards. The female to male ratio found to be near 1:1.78 .The small sizes of males may be due to the lack of sperm

competition because females are more in number within in the population. In *Puntius sarana subnasutus* male female length difference was not so prominent even though males are slightly shorter than females. But the male to female ratio is significantly low, lies in the range of 1:1.9

Size dimorphism is also affected by the ratio growth rate and mortality rate in each sex. If this is higher in one sex, its size increases (Parker, 1992) .The larger body size is said to be a protective adaptation (Nikolsky, 1963) and usually the larger parent protects the offspring (Lowe-McConnell, 1969).Body size dimorphism was often relate to differences in gonad size in fishes (Downhower *et al.*, 1983). Female gonads were significantly longer than testis in *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus*

The extent of sexual dimorphism in nuptial colouration may depend on the seasonability of breeding. Most of the *Puntius* species members show nuptial colouration during breeding season .In *Puntius amphibius* the main differences in nuptial colour were the following., the opercular bone in the male has a dark red colouration, but opercular bone in female has no prominent red colour, the male eyes were brightly orange-red and about eight out of ten part of orbital margin , the caudal black spot was more bright in males than females the scarlet coloured band is more sharp and bright in males than females and the central part of caudal fin is more coloured in males than females . Harikumar (1992) reports that in *Puntius melanampyx* males are generally darker with blackish dorsal and anal fins., females have dark fin tips only. In *Puntius parrah* in males

the pelvic fins are more yellowish in males than females. The tip of pelvic fins are bright yellow in males where they are little pale in females. The anal fins are a yellowish shade in males where they are pale in females. In females the scarlet colour is extended up to the black spot at the caudal region where in males the colour spread more around the black spot. Caudal black spot is brighter in males than females. The central part of caudal fin is more coloured in males than females. In *Puntius sarana sabnasutus* In male there is a special golden orange mark present on the gill opening of both sides. In male the pectoral fins are with Orange colour in their throughout length where in female the Pectoral fins are very pale yellow in colour and. Male ventral fins and anal fins are deep orange in colour throughout the length, where female ventrals and the anals are orange at the tip only. The nuptial colouration in these species well marked during the breeding season as reported in three spine sticklebacks *Gasterosteus aculeatus* (Wootton,1979).This may partly because of the signals are energetically costly to produce .Thus it can be seen that practically every part of the male affected, naturally different parts in different groups of fishes. Morphological differences especially intense nuptial colouration, between the sexes may be attributed to over activity of male hormone. Sexual dimorphism and colouration are two important aspects of orientation and selection of mates and therefore, both play important roles in courtship behavior in fishes.

Table 5.1 Comparison of morphometric details of male and female *Puntius amphibius*

Morphometric details	Male	Female
Total length (mm)	71	105
Total weight (g)	10.652	14.568
Maximum width (mm)	19	25
Eye diameter (mm)	5	6
Inter orbital space (mm)	7	8

Table 5.2 Morphological differences between two sex groups of *Puntius amphibius*

MALE	FEMALE
Short and slender	Tall and heavy
Operculum has a dark red colouration	No red colour on operculum
Eyes are in bright orange red	Eyes are dull coloured
The scarlet band is prominently bright and sharp	The scarlet band is less bright and sharp



Plate 5.1 *Puntius amphibius* – Male



Plate 5.2 *Puntius amphibius* - Female



Plate 5.3 *Puntius amphibius* – Female and Male

Table 5.3 Comparison of morphometric details of male and female *Puntius parrah*

Morphometric details	Male	Female
Total length (mm)	75	110
Total weight (g)	12.358	16.954
Maximum width (mm)	21	27
Eye diameter (mm)	5	6
Inter orbital space (mm)	7	9

Table 5.4 Morphological differences between two sex groups of *Puntius parrah*

MALE	FEMALE
Short and lean	Tall and heavy
The orange red colouration along the lateral line is bright	The colouration is less bright
The pelvic fins are brightly yellowish	Pelvic fins are in pale yellow
Caudal black spot is very bright	Caudal black spot less bright
The anal fins yellow shade	Anal fins are pale



Plate 5.4 *Puntius parrah* - Male



Plate 5.5 *Puntius parrah* - Female



Plate 5.6 *Puntius parrah* – Female and Male

Table 5.5 Comparison of morphometric details of male and female *Puntius sarana subnasutus*

Morphometric details	Male	Female
Total length (mm)	141	173
Total weight (g)	60.85	89.35
Maximum width (mm)	42	51
Eye diameter (mm)	8	9
Inter orbital space (mm)	13	14

Table 5.6 Morphological differences between two sex groups of *Puntius parrah*

MALE	FEMALE
A bright golden orange red mark on gill opening	The gill openings are less bright
The pectoral fins are orange in colour	The pectoral fins are orange at the tip only
The ventral and anal fins are orange in colour	Ventral and anal fins are orange at the tip only
The caudal fin is dull coloured	The inner margin of caudal fin is tinted with orange.



Plate 5.7 *Puntius sarana subnasutus* – Female



Plate 5.8 *Puntius sarana subnasutus*- Male



Plate 5.9 *Puntius sarana subnasutus* –Female and Male

CHAPTER- 6

SEX RATIO

CHAPTER- 6

SEX RATIO

INTRODUCTION

Sex - ratio indicates the comparison of number of males and females in a population. It is an important parameter largely determining adaptation, as well as the direction and rate of genetic processes in animal population. An understanding of the sex ratio in a fish in different months and seasons is essential for obtaining information on seasonal segregation of the sexes and their relative abundance in spawning season and reveals the differential growth of the two sexes also. It indicates features such as the movement of sexes in relation to season, strength of spawning stock, catch composition etc and also an indicator of population behavior and fecundity (Panthalu, 1961). A proper knowledge sex ratio is important in the management of fishery.

In natural environment the optimum sex -ratio is usually close to 1:1, however, it is some times shifted towards predominance of one or the other sex. In the younger groups, the males predominate because they tend to mature earlier than females. Departure from 1:1 sex ratio is not expected for most fish species, although some fish populations may present a strong bias in this ratio. Such difference may be attributed to various causes, namely temperature influences on sex determination, selective mortality by sex through differential predation, and differentiated sexual behavior, growth rate, or longevity expectations. Lower oxygen levels in polluted waters could lead to a higher ratio of male

fish that may threaten certain species with extinction. The changing sex ratio may be associated with the shoaling habits of fish which might be a contributing factor for the dominance of either of sex in catch composition of different days.

Sex ratio constitute information basic in assessing reproductive potential, the movement of sexes in relation to season, strength of spawning stock, catch composition and estimating stock size populations (Vazzoler,1996). According to Nikolsky (1980), the optimum sex ratio may vary drastically as a result of being affected by numerous factors. He reported the predominance of females in fishes, especially in fishes where the males produce several batches of sperms but females produce only one batch of ova. Different populations inhabiting different regions show different sex ratios (Nikolsky, 1963).A higher sex ratio has been reported during the first breeding season and a lower sex ratio in second breeding season when the water parameters are their peak.The sex ratio distribution in different months or seasons may help in estimating stock size by fecundity method. Sex composition in a fish population might be affected by many factors like segregation of sexes through periods of the year including segregation resulting from sex differences in age size at maturity and the sex which exhibits a faster growth rate, will be less affected by predation and this would influence sex ratio. In a population both intra and inter specific competition for food and space may also affect the sex ratio. Arising temperature and moderate water velocity, vulnerability of females to their predators and other natural hazards, migratory phase in brooder population are some of the reasons for the changes in the sex ratio in fishes. Higher temperatures favor the formation of males whereas lower temperatures favor the formation of females (Conover and Kynard, 1981.,

Middaugh and Hemmer, 1987.,Strussmann *et al.*, 1995). Sex ratio was calculated out to know the ratio in the number of male and female fishes, so that appropriate number of male and female fishes can be mated during artificial spawning and to devise means of ensuring a proportional fishing of the sexes. Knowledge on the identification of the two sexes is very essential in ornamental fish management and marketing since fishes are kept in aquaria in pairs.

Many workers like Sobhana and Nair (1976), De Silva and Kortmulder (1977), Premkumar and Subramanian (1984), Islam and Hussain (1990), Kumar and Siddiqui (1991),and Rao *et al.*(1999)studied the sex ratios in freshwater fishes. The present study is aimed at an understanding the sex composition of the three species of fishes *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.)

MATERIALS AND METHODS

The collected fishes were examined and sort out as males and females in the lab to determine the sex ratio. The total length was measured to the nearest 0.1 mm. For the sex-ratio (S_R) evaluation, the following ratio was used,

$$S_R = M / M + F$$

(M = number of males, F = number of females, M + F = total number of individuals)

The sex-ratio distribution was studied to test whether the observed sex ratio in each month and individual length group differs significantly from the expected ratio, the chi-

square test applied to check the sex ratio. The equation for Chi-square test is the following

$$\text{Chi square} = (O - E)^2 / E$$

Where E = Expected number of males and females in each month

O = Observed number of males and females in each month

RESULTS

Sex Ratio in *Puntius amphibius* (Val.)

There were 1478 specimens of *Puntius amphibius* (Val.) were examined in the lab to determine sex ratio. Out of these 1478 fishes examined, 517 were males and 961 females (Table 6.1). The month wise distribution of the two sexes Fig 6.1 revealed that the sexes were disproportionate on the sample population. Females outnumbered males in almost all months. Chi-square test confirmed that females showed dominance in most of the months. Though there was considerable variation in the distribution of sexes in some of the months of both the years the overall sex-ratio showed significant dominance of females ($p < 0.01$). The mean ratio of male to female was 1:1.85 for the pooled data for the period April 2003 - March 2005 and the respective chi-square value 66.6901 lend support to the above observations that the sex-ratio significantly skewed from the expected 1:1 ratio ($p < 0.01$). During spawning season, the ratio skewed significantly in the months of June and October. The ratio significantly skewed in almost all the months except December, January and August ($p < 0.01$) Table 6.2 shows the variation in sex ratio among the various size groups. Males were dominant up to 80 mm TL and thereafter females become dominant. Beyond 90 mm mainly females were encountered. Chi-

square values indicated that there was significant variation from 1:1 ratio in most of the size groups. Between 81-85 mm T l the ratio did not skew significantly from the expected values. The chi-square values of 66.69 for the overall sex ratio showed that the variations were highly significant.

Sex ratio in *Puntius parrah* (Day)

There were 1053 specimens of *Puntius parrah* (Val.) were examined in the lab to determine sex ratio. Out of these 1053 fishes examined, 377 were males and 676 females. The month wise distribution of the two sexes (Table 6.3) revealed that the sexes were disproportionate on the sample population. Females outnumbered males in almost all months. Chi-square test confirmed that females showed dominance in most of the months. Though there was considerable variation in the distribution of sexes in some of the months of both the years the overall sex- ratio showed significant dominance of females ($p < 0.01$). The mean ratio of male to female was 1:1.79 for the pooled data for the period April 2003 - March 2005 and the respective chi-square value 42.45 lend support to the above observations that the sex-ratio significantly skewed from the expected 1:1 ratio ($p < 0.01$). During peak spawning months of May, June and October the females predominantly dominates over males.

Male dominant up to the length group of 76-80 mm T l .Beyond 90 mm mainly females were encountered (Table 6.4) .Chi-square values indicated that there was significant variation from 1:1 ratio in the all the size groups except between 86-90 mm T l .The chi-square values of 42.4506 for the overall sex ratio showed that the variations was highly significant.

Sex ratio in *Puntius sarana subnasutus* (Val.)

A total of 568 specimens of *Puntius sarana subnasutus* (Val.) were examined in the lab to determine sex ratio. Out of these 568 fishes examined, 196 were males and 372 females (Table 6.5). The month wise distribution of the two sexes (Fig 6.3) revealed that the sexes were disproportionate on the sample population. Females outnumbered males, in all months. Chi-square test confirmed that females showed dominance in all the months. The overall sex-ratio showed, significant dominance of females ($p < 0.01$). The mean ratio of male to female as 1:1.9 and the respective chi-square 56.81647 lend support to the above observations that the sex-ratio significantly skewed from the expected 1:1 ratio ($p < 0.01$). During peak spawning seasons of July October and November, the ratio skewed significantly. Table 6.6 shows the variation in sex ratio among the various size groups. Males were dominant up to 140 mm TL and thereafter females become dominant. Beyond 200 mm mainly females were encountered. Chi-square values indicated that there was significant variation from 1:1 ratio in almost all the size groups except between 161-170 mm TL. The chi-square values of 27.26761 for the overall sex ratio showed that the variations were highly significant.

DISCUSSION

Considerable variation from the ideal sex-ratio 1:1 was observed in the cases of *Puntius amphibius*, *Puntius parrah* and *Puntius sarana subnasutus*. It was found that the number of females were more than that of males which is supported by the findings of other

authors working with different species such as Afroz *et al.* (1990) in case of *Amblypharygodon mola*, Mortuza and Mokarrama (2000) on *Botia lohachata*. Chandrasoma and De Silva *et al.* (2008) reported a slightly female dominant sex ratio in *Puntius sarana sarana* (1:1.5) and Nasar and Biswas (1987) reported a 1:1.4 male female sex ratio in *Puntius clavattus*, where highly female dominant sex ratio was present in *Puntius stigma* (1: 2.12).

The month wise distribution of the two sexes revealed that the sexes were disproportionate on the sample populations. In *Puntius amphibius* male female sex ratio was found to be 1:1.85 which was significantly different from the ideal ratio 1:1 where the male female ratio was 1:1.79 and 1:1.9 in *Puntius parrah* and *Puntius sarana subnasutus* respectively. This was in agreement with the findings of (Cek *et al.* 2001) in *Puntius conchoniis*. In *Puntius amphibius* a preponderance of females over the males was observed throughout the year except in August, January and February when the ratio was nearly 1:1. Out of 1478 specimens sampled during the study, 961 were females and 517 were males, giving an overall sex ratio of 1: 1.85 which significantly deviated from the hypothetical distribution of 1:1. The chi-square test at 0.95 significant levels and at 1 degree freedom revealed that the chi-square values were significant during most of the months except August, January and February. The insignificant chi square values were corresponded to the resting phase of the gonads.

In *Puntius parrah* a dominance of females over the males was observed throughout the year except in January when the ratio was nearly 1: 0.85. Out of 1053 specimens sampled during the study, 676 were females and 377 were males, giving an overall sex ratio of 1: 1.79 which significantly deviated from the hypothetical distribution of 1:1. The chi-

square test at 0.95 significant levels and at 1 degree freedom revealed that the chi-square values were significant during most of the months except January. The significant chi square values were corresponded to the resting phase of sexual cycle. In *Puntius sarana subnasutus* a preponderance of females over the males was observed throughout the year. Out of 568 specimens sampled during the study, 372 were females and 196 were males, giving an overall sex ratio of 1: 1.90 which significantly deviated from the 1:1 ratio. The chi-square test at 0.95 significant level and at 1 degree freedom revealed that the chi-square values were significant during most of the months except January and February where the corresponding values were 0.6944 and 0.6097 respectively.. The significant chi square values were corresponded to the reproductive rest. The predominance of males during these months may be due to the faster rate of growth of males. The out numbering of females may be probably due to sexual segregation, selective migration, differential collection or perhaps genetic conditions.

The sex ratio in the spawning population and in the various age and size groups varies with the species, reflecting the relation ship of that species to its environment. The sex structure is also adaptive to the food supply, which thereby influence the reproductive rate and the variability of the offspring. The sex distribution is very important to the reproduction of a population, and consequently there were mechanisms for adjusting this structure to any changes and specially changes in food supply. The last is itself dependent on the relationship of sex ratio to density .

In *Puntius amphibius* and *Puntius parrah* during the months of May, June, October and November which are the peak breeding periods the sex ratio highly skewed towards

female and the corresponding chi square values were very high and significant considerably. In *Puntius sarana subnasutus* the months of July and November showed high female male ratio and the corresponding chi-square values were also high. Relative abundance of females in comparison to males in the catch staics during the breeding season has been reported for another cyprinid fish, *Labeo fimbriatus* (Bhatnagar, 1972). During the spawning season, the females of these three species perhaps become heavy, due to the bulkier ovaries and get trapped easily, when a medium sized cast net is operated. Further it is said that during the spawning season female become lethargic and hence get caught more easily than the males which are more agile and comparatively slender (Bhatnagar, 1972). A definite ratio of males and females during the spawning season is a prerequisite for most effective fertilization of eggs deposited by spawning females.

The higher occurrence of males in lower and females in higher size groups was observed in *Puntius amphibius*, *Puntius parrah* and *Puntius sarana subnasutus* were corroborating with the findings in a number of fish species. (Bennet, 1962., Bailey, 1964., Bhatnagar, 1972., Chaturvedi, 1976., Siddiqui *et al* .1976 , Somavanshi, 1985., Vinci and Sugunan, 1981). Variation in sex ratio at different sizes and age groups exists even in species with in overall 1:1 ratio. In *Puntius amphibius*, *Puntius parrah* and *Puntius sarana subnasutus* in size wise, males were found to mature earlier than females. Siddiqui *et al*. 1976 stated that the predominance of females in higher groups might be due to high mortality of males in smaller size groups either due to natural death or fishing pressure as they were more active and caught more easily or more exposed to predation. According to Qasim

(1966) the display in growth rate between sexes led to the preponderance of one sex and the preponderance of sex attains a bigger size.

The sex ratio analysis of the present study was in agreement with the findings of De Silva *et al.* (1985) indicating a predominance of males in the lower size classes and the predominance of females in the higher classes, as reported in *Puntius vitatus* (Jameela Beevi and Ramachandran, 1990) and *Puntius bimaculatus*. In *Puntius cumingi* and *Puntius dorsalis* (Sivakami, 1982), the sex ratio approximates to unity in the lower size classes, but females predominate in the higher length groups, where in *Puntius nigrofasciatus* male predominantly significant in the higher size classes.

In most cases a greater increase in length of one sex is associated with a greater longevity. This may in itself be due to the influence of intrinsic factors upon the individuals of the population. Faster growing sex may be that which is better adapted physiologically and thus the intrinsic factors will also affect the sex ratio in favor of the sex growing faster.

Table 6.1 Monthly distribution of sexes in *Puntius amphibius*

Month	Total No.	Male	Female	Ratio of males to females	Chi square test	Remarks
April	132	38	94	1:2.4736	11.87878788	S
May	164	58	106	1:1.8275	7.024390244	S
June	221	76	145	1:1.9078	10.77149321	S
July	162	58	104	1:1.7931	6.530864198	S
Aug	44	25	19	1:0.76	0.409090909	N S
Sep	76	30	46	1:1.5333	1.684210526	S
Oct	252	66	186	1:2.8181	28.57142857	S
Nov	210	70	140	1:2	11.66666667	S
Dec	42	17	25	1:1.4705	0.761904762	N S
Jan	32	18	14	1:0.7777	0.25	S
Feb	69	35	34	1:0.9714	0.007246377	N S
March	74	26	48	1:1.8461	3.27027027	S
Total	1478	517	961	1:1.8588	66.69012179	S

Table 6.2 Lengthwise distribution of sexes in *Puntius amphibius*

Total length (mm)	Male	Female	Expected value	Chi square test	Remarks
60-65	92	0	46	46	S
66-70	98	0	49	49	S
71-75	157	0	78.5	78.5	S
76-80	99	0	49.5	49.5	S
81-85	44	46	45	0.022222	N S
86-90	29	83	56	13.01786	S
91-95	0	184	92	92	S
96-100	0	161	80.5	80.5	S
101-105	0	209	104.5	104.5	S
106-110	0	194	97	97	S

111-115	0	64	32	32	S
116-120	0	20	10	10	S
	519	961	739	66.69012	S

Table 6.3 Monthly distribution of sexes in *Puntius parrah*

Month	Total No.	Male	Female	Ratio of males to females	Chi square test	Remarks
April	105	38	67	1:1.7631	11.87878788	S
May	110	34	76	1:2.2352	7.024390244	S
June	108	28	80	1:2.8571	10.77149321	S
July	88	26	62	1:2.3846	6.530864198	S
Aug	40	18	22	1:1.2222	0.409090909	N S
Sep	48	20	28	1:1.4	1.684210526	S
Oct	158	60	98	1:1.6333	28.57142857	S
Nov	120	40	80	1:2	11.66666667	S
Dec	80	28	52	1:1.8571	0.761904762	N S
Jan	76	41	35	1:0.8536	0.25	S
Feb	58	24	34	1:1.4166	0.007246377	N S
March	62	20	42	1:2.1	3.27027027	S
Total	1053	377	676	1:1.7931	66.69012179	S

Table 6.4 Lengthwise distribution of sexes in *Puntius parrah*

Total length (mm)	Male	Female	Expected value	Chi square test	Remarks
60-65	38	0	19	19	S
66-70	55	0	27.5	27.5	S
71-75	69	0	34.5	34.5	S
76-80	112	0	56	56	S
81-85	87	10	48.5	30.56186	S
86-90	16	18	17	0.058824	NS
91-95	0	112	56	56	S
96-100	0	89	44.5	44.5	S
101-105	0	151	75.5	75.5	S
106-110	0	170	85	85	S

111-115	0	64	32	32	S
116-120	0	37	18.5	18.5	S
125-130	0	25	12.5	12.5	S
Total	377	676	526.5	42.45062	S

Table 6.5 Monthly distribution of sexes in *Puntius sarana subnasutus*

Month	Total No.	Male	Female	Ratio of males to females	Chi square test	Remarks
April	38	13	25	1:1.9230	5.769231	S
May	41	14	27	1:1.9285	4.666667	S
June	37	12	25	1:2.0833	3.789474	S
July	92	30	62	1:2.0666	11.70968	S
Aug	47	16	31	1:1.9375	4.083333	S
Sep	42	15	27	1:1.8	3.428	S
Oct	48	16	32	1:2	11.70968	S
Nov	84	24	60	1:2.5	15.42857	S
Dec	32	12	20	1:1.6666	2.189189	S
Jan	34	12	22	1:1.8333	2	S
Feb	33	14	19	1:1.3571	0.694444	N S
March	40	18	22	1:1.2222	0.609756	N S
Total	568	196	372	1:1.8979	56.81647	S

Table 6.6 Lengthwise distribution of sexes in *Puntius sarana subnasutus*

Total length (mm)	Male	Female	Expected value	Chi square test	Remarks
120-130	44	0	22	22	S
131-140	68	0	34	34	S
141-150	33	6	19.5	9.346154	S
151-160	21	10	15.5	1.951613	S
161-170	12	13	12.5	0.02	N S
171-180	8	60	34	19.88235	S
181-190	6	119	62.5	51.076	S
191-200	4	77	40.5	32.89506	S
201-210	0	55	27.5	27.5	S
211-220	0	20	10	10	S
221-230	0	12	6	6	S
Total	196	372	284	27.26761	S

Fig 6.1 Monthly distribution of sexes in *Puntius amphibius*

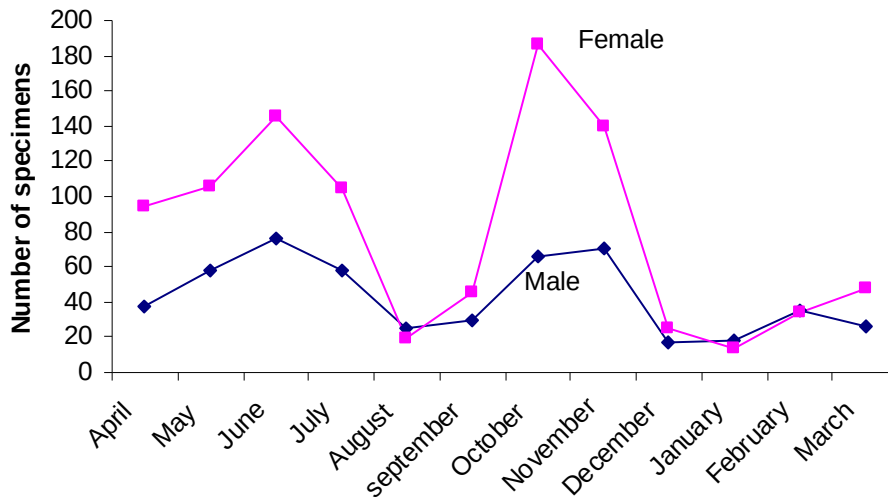


Fig 6.2 Monthly distribution of sexes in *Puntius parrah*

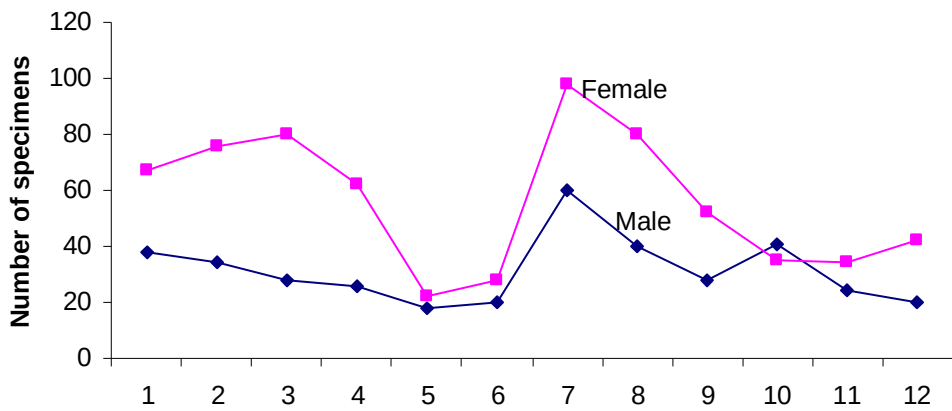
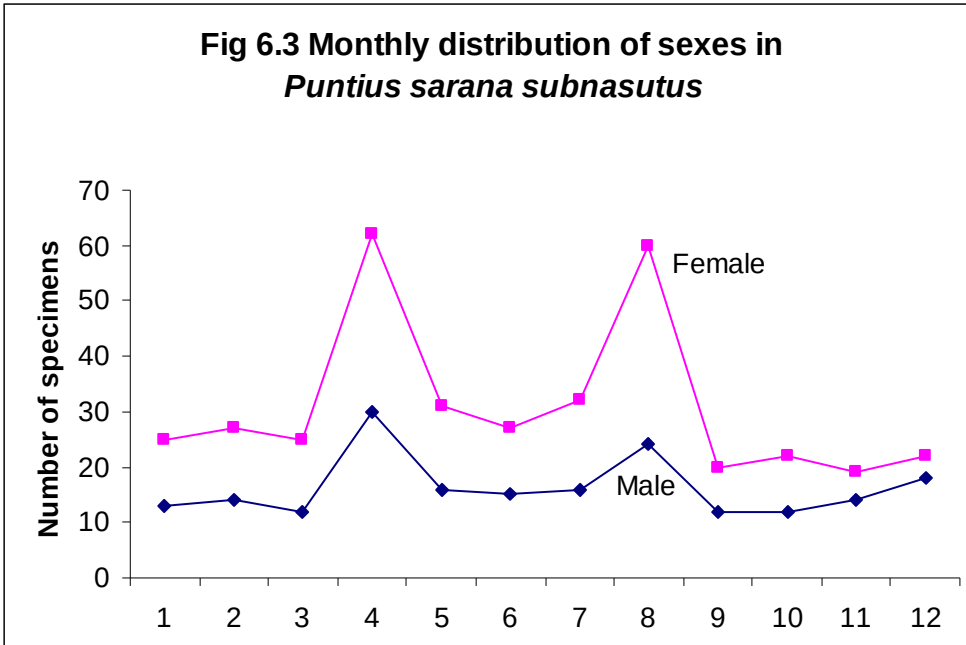


Fig 6.3 Monthly distribution of sexes in *Puntius sarana subnasutus*



CHAPTER - 7
LENGTH AT FIRST MATURITY

CHAPTER - 7

LENGTH AT FIRST MATURITY

INTRODUCTION

Mature fish are defined as those which have had a chance to spawn at least once. In teleost fishes length is considered as a better indicator of the first maturity and maturity occurs at different sizes. All the fishes do not mature at the same length. Some workers considered the earliest length at which the maturity was attained to be the minimum size of maturity. But due to the limited number of fishes collected, this may not give the true picture of a population and hence for population studies the minimum size at first maturity is considered at 50 % level. Length at first maturity is defined as the length at which 50 % of the fish of a given stock become sexually mature. Size at first maturity largely determined by the natural mortality rate and by the size that has to be attained before a fish can exploit its final food niche most efficiently (Jonsson and Jonsson,1993) It is considered as a mean to better operate a control on the fixed capture-size thus assuring the juveniles to reach the sexual maturity. It is accepted that knowledge on the fish length at first sexual maturity is indispensable in order to estimate size of the spawning stock. In a natural habitat size at first maturity is affected by environmental conditions, geographical barriers, water body area, overfishing, and food supply.

The length at first maturity may be different for the same species at different location. This variability might be attributed to the influence of various abiotic (temperature,

salinity, hydrodynamics) and biotic (nutrients and productivity) factors on different populations. Age and size at maturity are two of the most important life history traits affecting the fitness of fish (Mangel 1996). Early maturation increases the probability of survival to reproduction. By contrast, delayed maturation increases body size, which in turn increases fecundity. Therefore, there is a trade-off between the probability of survival to reproduction and fecundity. Several studies have shown that optimal solutions to maximize fitness under the tradeoff explain the inter- and intra-specific variation in size at maturity .

MATERIALS AND METHODS

For this purpose individual specimens were examined for their maturity stages. Based on the histological maturity stage of the ovaries and testis the proportion of specimens within each five mm size class that had gonads in stages II onwards were calculated. The immature fishes may form one group and the other maturity stages form another group. From these, the number of immature and mature fishes in each size group and the percentage occurrence of maturing males and females were determined. The average size at first maturity was determined by plotting the percentage of fish against the length group. Maturity curves were drawn to the scatter plots so as to estimate the length at which 50 % of the fish mature.

RESULT

A total of 961 females and 517 male specimens of *Puntius amphibius* were examined for the maturity stage of gonads. The minimum size at which *Puntius amphibius* female attains maturity was presented in Table 7.1. It can be seen from the table that those up to the length of 70 mm solely belongs to immature class. Above 106 mm all the specimens belonged to the mature class. The average size at first maturity was determined by plotting percentage of fish against length group. The length at which 50 % of the female fish attain maturity in *Puntius amphibius* was calculated as 83 mm (Fig 7.1). The minimum size at which *Puntius amphibius* male attains maturity was presented in Table 7.2. Out of 517 male specimens of *Puntius amphibius* examined, it can be seen from the table that those up to the length of 55 mm solely belongs to immature class. Above 80 mm all the specimens belonged to the mature class. The average size at first maturity was determined by plotting percentage of fish against length group. The length at which 50 % of the male fish attain maturity in *Puntius amphibius* was calculated as 63 mm (Fig 7.2)

Altogether 676 female and 377 male specimens of *Puntius parrah* were examined for the maturity stage of the gonads. The minimum size at which *Puntius parrah* attains maturity was presented in Table 7.3. It can be seen from the table that those up to the length of 65 mm solely belongs to immature class. The percentage of ripening fish increased rapidly from 71mm onwards. Above 106 mm all the specimens belonged to the mature class. The average size at first maturity was determined by plotting percentage of mature fish against length group. The length at which 50 % of the female fish attain maturity in *Puntius parrah* was calculated as 88 mm (Fig 7.3). A total of 377 male specimens of *Puntius parrah* were examined for the maturity stage of testis. The

minimum size at which *Puntius parrah* attains maturity was presented in Table 7.4. It can be seen from the table that those up to the length of 55 mm solely belongs to immature class. Above 80 mm all the males belonged to the mature class. The average size at first maturity was determined by plotting percentage of mature fish against length group. The length at which 50 % of the male fish attain maturity in *Puntius parrah* was calculated as 68 mm (Fig 7.4).

Altogether 376 female and 196 male specimens of *Puntius sarana subnasutus* were examined for the maturity stage of gonads. Total length was measured to the nearest millimeter and the specimens were grouped into 10 mm class intervals Table (7.5). The percentage occurrence of immature and mature fish in each size group was calculated. The specimens up to 130 mm belonged to the immature class. From 131 mm onwards there was gradual increase of mature females. Beyond the length of 181 mm all individuals were mature ones. The size at which 50 % individuals attain maturity in *Puntius sarana subnasutus* was found to be 155 mm (Fig 7.5). Out of 196 male specimens of *Puntius sarana subnasutus* were examined for the maturity stage of testis, the specimens up to 110 mm belonged to the immature class (Table 7.6). From 111 mm onwards there was gradual increase of mature females. Beyond the length of 161 mm all individuals were mature ones. The size at which 50 % individuals attain maturity in *Puntius sarana subnasutus* male was found to be 125 mm (Fig 7.6).

DISCUSSION

Usually fishes attain maturity at a particular length of the individuals. The onset of maturity differs considerably inter-specifically as well as intra-specifically (Nikolskii, 1963). Information on the size of maturation is essential for avoiding over exploitation of immature juveniles and ensuring the spawning of the individual fish at least once in life. The minimum size of maturity has been estimated earlier by several workers (Qayyum and Qasim, 1964., Paramswaran *et al.*, 1972., Somavanshi, 1980., Sunder, 1986., Kurup, 1994). In *Puntius amphibius* the females and males were found to be mature at 83 mm and 63 mm respectively. In *Puntius parrah* the females and males were found to be mature at 88 mm and 68 mm where in *Puntius sarana subnasutus* the length at first maturity of the females and males were found to be 165 mm and 125 mm respectively. Thus males attain sexual maturity at a smaller length than the female. Similar observations had been reported in many fresh fishes such as *Cyprinus carpio* (Parameswaran *et al.*, 1972,) *Barbus sarana* (Murthy, 1975) *Tort tor* (Chaturvedi, 1976) *Labeobata* (Siddiqui *et al.*, 1976) *Noemacheilus triangularis* (Ritakumari and Nair, 1979) *Schizothorax longipinnis* (Sunder, 1986) *Labeo dussumieri* (Kurup, 1994) . Jayaprakas and Nair (1981) reported almost similar situations in the *Etroplus suratensis*, where males (140 mm) attained maturity slightly ahead of females (144 mm). According to De Silva *et al.* (1985) the total length of *Puntius tittैया* at maturity ranged from 21mm to 40 mm. The first appearance of ripe and spent individuals of *Puntius amphibius* male seen in 56-60 mm group and in females it was between 71-75 mm size groups. In *Puntius parrah* the first occurrence of

ripe males and females lies in the range of 61-65 and 66-70 respectively. The first appearance of ripe and spent individuals of *Puntius sarana subnasutus* male seen in 111-120 mm group and in females it was between 131-140 mm size groups. These findings suggest that this roughly corresponds to the minimum size group at which the females and males attain ripeness and start spawning .It is a generalized fact that among fish males usually grow to a smaller size than females (Sivakami, 1982).In *Puntius amphibius* *Puntius parrah* and *Puntius sarana subnasutus* also the females are larger in size. The maximum size of the males and females encountered in *Puntius amphibius* during the present investigation is 85 mm and 120 mm respectively. The corresponding values of *Puntius parrah* were 90mm and 125 mm, where the maximum size attained by the males and females of *Puntius sarana subnasutus* were 200 mm and 230 mm respectively. The difference in size at first maturity and the maximum size attained in the two sex group may be due to the fact that female live longer and hence attain a larger size (Murthy, 1975).

Table 7.1 Percentage occurrence of immature and mature female *Puntius amphibius* of different length groups

No. of fish	Length group	% of immature female	% of mature female
7	56-60	100	0
12	61-65	100	0
18	66-70	100	0
20	71-75	70	30
23	76-80	56.52174	43.47826
85	81-85	48.23529	51.76471
78	86-90	30.76923	69.23077
121	91-95	23.1405	76.8595
176	96-100	6.25	93.75
201	101-105	2.487562	97.51244
151	106-110	0	100
48	111-115	0	100
21	116-120	0	100

Table 7.2 Percentage occurrence of immature and mature male *Puntius amphibius* of different length groups

No. of fish	Length group	% of immature male	% of mature male
32	51-55	100	0
92	56-60	81.52174	18.47826
98	61-65	48.97959	51.02041
157	66-70	37.57962	62.42038
99	71-75	10.10101	89.89899
44	76-80	4.545455	95.45455
29	81-85	0	100

Table 7.3 Percentage occurrence of immature and mature female *Puntius parrah* of different length groups

No.of fish	Length group	% of immature female	% of mature female
8	61-65	100	0
12	66-70	83.33333333	16.66667
16	71-75	68.75	31.25
18	76-80	61.11111111	38.88889
22	81-85	54.54545454	45.45455
39	86-90	48.7179487	51.28205
67	91-95	13.4328358	86.56716
74	96-100	2.7027027	97.2973
106	101-105	0	100
160	106-110	0	100
109	111-115	0	100
32	116-120	0	100
14	121-125	0	100

Table 7.4 Percentage occurrence of immature and mature male *Puntius parrah* of different length groups

No.of fish	Length group	% of immature male	% of mature male
9	51-55	100	0
12	56-60	75	25
30	61-65	60	40
55	66-70	49.09091	50.90909
64	71-75	29.6875	70.3125
105	76-80	16.19048	83.80952
86	81-85	0	100
16	86-90	0	100

Table 7.5 Percentage occurrence of immature and mature female *Puntius sarana subnasutus* of different length groups

No.of fish	Length group	% of immature female	% of mature female
11	120-130	100	0
18	131-140	77.77778	22.22222
14	141-150	57.14286	42.85714
27	151-160	48.14815	51.85185
32	161-170	31.25	68.75
48	171-180	12.5	87.5
108	181-190	0	100
55	191-200	0	100
38	201-210	0	100
15	211-220	0	100
8	221-230	0	100

Table 7.6 Percentage occurrence of immature and mature male *Puntius sarana subnasutus* of different length groups

No.of fish	Length group	% of immature male	% of mature female
4	101-110	100	0
9	111-120	77.77778	22.22222
30	121-130	46.66667	53.33333
30	131-140	36.66667	63.33333
35	141-150	20	80
32	151-160	12.5	87.5
28	161-170	0	100
16	171-180	0	100
8	181-190	0	100
4	191-200	0	100

Fig 7.1 Size at 50% maturity in *Puntius amphibius*-Female

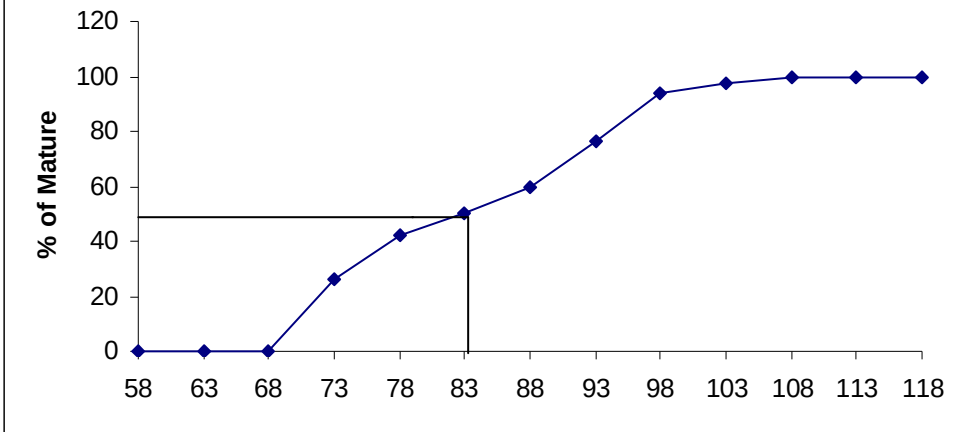
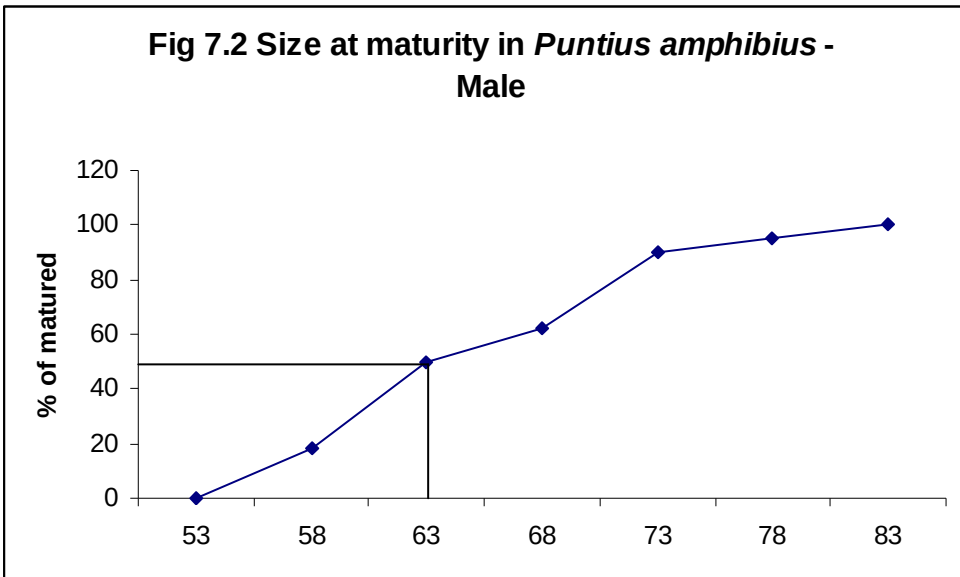
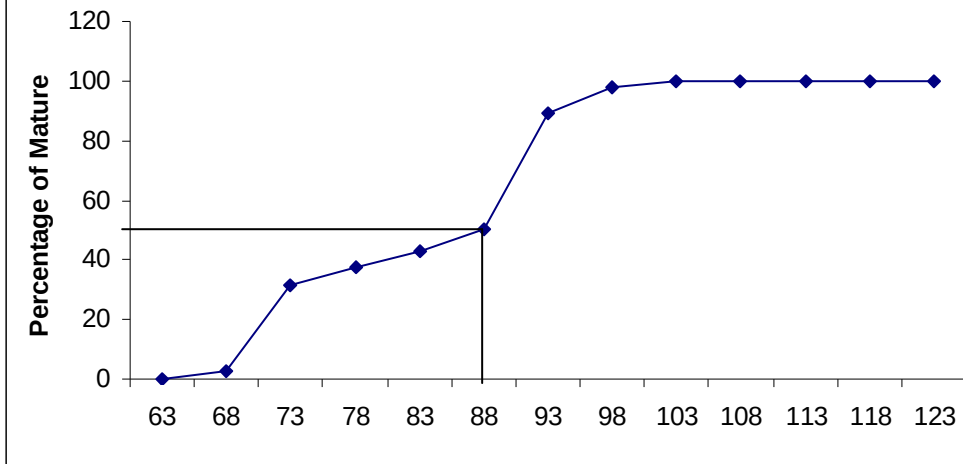


Fig 7.2 Size at maturity in *Puntius amphibius* - Male



**Figure 7.3 Size at first maturity in
Puntius parrah - Female**



**FIG 7.4 Size at first maturity in
Puntius parrah - Male**

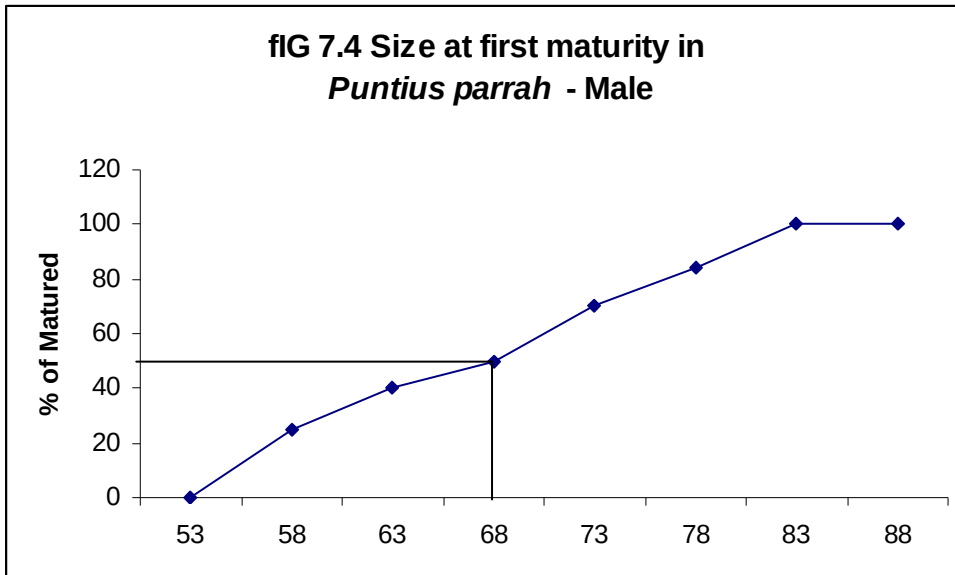


Fig 7.5 Size at first maturity in *Puntius sarana subnasutus*- Female

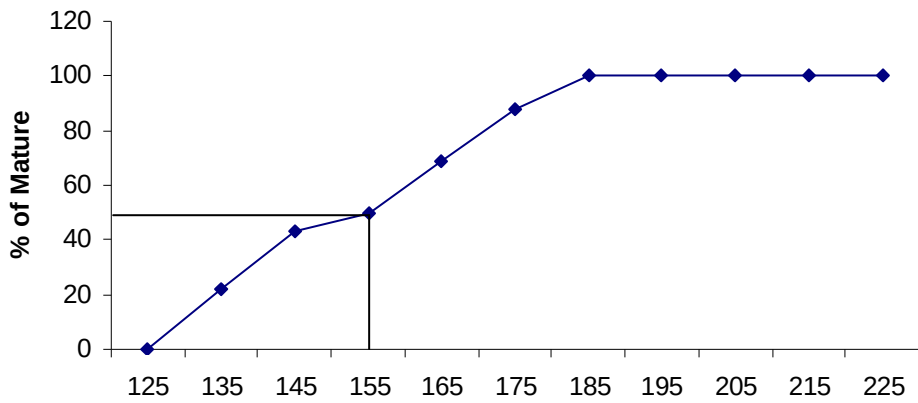
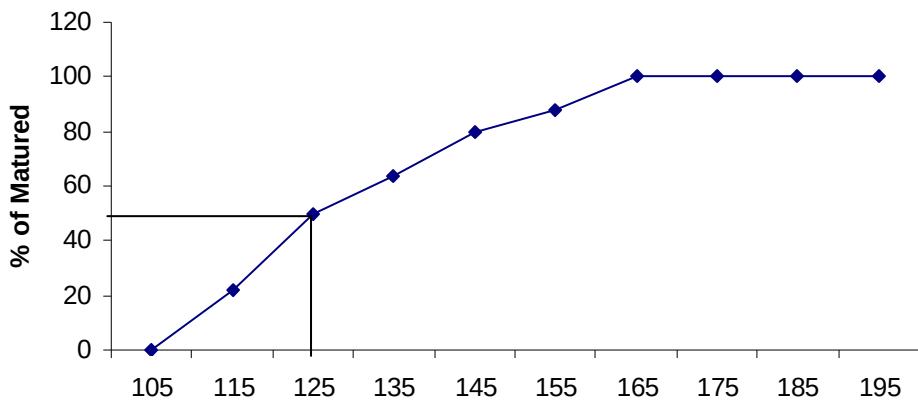


Fig 7.6 Length at Maturity in *Puntius sarana subnasutus* -Male



CHAPTER - 8

GONADO SOMATIC INDEX

CHAPTER - 8

GONADO SOMATIC INDEX

INTRODUCTION

Gonads undergo regular seasonal and cyclical changes in weight in relation to the total weight of the fish. Such changes are indicative of the spawning season of the fish and the relationship is termed as gonado somatic index. The gonado somatic index G.S.I. refers to the relationship between the gonad weight and the fish somatic weight (wootton, 1992). It is determined by expressing a given dimension of the ovary, for example weight, as a percent of the same dimension of the entire animal. The most commonly used method for quantifying seasonal ovarian changes is the gonado somatic index.

Reproductive effort is commonly expressed by the gonado somatic index. The variation in G. S. I. through out an annual cycle usually indicates the beginning and end of the fish reproductive cycle. The G. S. I. provides information on regression and recrudescence and is often used as an indicator of mating season. The gonado somatic index was observed to increase with the maturation of fish, being the maximum during the period of peak maturity and declining abruptly thereafter when the fish became spent (Parameswaran et al., 1972). The monthly changes in the G. S. I. reflect the ovarian

activity of a fish. Maximum G. S. I. values correspond with the spawning season of fish (Barnabe, 1996). The gonado somatic index is an indicator of the state of gonadal development and maturity of fish (James, 1967). It is extremely important to understand the mechanism linking gonad development and reproductive performance in order to improve culture techniques.

MATERIAL AND METHODS

Gonado somatic index (G.S.I) was estimated applying the method of June (1953). For this study fresh specimens of *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.) were collected twice a month to determine the G. S. I. during the period of April, 2003 to March 2005. The collected samples were brought to the laboratory for detailed studies. The total length and weight of the fish were recorded.

The ovaries of each specimen were removed very carefully and were preserved in 5% formalin with proper labeling to permit hardening of the ova to facilitate for subsequent studies. The ovary was cleaned properly and each pair of ovary was weighed separately to the nearest milligram. Monthly mean index values were calculated using the formula

$$\text{G. S. I.} = \text{Weight of gonad} / \text{Weight of fish} * 100$$

Correlation between the spawning cycle and the annual rainfall pattern was obtained by plotting (Neave and Worthington, 1992) the female G.S.I., and the rainfall data (collected from Hydrology section of Public Works Department, Govt. of Kerala) for

each month. The relationship between G. S. I. and different variables like fish length, fish weight was worked out by the least square method.

$$G S I = a X^b$$

Where G. S. I. = Gonadosomatic index, a = a constant and b = regression coefficient.

The exponential relationship was transformed into a straight-line relationship based on logarithms by the following equations

$$\text{Log G S I} = \text{Log a} + b \text{ Log X}$$

.

RESULT

In *Puntius amphibius* the mean monthly variation of G. S. I. values of females during April to March were depicted in Table 8.1 .The G. S. I. values started increasing from March and attained peak in June (17.4). A sharp decline was discernible in July and August which was followed by a slight increase in September .Again there was a straight increase during October (14.35).The month of November showed a steep decrease and the G. S. I. value remained low up to the month of February. G. S. I. values in female specimen were plotted against fish weight and length during different stages of gametogenesis.

The logarithmic relationship between mean G. S. I. and total length can be expressed in the formula

$$\text{Log G S I} = 0.950 + 0.9723 \text{ Log L} (r = 0.404)$$

The logarithmic relationship between mean G. S. I. and body weight can be expressed in the formula

$$\text{Log G S I} = 5.442 + 0.2309 \text{ Log B .W} (r = 0.3690)$$

Mean values of G. S. I. increased with increasing mean body weight and length during gameto genesis in females (Figs. 8.2 &3). When the monthly mean values of G. S. I. were compared with the month wise rainfall data (Table 8.1) ,it was found that G. S. I. values were maximum at the months of June and October where monthly rain fall was maximum (Fig. 8.4)

In *Puntius parrah* the mean monthly variation of G. S. I. values of females during April to March were depicted in Table 8.2. The G. S. I. values started increasing from March and attained peak in June (Fig 8.5). A sharp decline was discernible in July and August which was followed by a slight increase in September .Again there was a straight increase during October. The month of November showed a steep decrease and the G. S. I. value remained low up to the month of February. Later the the gonadosomatic value showed an increasing trend from March onwards. G. S. I. values in female specimen were plotted against fish weight and total length during different stages of gametogenesis.

The logarithmic relationship between mean G. S. I. and total length can be expressed in the formula

$$\text{Log G S I} = 2.8516 + 0.05793 \text{ Log L} (r = 0.3838)$$

The logarithmic relationship between mean G S I and body weight can be expressed in the formula

$$\text{Log G. S. I.} = 0.0004 + 2.2533 \text{ Log B. W} (r = 0.5112)$$

Mean values of G. S. I. increased with increasing mean body weight and length during gameto genesis in females (Figs. 8.6 & 8.7.). When the monthly mean values of G. S. I. were compared with the month wise rainfall data (Fig. 8.8), it was found that G. S. I. values were high at the months of June and October where monthly rain fall maximum.

In *Puntius sarana subnasutus* the mean monthly variation of G. S. I. values of females during April to March were depicted in Table 8.3. The G. S. I. values started increasing from March and attained peak in July (Fig 8.9). A sharp decline was discernible in August which was followed by a gradual increase in September and October. Again there was a straight increase during November. The month of December showed a steep decrease and the G. S. I value remained low up to the month of February. Later the gonadosomatic value showed an increasing trend from March onwards.

G. S. I values in female specimen were plotted against fish weight and total length during different stages of gametogenesis. Mean values of G. S. I increased with

increasing mean body weight and length during gametogenesis in females (Figs. 8.10 & 8.11.).

The logarithmic relationship between mean G. S. I. and total length can be expressed in the formula

$$\text{Log G. S. I.} = 3.5742 + 0.4991 \text{ Log L} (r = 0.369)$$

The logarithmic relationship between mean G S I and body weight can be expressed in the formula

$$\text{Log G. S. I.} = 4.5007 + 0.5247 \text{ Log B. W} (r = 0.7466)$$

When the monthly mean values of G. S. I. were compared with the month wise rainfall data (Fig .8. 12), it was found that G. S. I values were maximum at the months of June, July , October and November where monthly rain fall were comparatively high.

DISCUSSION

Gonado somatic index (G. S. I.) indicates the gonadal development and maturity of the fish. Nicklosky (1963) states that the effect of fish size on gonadal weight is defined by expressing gonadal weight as a percentage of body weight. The gonadosomatic index values of the three specie under study for a period of 24 months during 2003 April to 2005 March were assessed. The maximum average gonadosomatic index observed in *Puntius amphibius* and *Puntius parrah* were during June and October, while it was during the months of July and November in *Puntius sarana subnasutus*. It might be due to the

completion of maturity and subsequent steep fall in the index values clearly indicated spent condition of the fish.

During the peak periods of G. S. I., the percentage of mature and ripe fishes was very high when compared to other groups (Tables 10.4, 10.5, & 10.6). These two peaks represented the peaks of spawning in an year .Lowest value of G S I noted in the months of August, November, December, January and February in both the species of *Puntius amphibius* and *Puntius parrah*. In *Puntius sarana subnasutus* low G. S. I values were encountered during the months of August, December, January and February. After the first peak G. S. I. came down and then again G. S. I. was increased to the second peak. From these observations it was clear that the increase of ovary weight is associated with the progress of maturity of the ovary. This finding is similar to that reported for other species (Awaji and Hanyu, 1987., Delahunty and de Vlaming, 1980., Htun-Hun, 1978., Asahina et al., 1990).

G. S. I. values in female specimen were plotted against fish weight and length during different stages of gametogenesis. Mean values of G. S. I increased with increasing mean body weight and length during gametogenesis in females. Hence larger fish developed proportionately larger ovaries during gameto genesis .Since total fish length and weight are included in the calculation of G. S. I, they present an auto – correlation. De Vlaming *et al* (1982) assumed that gonadal weight depends on animal size and stage of gonadal development.

The gonado somatic index was observed to increase with the maturation of fish, being the maximum during the period of peak maturity and declining abruptly thereafter, when the fish become spent.The monthly changes in the G. S. I reflect the ovarian activity of a fish.

The G S I count showed three phases correlated with structural changes in the ovary. In *Puntius amphibius* and *Puntius parrah* an inactive phase of the first breeding season was characterized by low G. S. I lasted from November to February. But in *Puntius sarana subnasutus* it was from December to February. The enlargement phase begins in mid-March and is followed by peak in ovarian growth by June. In *Puntius sarana subnasutus* the process is late for a month, the peak ovarian growth was occurred in July. The diminution phase occurs from July to August in *Puntius amphibius* and *Puntius parrah* where in *Puntius sarana subnasutus*, it occurred during August. In the second breeding season ovarian development begins from the month of September onwards. The peak gonado somatic index marked the full maturation of ovary, and it occurred during the month of October in *Puntius amphibius* and *Puntius parrah* and during November in *Puntius sarana subnasutus*.

According Nickolskii (1963) G. S. I of fishes is widely used as an index of gonadal activity and as an index for spawning preparations. When assessing gonadal activity, fish of different sizes were frequently sampled and it is generally assumed that gonadal weight depends upon animal size and stage of gonadal development (De Vlaming *et.al*, 1982) the gonado somatic index is used as a criterion for determining the duration and intensity of spawning in fishes (June, 1953, Thomas, 1969) during the development of gonads the G. S. I increases gradually and attain a peak value and declines at the commencement of spawning. This statement was in agreement with the present study. The two seasons for spawning for *Puntius amphibius* and *Puntius parrah* were considered to be June and October and for *Puntius sarana subnasutus* it was July and November.

To understand whether there exists any correlation between rainfall and gonadosomatic index, G. S. I values were compared to that of rainfall .The rainfall was maximum in June and a noticeable increase in gonadosomatic index was observed in June and July .From the data it was clear that the peak G. S. I values coincided with the monsoon season as the rainfall diminishes during post monsoon season the value attained its lowest position. Later the second peak in G. S. I values coincided with the north east monsoon during October and November. The above observations revealed that the peak period of spawning were in June and October in *Puntius amphibius* and *Puntius parrah* and during July and November in *Puntius sarana subnasutus*. Since these two spawning periods were connected with southwest monsoon and north east monsoon ,the first ovulation period points out the early arrival of southwest monsoon which can be considered as a factor for the prediction of the south west monsoon in kerala.

Table 8.1 Mean and \pm SD of monthly rainfall, body weight, total length, ovary weight and G. S. I. for *Puntius amphibius*

Month	Rainfall (cm)	No.of fish examined	Av.total length (mm)	Av. Body weight (g)	Av.ovary weight (g)	Av.G S I
April	11.8	54	99 \pm 2	17.12 \pm .8	1.95 \pm .3	6.8 \pm .3
May	17.4	56	104 \pm 3	18.6 \pm .9	2.5 \pm .2	12.5 \pm .2
June	73.4	50	108 \pm 3	19.5 \pm .5	2.89 \pm .5	17.4 \pm .5
July	67.6	126	111 \pm 4	16.35 \pm .7	1.25 \pm .2	3.9 \pm .2
August	46.1	62	112 \pm 3	16.02 \pm .6	1.42 \pm .3	2.3 \pm .3
September	30.6	54	108 \pm 2	17.15 \pm .3	1.98 \pm .1	4.9 \pm .3
October	32.5	67	111 \pm 4	18.75 \pm .5	2.64 \pm .5	14.3 \pm .4
November	12.7	120	112 \pm 5	16.35 \pm .4	1.65 \pm .4	3.2 \pm .2
December	5.8	43	114 \pm 2	15.36 \pm .4	.98 \pm .3	2.3 \pm .1
January	4.5	46	112 \pm 4	15.86 \pm .6	1.2 \pm .2	2.2 \pm .2
February	0.8	41	113 \pm 3	15.97 \pm .3	1.3 \pm .3	1.8 \pm .1
March	1.3	46	115 \pm 4	16.4 \pm .3	1.8 \pm .3	4.2 \pm .2

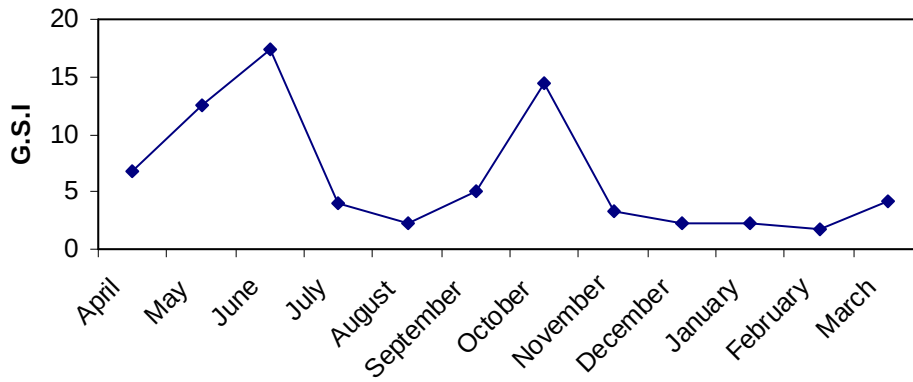
Table 8.2 Mean and \pm SD of monthly rainfall, body weight ,total length ,ovary weight and G. S. I for *Puntius parrah*

Month	Rainfall (cm)	No.of fish examined	Av.total length (mm)	Av. Body weight (g)	Av.ovary weight (g)	Av.G S I
April	11.8	67	113 \pm 3	18.54 \pm .5	2.32 \pm .3	7.2 \pm .2
May	17.4	76	114 \pm 4	19.35 \pm .6	2.91 \pm .2	13.2 \pm .2
June	73.4	80	118 \pm 5	20.52 \pm .5	3.22 \pm .5	18.2 \pm .4
July	67.6	62	117 \pm 5	18.35 \pm .6	2.13 \pm .2	4.3 \pm .2
August	46.1	22	117 \pm 3	18.02 \pm .4	1.68 \pm .3	3.3 \pm .3
September	30.6	28	118 \pm 2	18.15 \pm .3	2.25 \pm .1	5.32 \pm .3
October	32.5	98	118 \pm 4	19.89 \pm .6	2.99 \pm .5	15.32 \pm .5
November	12.7	80	115 \pm 5	18.35 \pm .4	1.75 \pm .4	3.9 \pm .3
December	5.8	52	116 \pm 2	16.16 \pm .2	1.24 \pm .3	2.9 \pm .1
January	4.5	35	114 \pm 3	16.06 \pm .5	1.42 \pm .2	2.6 \pm .2
February	0.8	34	116 \pm 3	16.27 \pm .3	1.62 \pm .3	2.5 \pm .2
March	1.3	42	114 \pm 2	17.3 \pm .3	2.01 \pm .3	4.9 \pm .2

Table 8.3 Mean and \pm SD of monthly rainfall, body weight ,total length ,ovary weight and G. S. I for *Puntius sarana subnasutus*

Month	Rainfall (cm)	No.of fish examined	Av.total length (mm)	Av. Body weight (g)	Av.ovary weight (g)	Av.G S I
April	11.8	54	191 \pm 3	91.33 \pm .5	9.44 \pm .3	9.3 \pm .2
May	17.4	56	189 \pm 4	96.42 \pm .6	13.5 \pm .2	13.29 \pm .2
June	73.4	50	192 \pm 5	98.25 \pm .5	17.96 \pm .5	17.68 \pm .4
July	67.6	126	196 \pm 5	101.56 \pm .6	19.89 \pm .2	19.58 \pm .2
August	46.1	62	194 \pm 3	90.21 \pm .4	4.57 \pm .3	4.5 \pm .3
September	30.6	54	195 \pm 2	91.35 \pm .3	6.62 \pm .1	6.52 \pm .3
October	32.5	67	193 \pm 4	97.61 \pm .6	12.17 \pm .5	11.98 \pm .5
November	12.7	120	195 \pm 5	103.57 \pm .4	18.94 \pm .4	18.65 \pm .3
December	5.8	43	179 \pm 2	87.66 \pm .2	2.844 \pm .3	2.8 \pm .1
January	4.5	46	1176 \pm 3	88.39 \pm .5	2.72 \pm .2	2.7 \pm .2
February	0.8	41	178 \pm 3	90.63 \pm .3	3.556 \pm .3	3.5 \pm .2
March	1.3	46	189 \pm 2	93.25 \pm .3	5.181 \pm .3	5.1 \pm .2

**Fig.8.1 Monthly variations of G.S.I
in *Puntius amphibius***



**Fig.8.2 Relationship between GSI and
body weight in *Puntius amphibius***

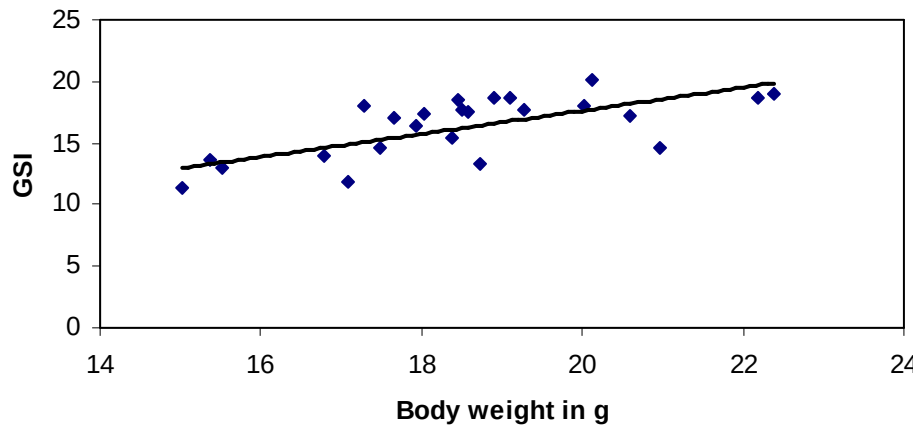


Fig.8.3 Relationship between GSI and Total length in *Puntius amphibius*

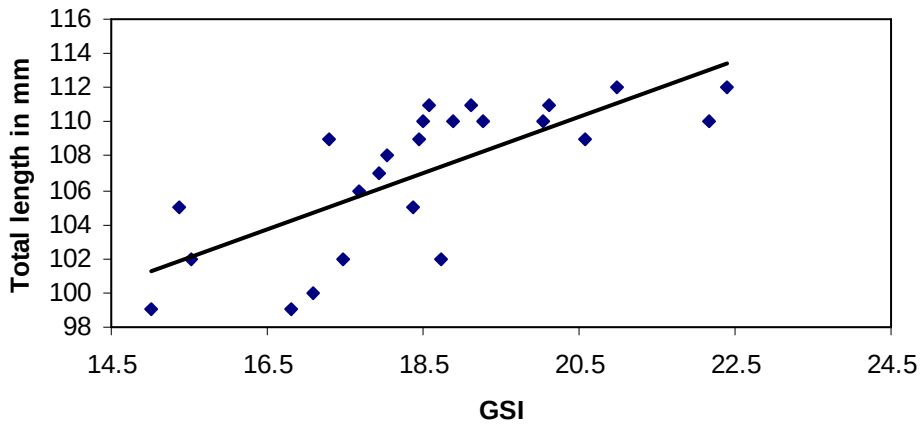
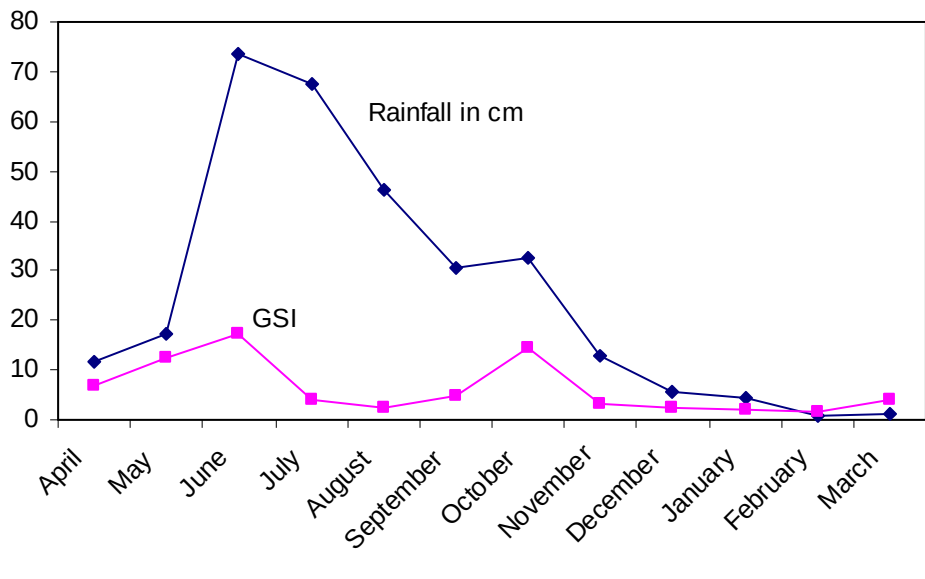
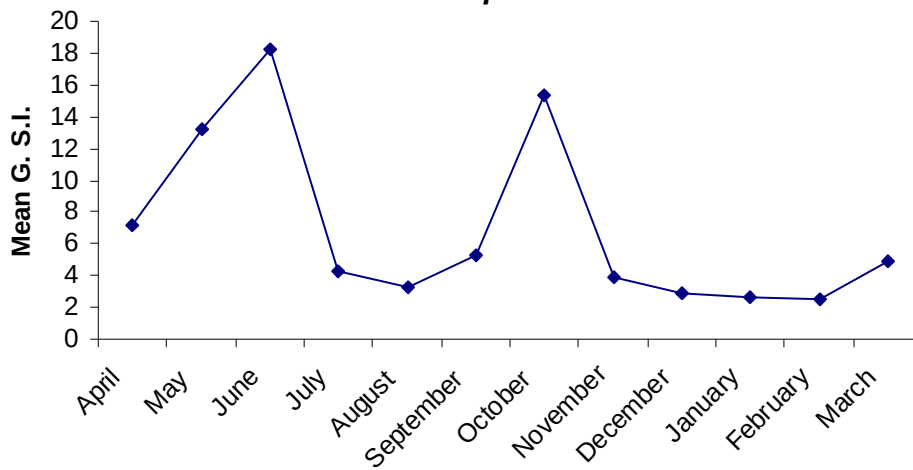


Fig.8.4. Relationship between monthly rainfall and GSI in *Puntius amphibius*



**Fig.8.5 Monthly variations of G S I
in *Puntius parrah***



**Fig 8.6 Relationship between body weight and
G.S.I. in *Puntius parrah***

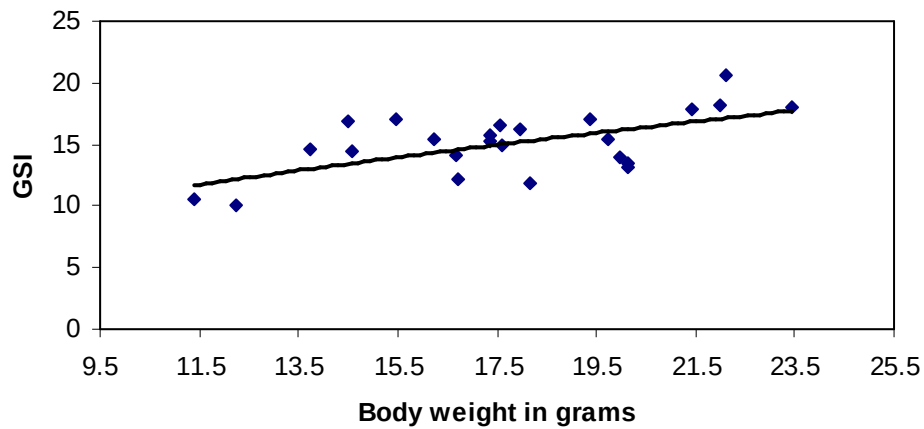


Fig.8.7 Relationship between Total length and G S I in *Puntius parrah*

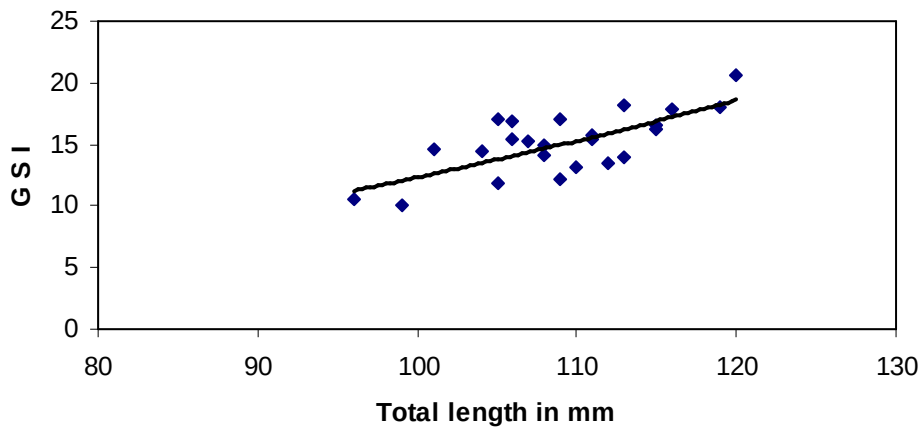
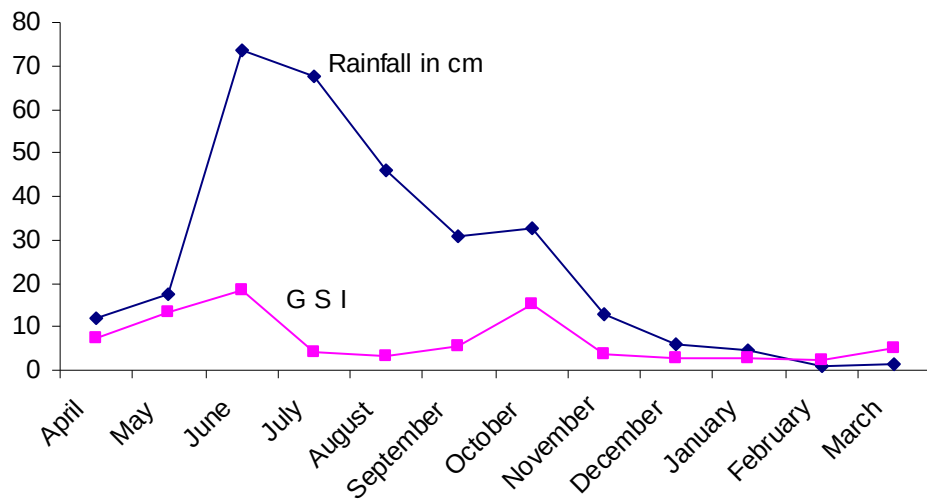
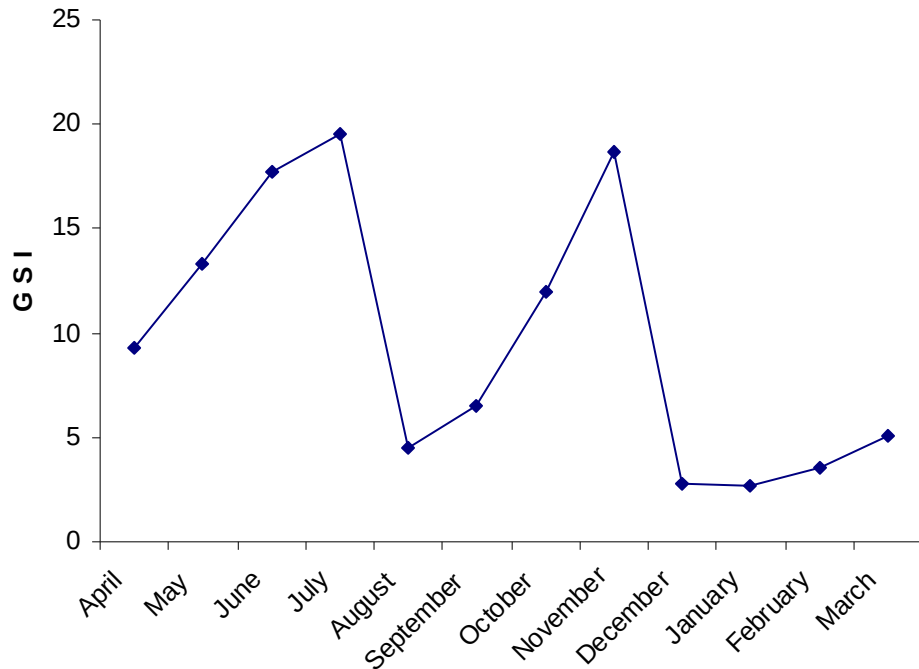


Fig 8.8 Relationship between monthly rainfall and G. S. I.in *Puntius parrah*



**Fig 8.9 Monthly variations of G S I
in *Puntius sarana subnasutus***



**Fig.8.10 Relationship between Total length and
G. S. I. in *Puntius sarana subnasutus***

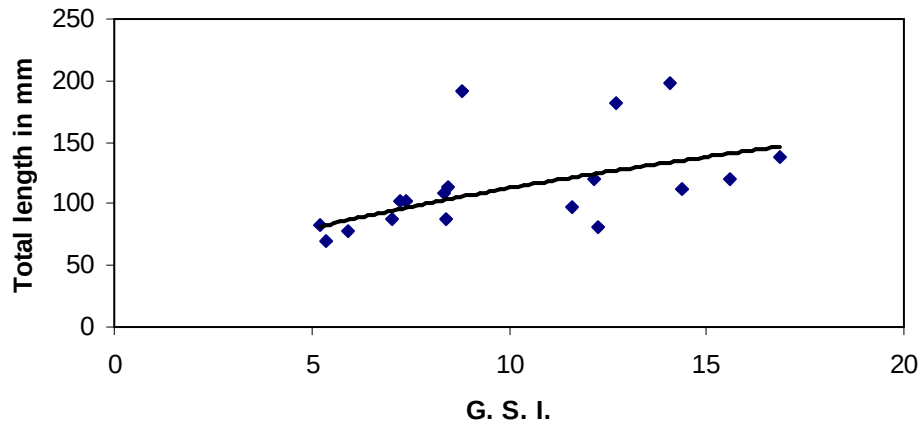


Fig 8.10 Relationship between body weight and G S I in *Puntius sarana subnasutus*

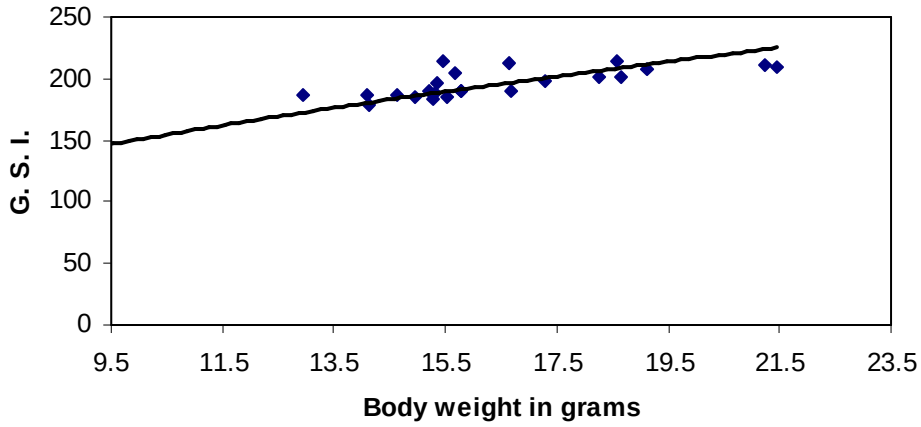
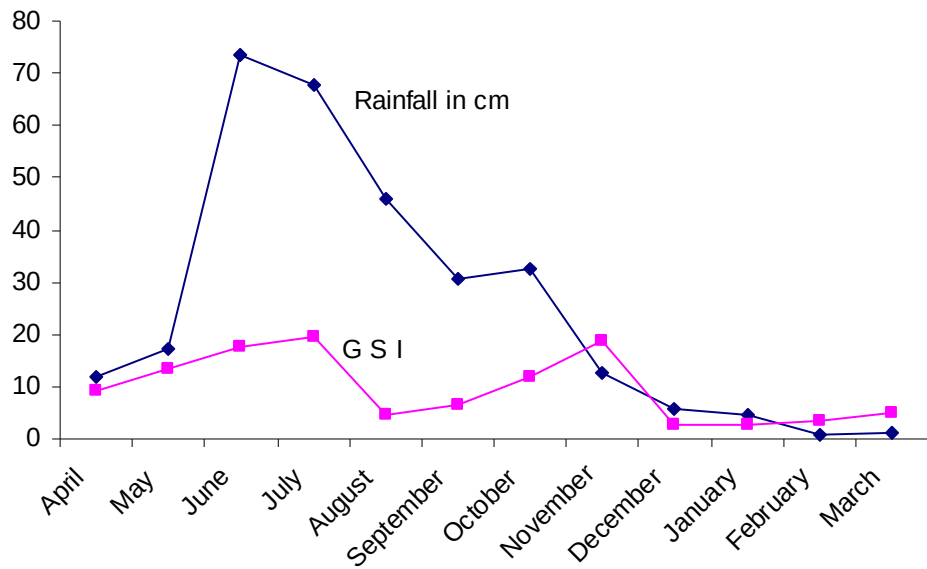


Fig.8.12 Relation between rainfall and G. S. I. in *Puntius sarana subnasutus*



CHAPTER - 9

FECUNDITY

CHAPTER - 9

FECUNDITY

INTRODUCTION

Fecundity is one of the most common measure of reproductive potential in fishes, because it is a relatively easy measurement to make. It is the number of eggs in the ovaries of a female fish. Fecundity varies from one species to another, depending on the environmental conditions, length, age etc. In oviparous teleosts, ovarian cavity has been regarded as merely a “stock room” in which ovulated eggs are kept temporarily until they are spawned (Nagahama, 1983).

Even the success or failure of a fish species largely depends upon its spawning potential, the knowledge of fecundity become extremely important from the viewpoint of successful management and exploitation of its fishery. Lowe McConnell (1958) defined fecundity as the number of eggs produced by an individual fish in its life time. Fecundity estimates are generally evaluated on the basis of absolute and relative fecundity. The absolute fecundity is defined as the number of eggs in a female fish prior to spawning and the relative fecundity as the number of eggs per 1g of female. Both estimates depend largely on the egg size. Fecundity appears to bear some broad relationship to the care of environment accorded to the eggs (Lagler *et al.*, 1967). 361 Knowledge about fecundity of a fish is essential for evaluating the commercial potentialities of its stock, life history, practical culture and actual management of the fishery (Lagler 1956., Doha and Hye, 1970).

Fecundity is often correlated with length weight and age of fish and also with the length and weight and volume of ovary. The relationship between length and fecundity differ in different species of fish. Clark (1934) opinioned that, the fecundity of a fish increased in proportion to the square of its length. Simpson (1951) established that the fecundity of plaice was related to the cube of its length and was thus directly proportional to fish weight. Many authors have supported Simpson's view of fecundity being related to fish length by a factor close to the cube. (Bagenal, 1957., Sarojini., 1957., Pillay, 1958., Pantalau, 1963., Varghese, 1973, 1976., Kurup, 1994). Bagenal (1978) stated that the exponent often ranged from 2.3 to 5.3 and frequently exceeds 3. After surveying 62 species, Wooten (1979) concluded that the exponent value varied from 1 to 5 with most of the values lying between 3.25 and 3.75 and invariably higher values were reported in marine species than in fresh water forms

. From a review of the literature, it is apparent that, studies on the reproductive biology of fish are vast and mostly concerning the marine teleosts. Witthames and Walker (1987) developed an automated method for counting and sizing fish eggs. Highlighting a few studies on reproductive biology of fresh water fish, Devaraj (1973), Babiker and Ibrahim (1979) have worked on gonadal maturation and fecundity of *Ophiocephalus marulius* (Ham.) and *Tilapia nilotica* respectively. The reproductive biology and fecundity studies were worked out on *Labeo calbasu* (Khan, 1954., Rao and Rao, 1972., Vinci and Sugunan, 1981), *Labeo rohita* (Varghese, 1973) *Cirrhinus mrigala* (Chakrabarty and Singh, 1967) *Labeo bata* (Alikunhi, 1956., Siddiqui *et al* ,1976) *Cyprinus carpio* (Parameswaran *et al* ,1972), *Labeo fimbriatus* (Bhatnagar ,1972), *Labeo gonius* (Joshi and Khanna, 1980) *Labeo dusmieri* (Kurup, 1994) *Puntius vittatus* (Desai and

Karamchandani, 1967), *Glyptothorax Kashmirensis* (Kaul, 1994), and *Noemacheilus triangularis* (Ritakumari and Nair, 1979).The fecundity studies were also carried out on other cyprinids like *Puntius ticto* (Ibrahim, 1957) *Garra mullya* (Somavanshi, 1985) *Crossocheilus latiusdiplocheilus* (Kaul, 1994) *Puntius stigma* (Ibrahim, 1957) *Puntius dorsalis* (Sivakami, 1982) and *Puntius sarana sarana* (Sinha, 1975).There are very few studies available on the reproductive aspects of *Punctius amphibius* and *Puntius parrah Puntius sarana subnasutus*. Available literature indicates that these species have not been considered for any study on an important aspect of its reproduction viz. ,fecundity .

MATERIALS AND METHODS

The fish samples were collected fortnightly and they were brought to the lab and freeze. After removing the moisture, the total length, standard length and weight of each individual fish was noted in fresh condition. Then the ovaries were removed and weighed separately. The potential fecundity or the number of eggs available to be spawned in a single breeding season was estimated from ovaries of fishes in late mature stage and early ripe stage. The ovaries were weighed in a mono pan electronic balance and then a small portion of ovary was separated and weighed to the nearest 0.001 gm. The sampled portion was placed on a micro slide and ova were teased out and these ova were then transferred to a measuring cylinder containing a known volume of water and the total number of ova in the sample was counted. The fecundity was estimated by employing the formula.

$$F = (n / v) V$$

Where n = number of ova in the sub sample. V = volume to which the total number of ova is made up and v volume of sub sample.

The relationship between fecundity and different variables like fish length, fish weight, and ovary weight was worked out by the least square method.

$$F = a x^b$$

Where F = fecundity, x = fish length or fish weight or ovary weight, a = constant and b = regression coefficient. The exponential relationship was transformed into a straight-line relationship based on logarithms by the following equations.

$$\text{Log Fecundity} = \text{Log } a + b \text{ Log } x$$

RESULTS

Fecundity estimates were based on the enumeration of mature eggs from 24 specimens of each species with mature or ripe ovaries. In *Puntius amphibius* absolute fecundity varied from 2322 to 13710 ova in individuals of total length from 97 mm to 116 mm, whole body weight between 11.778 g and 20.112 g, and ovary weight between 2.002 g to 3.628 g. The mean fecundity was 7172. The relative fecundity was estimated to be between 174 and 734 with an average of 454, while the number of ova per gram ovary weight varied from 1159 to 3844 with an average of 2338. The maximum fecundity was found to be 13710 for a female fish of length 111 mm, weight 18.668 gm and ovary weight 3.56g

(Table 9 .1).

In *Puntius parrah* fecundity varied from 2499 to 16303 in individuals of total length from 96 mm to 120 mm, whole body weight between 11.397 g and 22.127 g, and ovary weight between 1.999g to 4.891 g. The mean fecundity was 9424. The relative fecundity was estimated to be between 219 and 737 with an average of 531. The number of ova per gram ovary weight varied between 2084 and 4343 with an average of 3483 ova. The maximum fecundity was 16303 for a female fish of length 120 mm, weight 22.127 g and ovary weight 4.891 g (Table 9.2).

Fecundity estimates of *Puntius sarana subnasutus* were based on the enumeration of mature eggs from 24 specimens with mature or ripe ovaries .Fecundity varied from 24970 to 87894 in individuals of total length from 179 mm to 234 mm, whole body weight between 75.842 g m and 216.743 gm, ovary weight between 11.098 gm to 40.276 gm. The mean fecundity was 39575. The maximum fecundity was 87874 for a female fish of length 234 mm, weight 216.743 gm and ovary weight 40.276 gm (Table 9.3) Average fecundity per gram body weight was 384 and per 0. 1g ovary weight was 237. The fecundity tends to increase along with an increase in total body weight and ovary weight.

Fecundity Relationships In *Puntius amphibius*

Relationship between fecundity and total length

To find out this relationship, the absolute fecundities estimated for the 24 fish were plotted against their total lengths (Fig 1). The relationship was calculated by the least square method .The logarithmic values based on the formula

$$\text{Log F} = 3.73662 + 2.7017 \text{ Log L} \quad (r = 0.783743)$$

Relationship between fecundity and total body weight

The fecundities of the 24 fish were plotted against their total body weight (Fig 2). A regression line was fitted to the data, which gave a linear relationship according to the formula

$$\text{Log F} = 2.520489 + 1.031709 \text{ Log BW} \quad (r = 0.836364)$$

Relation between fecundity and ovary weight

For this study, fecundity of the 24 fish were plotted against their Ovary weight (fig .3).A regression line was fitted using the formula

$$\text{Log F} = 3.336749 + 1.033344 \text{ Log O W} \quad (r = 0.961037)$$

FECUNDITY RELATIONSHIPS IN *PUNTIUS PARRAH*

Relation between fecundity and total length

The fecundity of the 24 fishes were plotted against their total length (fig. 4)

The scatter diagram showed a linear relationship according to the formula

$$\text{Log F} = 5.59665 + 2.8323 \text{ Log L} \quad (r = 0.818445)$$

Relationship between fecundity and body weight

The scatter diagram on the fecundity and total body weight Relationship of *Puntius parrah* gave a linear relationship (fig.5) between the variables according to the formula.

$$\text{Log F} = 1.41898 + 1.898668 \text{ Log B.W (r} = 0.815335)$$

Relationship between ovary weight and fecundity

For this study of relationship fecundity of 24 fish were plotted against their respective ovary weight, which gave a linear relationship (fig .6) between the variables according to the formula

$$\text{Log F} = 0.511127 + 1.075317 \log \text{O .W (r} = 0.916817)$$

Fecundity Relationships In *Puntius sarana subnasutus*

Relation between fecundity and total length

To find out this relationship, the absolute fecundities estimated for the 24 fish were plotted against their total lengths (Fig 1).the relationship was calculated by the least square method .The logarithmic values based on the formula

$$\text{Log F} = 1.005 + 3.1557 \log L \quad (r = 0.6309)$$

Relation between fecundity and total weight

The fecundities of the 24 fish were plotted against the total weight of these fish. (Fig 2). A regression line was fitted to the data, which gave a linear relationship according to the formula

$$\text{Log F} = 8.0245 + 1.8568 \log W \quad (r = 0.7716)$$

Relation between fecundity and ovary weight

For this study, fecundity of the 24 fish were plotted against their ovary weight (fig .3). A regression line was fitted using the formula

$$\text{Log F} = 1.739 + 1.1252 \log O. W. \quad (r = 0.9192)$$

DISCU

SSION

As is true of most teleosts, ovaries of *Puntius amphibius*, *Puntius parrah* and *Puntius sarana subna sutus* are paired and of the `cystovarian` type, which have coelom derived ovarian lumen (ovocoel) continuous with the oviduct . Fecundity is generally regarded as the number of ova in an organism which has the potential to give rise to the offspring thus the reproductive potential is a function of the fecundity of fish. Fecundity varies both within and between fish population and numerous factors such as nutritional state (Scott, 1979., Stauffer, 1976), time of sampling, maturity stage (Healey et al. 1975) racial characteristics and environmental conditions such as rainfall and salinity (Joshy and

Khanna, 1980) have been proposed to explain such variations. Fecundity in teleosts ranges from a few hundreds to several lakhs. The fecundity estimates of important fresh water cyprinids have been reported by several authors. Fish such as *Labeo calbasu* (Khan, 1954., Rao and Rao ,1972.,Vinci and Sugunan ,1981), Varghese, 1973) *mrigala* (Chakrabarty and Singh, 1967) *Labeo bata* (Alikunhi, 1956., Siddiqui *et al* ,1976) *Cyprinus carpio* (Parameswaran *et al* ,1972), *Labeo fimbriatus* (Bhatnagar ,1972),*Labeo gonius* (Joshi and Khanna, 1980) and *Labeo dusmieri* (Kurup, 1994) are highly fecund fish with several lakhs eggs. *Puntius vittatus* (Desai and Karamchandani,1967)with 305-1168 ova, *Glyptothorax kashmirensis* (Kaul, 1994) with 692-1392 ova and *Noemacheilus triangularis* (Ritakumari and Nair, 1979) with 800-2126 ova are some fresh water fish species with less number of ova in their mature ovaries. The fecundity of other cyprinids are 2368-8590 in *Puntius ticto* (Ibrahim, 1957)1700-6259 ova in *Garra mullya* (Somavanshi, 1985) 3340-6160 in *Crossocheilus latiusdiplocheilus* (Kaul, 1994) 3416-58330 ova in *Puntius stigma* (Ibrahim, 1957)14245-58330 ova in *Puntius dorsalis* (Sivakami, 1982) and 58327-139934 ova in *Puntius sarana sarana* (Sinha, 1972).

In *Puntius amphibius* the fecundity ranged from 2322 to 13710 where in *Puntius parrah* and *Puntius sarana subnasutus* it was between 2499 and 16303 and 24970 and 87894 respectively. The reproductive potential of fish of different size groups had been expressed as the number of ova produced per gram body weight called relative fecundity (Bagenal., 1978., DeSilva, 1973)or comparative fecundity (Das, 1964).Relative fecundity provides a better comparison of the fecundities eliminates the alteration in absolute fecundity with fish age and size (Sheila and Nair, 1983).The

present study revealed that the average relative fecundity of *Puntius amphibius* was 454 and that of *Puntius parrah* was 531 where it was 384 in *Puntius sarana subnasutus*. These values are high, when compared to the fecundity estimates of 252 ova in *Labeo calbasu* by Pathak and Jhingran (1977) and 256 ova in *Labeo rohitha* by Varghese (1973). Reports on the relative fecundity of other cyprinids included that of *Labeo bata* with 285 ova (Alikunhi, 1956) *Labeo gonius* with 271 ova (Joshi and Khana, 1980) *Puntius vittatus* with 228 ova (Ibrahim, 1957) *Labeo calbasu* with 201 eggs (Vinci and Sugunan, 1981) and *Labeo dussumieri* with 180 eggs (Kurup, 1994). *Osteobrama cotio* (Parameswaran et al, 1971) was found to possess 514-599 ova per gram body weight. The average number of ova per gram weight of ovary was found to be 2338 in *Puntius amphibius*, 2370 in *Puntius sarana subnasutus* where in *Puntius parrah* it was 3483 which is high when compared to the other two and slightly higher than that *Puntius dorsalis* (Sivakami, 1982). The fecundity estimates reveal that *Puntius parrah* has more reproductive potential than *Puntius amphibius* and *Puntius sarana subnasutus*.

Fecundity is often correlated with length, weight and age of fish and also with the length and weight and volume of ovary. The relationship between length and fecundity differs in different species of fish. Clark (1934) opined that the fecundity of a fish increased in proportion to the square of its length. Simpson (1951) established that the fecundity of plaice was related to the cube of its length and was thus directly proportional to fish weight. Many authors have supported Simpson's view of fecundity being related to fish length by a factor close to the cube. (Bagenal, 1957, Sarojini, 1957, Pillay, 1958, Pantalau, 1963, Varghese, 1973, 1976, Kurup, 1994). Jhingran (1961) and Quasim and

Quaum (1963) have reported the exponential value of range around 3. In the present study the exponential value of *Puntius amphibius* was observed to be 2.7017 where in *Puntius parrah* it was 2.8323 and in *Puntius sarana subnasutus* it was 3.1557 which did not deviate significantly from the value of “3” and this finding is in total agreement with the above reports. Fecundity was found to have a linear relationship to weight. In other words the number of ova increased in proportion to body weight. Egg production was explained by the changes in weight .Smith (1947) stated that the number of ova was related to the weight or volume of fish than the length .Linear relationship between fecundity and body weight has been reported in *Labeo fimbriatus* (Bhatnagar, 1972), *Puntius sarana sarana* (Sinha, 1972), *Labeo rohitha* (Khan and Jhigran ,1975), *Labeo bata* (Siddiqui *et al.* ,1976), *Labeo dero*(Raina and Bali, 1982) and *Labeo dussumieri* (Kurup, 1994).The observations of some early workers (Bagenal, 1957., Srojini, 1957., Gupta, 1968., Varghese, 1973) also lend support to the linear relationship between fecundity and body weight.

The coefficient of correlation of the various statistical relationships derived between fecundity, body length, body weight, ovary weight revealed significant relation between fecundity and the body parameters. In *Puntius amphibius*, *Puntius sarana subnasutus* and *Puntius parrah* the highest degree of correlation was seen between fecundity and ovary weight. This is in agreement with the observations of Chaturvedi (1976) in *Tor tor* , Joshi,Khanna (1980) in *Labeo gonius*, Quadri *et al* (1983) in *Schizothorax richardsonii*, Sunder (1986) in *Sardinella longipinnis* and Kurup (1994) in *Labeo dussumieri*. It is well known that the weight of ovaries of a fish is mainly influenced by the ova contained

in them. The 'r' value between ovary weight and body length and ovary weight and body weight exhibited a fair correlation between the variables. But body weight was more closely related to ovary weight than length, as observed in *L.dussumieri* by Kurup (1994). From the multi variant analysis ovary weight was identified as the most appropriate predictor of ovarian egg count. In these three species, near 96 % of the variation in fecundity being explained by the changes in ovary weight. But it is undesirable to sacrifice the fish to determine the gonad weight .Bagenal (1957) had stated that fish length, being easier to measure in the field is more suitable to make prediction of fecundity when large samples are to be dealt with within limited time. Fecundity in *Puntius amphibius* , *Puntius sarana subnasutus* and *Puntius parrah* were found to be almost close to the cube of length and directly proportional to the fish weight and these results would be is valuable in enumerating the fecundity without sacrificing the specimens.

Table 9.1 Fecundity of *Puntius amphibius*

Sl.No	Total length (mm)	Body weight (g)	Ovary weight (g)	Fecundity
1	112	18.6	3.514	12802
2	116	20.122	3.628	13404
3	110	16.514	3.662	11666
4	105	15.348	2.82	5405
5	113	17.64	3.262	9786
6	111	17.574	3.264	10958
7	102	14.258	2.213	3428
8	111	18.668	3.567	13710
9	106	16.998	3.003	8335
10	108	15.886	2.865	6440
11	101	12.354	2.543	4702
12	102	11.876	2.223	2486
13	104	12.002	2.687	4884
14	101	13.224	2.774	5104
15	99	13.328	2.002	2322
16	109	18.002	3.112	7560
17	99	13.89	2.333	3008
18	97	11.778	2.012	2742
19	110	17.974	3.6	12980
20	105	17.442	2.68	8698
21	109	18.554	3.422	11428
22	107	16.442	2.948	7908
23	99	10.58	2.225	2508
24	102	14.567	2.546	3552

Table 9.2 Fecundity of *Puntius parrah*

Sl. No	Total length (mm)	Body weight (g)	Ovary weight (g)	Fecundity
1	113	22.001	4.002	15029
2	116	21.416	3.82	12533
3	108	17.586	2.624	7554
4	111	19.73	3.058	10587
5	112	20.14	2.705	8544
6	109	19.357	3.301	12366
7	109	16.721	2.047	6584
8	107	17.359	2.65	5589
9	105	18.148	2.163	6276
10	104	14.561	2.107	4916
11	105	15.483	2.648	9930
12	115	17.547	2.898	9124
13	115	17.968	2.911	11548
14	111	17.376	2.74	9316
15	96	11.397	1.199	2499
16	120	22.127	4.891	16303
17	106	16.227	2.494	8425
18	119	23.43	4.225	15029
19	110	20.122	2.648	9652
20	99	12.232	1.235	4998
21	113	19.988	2.801	11258
22	108	16.685	2.368	9223
23	101	13.714	2.002	8695
24	106	14.511	2.451	10217

Table 9.3 Fecundity of *Puntius sarana subnasutus*

Sl. No	Total length (mm)	Body weight (g)	Ovary weight (g)	Fecundity
Sl no	Total length(mm)	Body weight(gm)	Ovary weight(gm)	fecundity
1	222	121.578	10.809	26146
2	187	88.033	11.412	26628
3	232	150.809	25.118	42519
4	208	96.266	18.408	62587
5	203	95.664	16.524	41258
6	190	89.338	14.91	32410
7	202	91.224	17.003	48256
8	194	98.368	21.112	55479
9	198	104.022	22.11	56002
10	189	92.558	13.365	31552
11	179	75.842	11.098	24970
12	216	126.026	19.333	26666
13	218	138.2	40.276	87874
14	185	80.125	12.446	29665
15	185	81.225	12.824	30112
16	189	94.698	13.374	32004
17	183	79.221	12.114	27118
18	196	101.224	15.987	39451
19	186	87.551	12.335	27002
20	190	98.698	15.001	36214
21	215	120.654	18.664	52114
22	205	104.226	16.335	44335
23	199	87.558	13.11	25113
24	188	90.667	16.552	44324

Fig 9.1 Relationship between Total length and Fecundity in *Puntius amphibius*

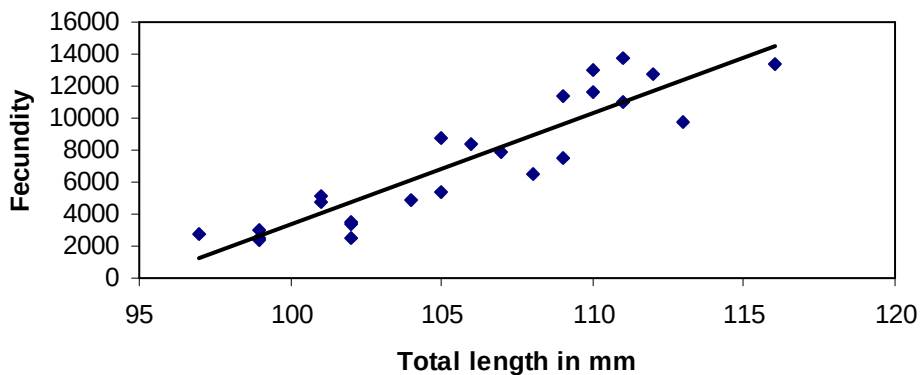


Fig 9.2 Relationship between Total length and Fecundity in *Puntius parrah*

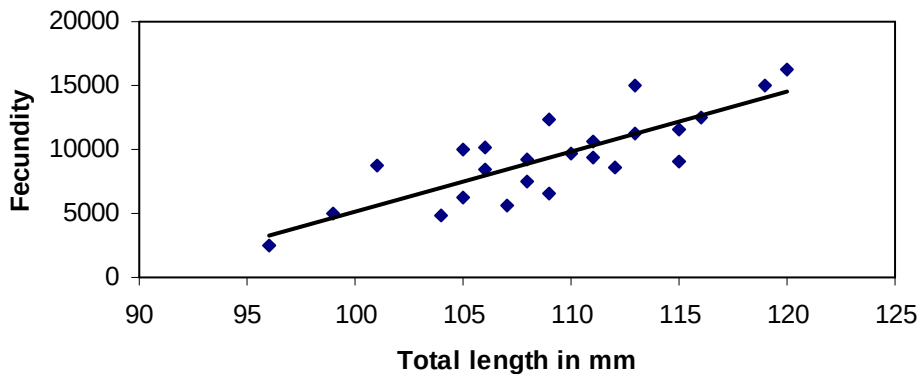


Fig 9.3 Relationship between Total length and Fecundity in *Puntius sarana subnasutus*

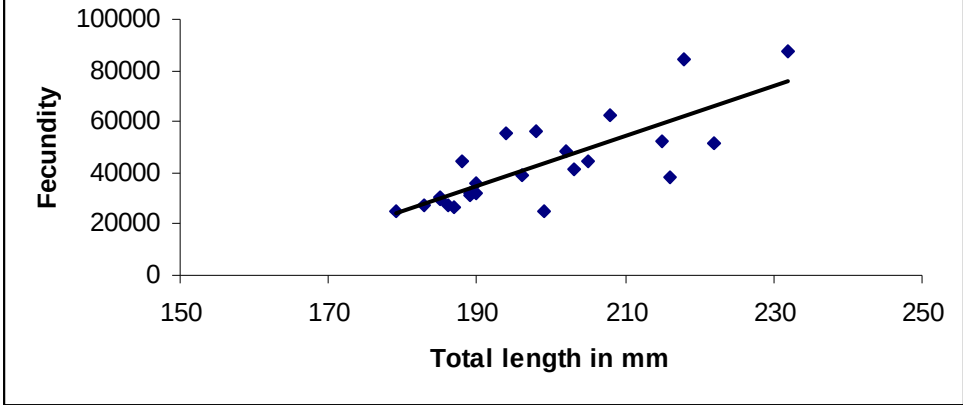


Fig 9.4 Relationship between Body weight and Fecundity in *Puntius amphibius*

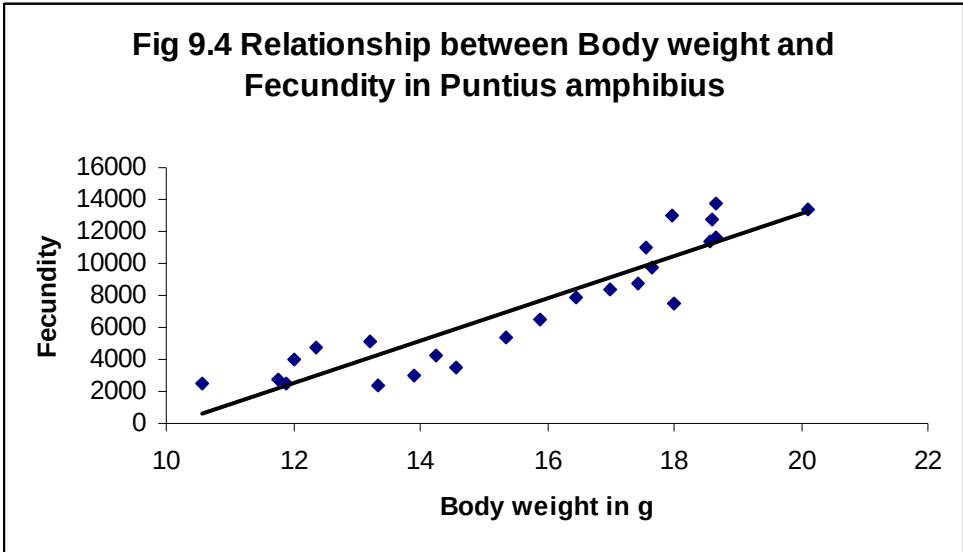


Fig 9.5 Relationship between Body weight and Fecundity in *Puntius parrah*

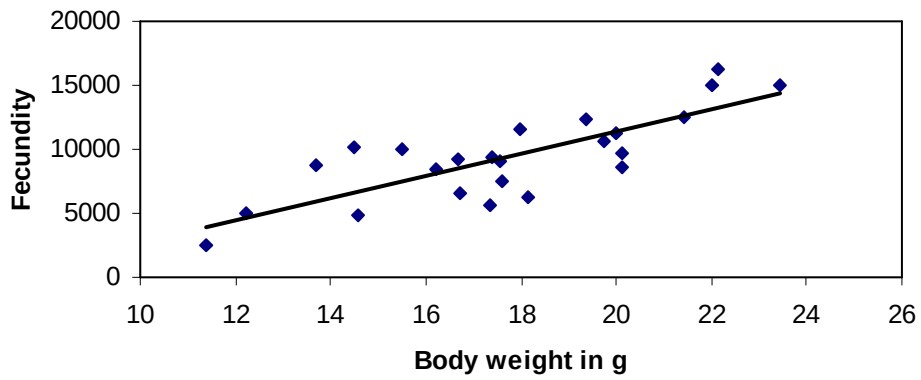


Fig 9.6 Relationship between Body weight and Fecundity in *Puntius sarana subnasutus*

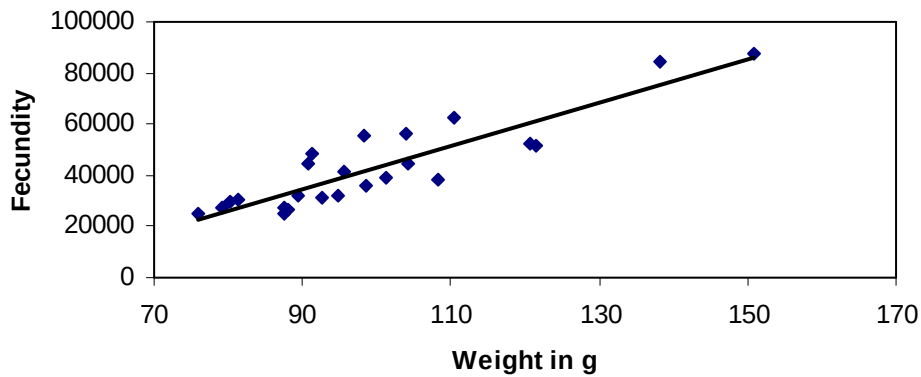


Fig 9.7 Relationship between Ovary weight and Fecundity in *Puntius amphibius*

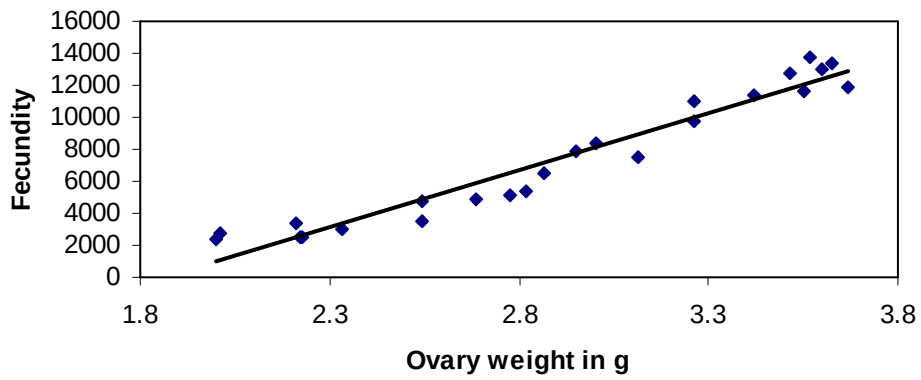


Fig 9.8 Relationship between Ovary weight and Fecundity in *Puntius parrah*

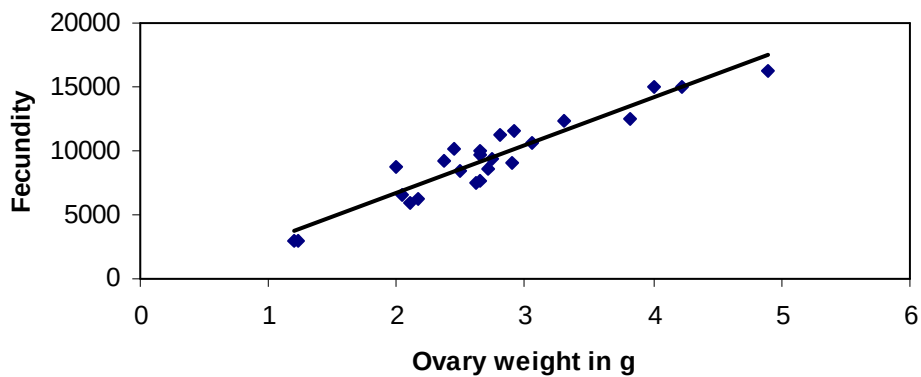
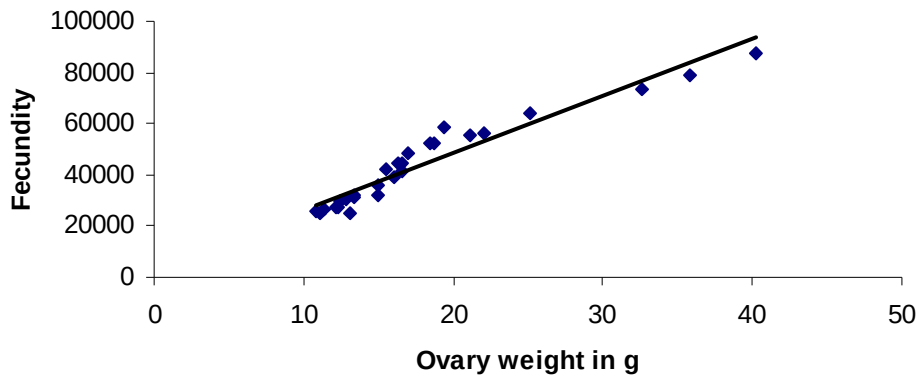


Fig 9.9 Relationship between Ovary weight and Fecundity in *Puntius sarana subnasutus*



CHAPTER - 10

SPAWNING SEASON

CHAPTER – 10

SPAWNING SEASON

INTRODUCTION

Fishes have a fascinating group of reproductive behavior patterns. Such behavior is highly adaptive and strongly related with the morphological adaptations. Environmental parameters like day light, temperature, availability and nature of food and social relations may influence the nature and pattern of reproduction. Gonads undergo regular seasonal and cyclical changes in weight in relation to the total weight of the fish. Such changes are indicative of the spawning season of the fish. Spawning of fish occurs during a particular phase of the reproductive cycle. Some of them breed once a year while some at regular intervals through out the year. Information of gonadal development and the spawning season of a species make subsequent studies on spawning frequency of its population easier, which is important for its management.

Every living organism has immense power of reproduction and recruitment. Under favorable conditions tremendous increase in their number may lead to population explosion. However this doesn't happen in nature, because right from the beginning of gametogenesis to the attainment of maturity there are several factors adversely affecting organisms in different stages of reproduction and growth. A majority of the offspring perishes before reaching maturity. During recent past, the natural and anthropogenic stress has been bringing about drastic reduction in the population of many fish species,

even leading to the endangerment of some of them. If any species is to be managed, conserved and exploited scientifically, a thorough knowledge on the various intricacies of reproduction is of paramount importance. Quasim (1973) while explaining the importance of studying the maturation and spawning of fish has stated that the main purpose of such studies is to understand and predict the biological changes, undergone by the population as a whole, during the year. Information on related aspects such as ecological conditions, which lead to the synchronization of maturity and breeding activity are having immense application for the conservation and management of fish stocks and also for developing capture breeding techniques and understanding aquaculture programs. A precise knowledge on maturing stage, breeding period is of great practical utility in fish culture programs, for proper planning of successful hatching, and nursing operations.

Under natural conditions, reproduction in fish is timed by changes in the external environment (Bromage, 2006). Seasonal reproduction is of, considerable adaptive significance to fish stocks, and were typically linked with the hydrological cycle. Spawning most commonly occurs during the flood season (Lowe-McConnell 1969) although alternate patterns have been noted (McKaye 1977, Kramer 1978, Harikumar *et al.* 1994). 'Flood spawning' is adaptive for many species, as flooding leads to increased food availability and allows dispersal and predator avoidance (Lowe-McConnell 1955). The critical importance of floods to tropical riverine fish production is emphasized by the positive relationship between flood intensity and subsequent catch (Welcome 1967) and the failure of spawning when floods fail (Welcome, 1973).

The activity of all the internal system is cued by environmental variable to ensure that reproductive development, ovulation and spawning occur at the most appropriate time. In tropical fish reproductive seasonality is in relation to day length and short term changes in temperature stimulate spawning episodes. Water flow or level may be important in tropical fresh water species, particularly in areas where juvenile nursery areas are depend on seasonal inundation.

The tropical fishes can be classified as continuous breeders, discontinuous breeders, annual breeders, and breeders which breed more than once a year. Most tropical fresh water fishes spawn seasonally during the rainy periods (Lowe-Mc Connell, 1969.).This certainly applies to tropical cyprinids which lack parental care, viviparity and adaptations for aestivation. Exceptions however do occur. Spawning may be throughout the year as in *Puntius magdalenae* (Welcome, 1969) or even confined to dry season as in *Barbus melanampyx* (Harikumar et al. 1994).

Studies on breeding seasons and factors associated with it are needed to protect new recruits and predict recruitment variability. Knowledge on reproductive biology of fish is essential for evaluating the commercial potentialities of its stock, life history, culture practice and management of its fishery (Doha and Hye 1970).Reproductive potential of a population is one of the basic exigencies to designate the individuals of that population in respect to their gonadal conditions (Jhingran and Verma 1972). In order to achieve success in fish culture, it is important to assess the yearly breeding cycle of culturable fishes. Information of gonadal development and the spawning season of a species make subsequent studies on spawning frequency of its population easier, which is important for

its management. A thorough understanding of the early development of a fish species is also considered to be an important step for the fish culturists.

The actual number of times a female spawns and the number of eggs released each time, however, may ultimately depend on the amount of energy allotted for reproduction given the prevailing physical and biological conditions. In smaller females, more of the energy would be utilized for growth or basic metabolic needs, hence, less energy would be available for egg production.

The spawning season of different Indian fresh water fishes were worked by many authors like Ahamed, 1991., Ganapathi and Alikunhi ,1950.,Alikunhi, 1956., Prabu, 1956.,David, 1959., Sathyanesan, 1962., Qasim and Qayyum, 1961.,Belsare, 1962.,Das, 1964.,Saigal 1967.,Malhotra,1967., Bhatnagar, 1964,1972., Parameswaran *et al.*, 1972., Desai, 1973., Varghes, 1973., Sinha, 1975., Murty, 1975., Chaturvedi, 1976., Siddiqui *et al.* ,1976., Chondar, 1977., Raina, and Bali1982., Ritakumari and Nair, 1979., Joshi and Khanna, 1980., Somavanshi, 1980, 1985., Thakre and Bapat, 1981., Singh *et al.*, 1982., Sheila and Nair 1983., Shrestha, 1986., Sunder, 1986., Joshi, 1987., Pisca and Waghray, 1989., Rao et al. 1999., Jyoti and Malhotra 1972., Kaul, 1994 and Kurup, 1994. A review of literature showed that hitherto no detailed information is available on the spawning biology of *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.).However ,some preliminary observations were reported by Sobhana and Nair (1976)on *Puntius sarana subnasutus* (Val.)and Premkumar etal on *Puntius amphibius* (Val.).

Materials and methods

From 5 to 10 females of each species were sampled each month so that the progression of ovarian development could be followed throughout the study period and spawning frequency could be determined. Entire ovaries were excised, weighed to the nearest 0.1g. and put in gauze bags, placed in modified Gilsonn's solution to harden the eggs and to break down ovarian tissue (Simpson, 1951)

The maturity stages in females were assessed by cross examination of ovaries. The ovaries were preserved in 5 % formalin for ova diameter study. On the basis of their gonadosomatic index and gross examination , three ovaries were selected from monthly samples for detailed ova diameter study. In this study the diameter of 100 ova were measured at random from each ovary adopting the method followed by Clark (1934) and Prabhu (1956).The ova diameter measurements were taken under microscope with an ocular micrometer at a magnification which give a value of 0.0074 mm to each micrometer division. Test measurements of ova from anterior, middle and posterior parts of the ovary indicated no difference in distribution of ova in different regions of the ovary. Hence 100 ova from the middle portions of each ovary of different stages of maturity were measured to study the maturation of ova through the different stages.

The criteria used for quantification of maturity were colour, shape and size of the ovary, diameters of the unspawned eggs and their general appearance, particularly the extent of yolk formation . Specimens were categorized into different length groups on the basis of their stages of maturity. To delineate the spawning season, the ova diameter

measurements and percentage occurrence of gonads in the different stages of maturity were studied during the different months. Female alone were used since male gonads exhibited only microscopically discernible changes, apart from minor alterations in size.

RESULT

The structure of ovaries at different maturity stages follows more or less similar pattern in *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.). The ovaries have been classified into 5 stages, as suggested by Qasim (1973) for tropical fish. Stage I (immature) ovaries were slender, elongated, jelly like flesh coloured, occupy a little more than $\frac{1}{4}$ of the body cavity. Ova invisible to the naked eye appear oval or irregular shaped under microscope, transparent with a distinct central nucleus, devoid of yolk. Stage II (maturing) ovaries were somewhat flattened pale yellow occupy $\frac{1}{2}$ of the body cavity. Ova more or less spherical and translucent, yolk deposition commences. Stage III (ripening) ovaries were slightly cylindrical, yellow opaque, occupy $\frac{3}{4}$ of the body cavity. The inner side slightly depressed to accommodate the gut asymmetry in the lobes, the right lobe slightly longer than the left, yolk deposits increases, spherical ova. Stage IV (ripe) ovaries were considerably enlarged, occupy nearly the entire length of the body cavity, dark red in colour in *Puntius amphibius* (Val.) where in *Puntius parrah* (Day) it is brownish yellow. In *Puntius sarana subnasutus* (Val.) the ripe ovary is in pale yellow. The distended outer membrane of ovary was loosely arranged and the ova were clearly visible, almost transparent. Stage V (spent) ovaries were appeared as shrunken, flaccid, blood shot, translucent, occupies a little more than $\frac{1}{2}$ of the body cavity. Ova were small, invisible, except a few large whitish ones. Immature stocks of

oocytes along with a few residual eggs and empty follicles were present in the spent ovary.

The classification of maturity stages of female *Puntius amphibius* on the basis of ova measurements were depicted in Table 10.1. All the ova less than .2 mm diameter were immature. In the next group of ova above 0.6mm, yolk deposition had just commenced and was thus found to be maturing ova. The ova ranging between 0.8-0.88 were yolked, represented in the ripening eggs. Ova measuring 0.9 mm and above were in fully ripe condition.

In *Puntius parrah* (Day) all the ova less than .2 mm diameter were immature. In the next group of ova above 0.7 mm, yolk deposition had just commenced and was thus found to be maturing ova. The ova ranging between 0.85-0.95 mm were yolked, represented in the ripening eggs. Ova measuring 0.95 mm and above were in fully ripe condition (Table 10.2).

The maturity stages of female *Puntius sarana subnasutus* on the basis of ova measurements were depicted in Table 10.3. All the ova less than 0.5 mm diameter were immature. In the next group of ova above 0.9 mm, yolk deposition had just commenced and was thus found to be maturing ova. The ova ranging between 0.9 -1.1 mm were yolked, represented in the ripening eggs. Ova measuring 1 mm and above were in fully ripe condition.

The development of ova during different months in *Puntius amphibius* (Table 10.4) showed the preponderance of immature ova during August, December, January and

February .From March onwards ova diameter increase gradually till April. There after the progression of ova was very rapid with the result that ripening oocytes were very prominent in May. The ova diameter was maximum during the months of June and October.

In *Puntius parrah* there was a preponderance of immature ova during August, December, January and February (Table 10.5). From March onwards ova diameter increase gradually tills April. There after the progression of ova was very rapid with the result that ripening oocytes were very prominent in May. The ova diameter was maximum during the months of June and October.

The pattern of progression of ova during different months in *Puntius sarana subnasutus* (Table 10.6) showed the preponderance of immature ova during September, January and February .From March onwards ova diameter increase gradually till April. There after the progression of ova was very rapid with the result that ripening oocytes were very prominent in June. The ova diameter was maximum, during the months of July and November.

Percentage occurrence of female fish with gonads of various stages of maturity in *Puntius amphibius* (Val.) (Table 10.7) reveals that except August , December, January, February and march , ripe females are present during all the months and a distinct periodicity in breeding is discernible. A major peak in gravid female number is recorded in May and June which then gradually falls, reaching a minimum in August. The spent fish number is high in these months with a maximum in June and July, after spawning. The number of recovered spent is high in July and August, indicating the conversion of at

least a part of spent s into the recovering stage. In September, a second rise in ripe female number is visible with a peak in October and November .By December all specimens collected were in the recovered spent stage. From the month of January onwards till March , only immature specimens were collected.

In *Puntius parrah* immature females were predominant during the months of July, August, December, January and February. A major peak in gravid female number is recorded in May and June which then gradually falls, reaching a minimum in August. The spent fish number is high in these months with a maximum in June and July, after spawning. The number of recovered spent is high in July and August, indicating the conversion of at least a part of spent s into the recovering stage. In September ,a rise in ripe female number is visible with a peak in October and November .By December all specimens collected were in the recovered spent stage. From the month of January onwards till March, only immature specimens were collected.

Table 10.9 showed the predominance of immature stages of ovary during the months of January and February. A major peak in gravid female number is recorded in June and July which then gradually falls, reaching a minimum in September. The spent fish number is high in months of August and November, after spawning .By January all specimens collected were in the recovered spent stage. From the month of March onwards the maturing stages were gradually increasing.

DISCUSSION

Breeding season was ascertained by applying indirect methods such as quantification of maturity stages, monthly occurrence of gonads in different stages of maturity, monthly

progression of ova towards maturity and seasonal variations in the gonadosomatic index. During August all the specimens of *Puntius amphibius* (Val.) and *Puntius parrah* (Day) belonged to b immature stage. Thenceforth majority of the fish underwent ripening rapidly and interestingly by the end of September, most of them were in advanced degree of maturing and ripening and a small number had even reached the ripe condition .During October a majority part of the population had spawned as manifested by the appearance of spent fish in the population in November. Majority of the fish belongs to the maturing and ripening stages from March onwards and their number showed a rapid increase in the subsequent months to attain peak in June .The presence of spent fish was observed only from the month of May which would suggest that actual spawning might have commenced in May. . Both species might have completed their spawning by the end of July as manifested by the total absence of spent fish during August. Two distinct spawning seasons could be delineated, which were found preceding and succeeding the months of recrudescence .Based on the results of the present study it can well be concluded that *Puntius amphibius* (Val.) and *Puntius parrah* (Day) inhabiting the Chalakudy river spawn twice in an year the first spawning take place during May -June, with intense spawning June and while the second spawning occurs during October .November with intense spawning activity during October.

In *Puntius sarana subnasutus* by the end of October, most of them reached the ripe condition .From October to December the spent females were seen. Majority of the fish belongs to the maturing and ripening stages from March onwards and their number showed a rapid increase in the subsequent months to attain peak in July .The presence of

spent fish was observed only from the month of July which would suggest that actual spawning might have commenced in July. It might have completed the spawning by the end of August as manifested by the total absence of spent fish during September. Based on the results of the present study it can well be concluded that *Puntius sarana subnasutus* (Val.) and inhabiting the Chalakudy river spawn twice in an year the first spawning take place during June-July, with intense spawning in July and while the second spawning occurs during October –December with intense spawning activity during November.

It is well known that ova diameter measurements can give reliable evidence about the time of spawning and spawning periodicity of fish (Clark, 1934). This method has been successfully applied for delineating the spawning period of many Indian fishes by several authors (Prabhu, 1956., Qasim and Qayyum, 1961., Sathyanesan, 1962., Annigeri, 196., Desai and Karamchandani, 1967., Qasim, 1973., Murthy, 1975., James and Baragi, 1980., Thakre and Bapat, 1981., Kurup, 1994). In *Puntius amphibius* (Val.) all the ova measuring .9 mm and above were fully ripe while in *Puntius parrah* (Day) it was 0.95 mm, above which all the ova were ripe. From the appearance of largest oocytes in fully ripe condition in May, June and October, it can be reasonably concluded that these species starts spawning during the above months and this is in close agreement with the spawning season delineated for *Puntius amphibius* (Val.) and *Puntius parrah* (Day) in the present study. In *Puntius sarana subnasutus* (Val.) all the ova measuring 1 mm and above were fully ripe. From the appearance of largest oocytes in fully ripe condition in June, July and November, it can be reasonably concluded that it starts spawning during the

above months and this is in close agreement with the spawning season delineated for *Puntius sarana subnasutus* (Val.) in the present study. According to Jalbert and Zohar (1982) after completion of vitellogenesis, oocytes are maintained within the ovary for a variable period until a series of endocrinal events stimulates their final maturation and ovulation.

According to Prabhu (1956) all ova that are opaque full of yolk and with yolk spherules are mature ones. Accordingly in *Puntius amphibius* (Val.) and *Puntius parrah* (Day) large opaque and semitransparent ova above 0.8 mm were found to be mature. In *Puntius sarana subnasutus* (Val.) large opaque and semitransparent ova above 1 mm were found to be mature. The upper limits of the monthly ranges reflected the presence of individuals with mature ova in all the months except August, December, January, and February. Endogenous rhythm involved in sexual cycling in tropical fish (Scott, 1979., Lam, 1983) takes gonadal development to final maturity which is then maintained until spawning is triggered by sudden environmental fluctuations (Scwassmam, 1971). A relatively long lasting spawning readiness which could explain the continuous presence of mature females has been previously reported by Alkins-koo (2000) while studying the reproductive timing of fishes in a tropical intermittent stream in West Indies.

In *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.) ova diameter indicated the presence of oocytes in varying maturity stages in the ovaries .The wide size range of mature ova with indistinct minor modes within the group of these mature ova could manifest the tendency of the fish for fractional spawning within the season, according to Nikolskii (1963).Fractional spawning and prolonged spawning are mainly characteristics of tropical and subtropical species of fish and may

not only be just an adaptation to increased food supplies ,they also ensure the survival of the species under unfavorable spawning conditions .The succession of maturity group of ova, implied that each individual fish spawned more than once at intervals of several days or weeks. in such forms the breeding cycle of each individual takes an independent course (Qasim and Qayyum, 1961). Foulton (1989) 572 stated that the occurrence of large number ova of different sizes between immature and ripe ones in mature fishes can be considered as an evidence of its prolonged spawning period . The actual rate of extrusion of ova will vary in different species, in some majority of eggs become ripe more or less at the same time, while in others the process is comparatively slow, and only a part of the ova ripen and are released at a time. According to Hickling and Rutenberg (1936) a single group of ova will get differentiated when the spawning is short and definite while in the case of long and indefinite spawning, no distinct separation exists between the general stock of eggs and the maturing eggs.

.In *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.) all oocytes in the ovary develops synchronously and hence the fishes exhibited synchronism in oocyte maturation .It should be mentioned that the duration of breeding seasons is concerned Kramer(1978) suggested that it ranges from extremely brief (1-2 days).through moderately long(2- 4 months) to continuous spawning. Prabhu (1956) treated the duration of 2-3 months as prolonged breeding season. Qasim and Qayyum, (1961) stated that the breeding season is short when it lasts for 2-4 months relatively longer when it lasts for 4-5 months and non seasonal, occurring over a greater part of the year .In *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus*

(Val.) the breeding season lasts for 2-4 months and therefore these species can be categorized under moderately long following Kramer (1978).

The timing of annual spawning of for each species inhabiting a particular niche has evolved to ensure that the young hatch and commence feeding in a season which most conducive to their survival .Stacy (1984) reported that ovulation in most teleosts occurs rapidly in response to specific exogenous factors relevant to reproductive success. These factors include photoperiod, temperature, spawning substrate, visual and chemical stimuli, pH, turbidity of water and availability of food items .In Indian subcontinent most of the freshwater fishes are reported to be monsoon breeders (Jhingran,1982).The earlier reports of Kulkarni (1971), Alikunhi and Rao (1951), Khanna (1958) ,David (1959), Belsare (1962), Bhatnagar (1963) , Parameswaran *et al.* (1972), Rao and Rao (1972), Khan and Jhingran (1975), Murthy (1975), Siddiqui *et al.* (1976), Pathak and Jhingran (1977),Somavanshi (1980), Vinci and Sugunan (1981), Shreshtha (1986) and Kurup (1994) lend support to the above observation .Most of the factors triggering spawning in tropical fishes are supposed to be associated with onset of monsoon and flooding .Fishes are thought to be sensitive to the rising water levels (Alikunhi and Rao, 1951., Khanna, 1958., Kulkarni, 1971). Habitat expansion in the raining season leads to decreased crowding predation pressure, improved productivity and food availability and optimum temperature (Qasim and Q ayyum, 1961) during raining season are the other reported factors influencing the spawning of freshwater fishes .Qasim and qayyum (1961) stated that the breeding seasons in fresh water fishes are adapted to provide optimum conditions of temperature and shelter for the newly hatched fishes. The results of beginning of spawning in *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana*

subnasutus (Val.) coincided with the pre monsoon showers, however the juveniles would be present in the population at the time of peak flooding



Plate 10.1 *Puntius amphibius* –Ripe ovary



Plate 10.2 *Puntius parrah* –Ripe ovary



Plate 10.3 *Puntius sarana subnasutus* –Ripe ovary

Table 10.1 The classification of maturity stages of female *Puntius amphibius*

Stage of maturity	Description of the intra ovarian ova	Mode of the largest group of ova in mm	Size of the intra ovarian ova in mm
I	Immature	0.15 – 0. 2	0.1- 0.25
II	Maturing	0.7-0.75	0.6.5-0.78
III	Ripening	0.83-0.86	0.8-0.88
IV	Ripe	0.9-0.98	0.9 -1
V	Spent	0.80-0.82	0.75-0.85

Table 10.2 The classification of maturity stages of female *Puntius parrah*

Stage of maturity	Description of the intra ovarian ova	Mode of the largest group of ova in mm	Size of the intra ovarian ova in mm
I	Immature	0.18-0.19	0.17-0.2
II	Maturing	0.75-0.8	0.7 -0.85
III	Mature	0.89-0.9	0.85-0.95
IV	Ripe	0.95-0.1.02	0.9-1.04
V	Spent	0.80-0.82	0.75-0.85

Table 10.3 The classification of maturity stages of female *Puntius sarana subnasutus*

Stage of maturity	Description of the intra ovarian ova	Mode of the largest group of ova in mm	Size of the intra ovarian ova in mm
I	Immature	0.38-0.45	0.3-0.5
II	Maturing	0.9-0.98	0.88 -1

III	Ripening	0.95-1	0.9-1.1
IV	Ripe	1.1-1.3	1-1.5
V	Spent	.98-1.01	0.95-1.05

Table 10.4 Average ova diameter of various months

of *Puntius amphibius* of various months of *Puntius amphibius*

Month	Average ova diameter in mm
April	0.722
May	0.859
June	0.968
July	0.81
August	0.704
September	0.818
October	0.932
November	0.79

December	0.189
January	0.202
February	0.216
March	0.575

Table 10.5 Average ova diameter of various months of *Puntius parrah*

Month	Average ova diameter in mm
April	0.832
May	0.938
June	1.01
July	0.81
August	0.4
September	0.918
October	0.987
November	0.768
December	0.198
January	0.322
February	0.318
March	0.658

Table 10.6 Average ova diameter of various months of *Puntius sarana subnasutus* of

Month	Average ova diameter in mm
April	0.902
May	0.986
June	1.08
July	0.81
August	0.604
September	0.918
October	0.987
November	1.76
December	0.298
January	0.402
February	0.418
March	0.658

Table 10 .7 Percentage occurrence of female *Puntius amphibius* in different stages of maturity during the period from April 2003 to March 2005

Month	Percentage occurrence of different maturity stages				
	i	ii	iii	iv	v

April	2.127	76.59	19.14	2.12	0
May	0	0	67.92	21.69	10.3
June	0	0	1.379	75.17	23.65
July	11.64	0	0	6.7	82.1
August	89.05	1.98	0	0	8.97
September	2.36	56.521	39.13	2.17	0
October	0	0	2.14	74.65	20.89
November	23.3	0	0	2.14	74.65
December	100	0	0	0	0
January	100	0	0	0	0
February	53.6	47	0	0	0
March	44.82	53.56	2.083	0	0

Table 10.8 Percentage occurrence of female *Puntius parrah* in different stages of maturity during the period from April 2003 to March 2005

Month	Percentage occurrence of different maturity stages				
	i	li	iii	iv	v
April	0	65.78	32.25	2.63	0
May	0	0	66.36	31.42	2.94
June	0	0	3.57	71.42	25.1
July	19.23	0	0	7.68	73.16
August	83.4	5.5	0	0	11.1
September	5	50	40	5	0
October	0	0	5.65	69.53	24.82
November	26.88	0	0	1.65	71.47
December	100	0	0	0	0
January	100	0	0	0	0
February	45.84	54.16	0	0	0
March	40	55	5	0	0

Table 10.9 Percentage occurrence of female *Puntius sarana subna sutus* in different stages of maturity during the period from April 2003 to March 2005

Month	Percentage occurrence of different maturity stages				
	Immature	Maturing	Ripening	Ripe	spent
April	0	66.25	29.63	4.12	0
May	0	0	66.36	33.64	0
June	0	0	58.32	41.68	0
July	1.24	0	3.24	78.26	17.26
August	10.68	3.22	3.22	6.45	82.85
September	14.81	58.25	27	0	0
October	0	0	18.75	68.75	12.5
November	1.66	0	0	73.33	24.28
December	30	0	0	0	70
January	100	0	0	0	0
February	73.66	26.34	0	0	0
March	45.45	50	4.55	0	0

Fig 10.1 monthly variations of ova diameter measurements in *Puntius amphibius*

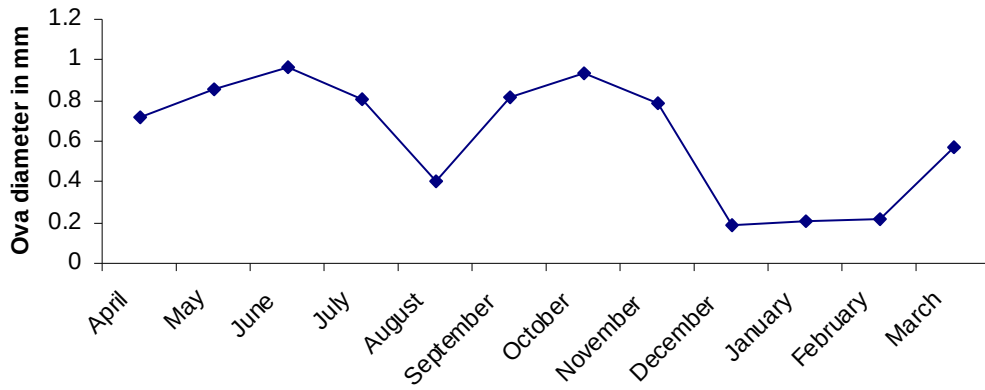


Fig 10.2 Monthly variations of ova diameter measurements in *Puntius parrah*

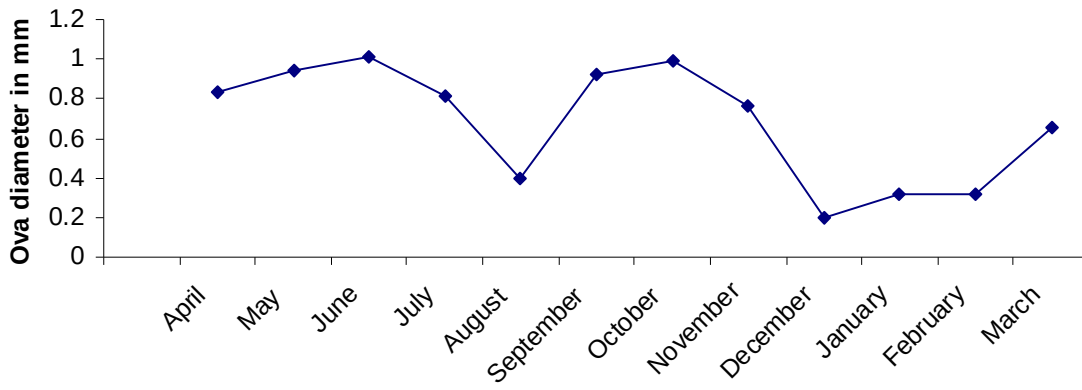


Fig 10.3 Monthly variations of ova diameter measurements in *Puntius sarana subnasutus*

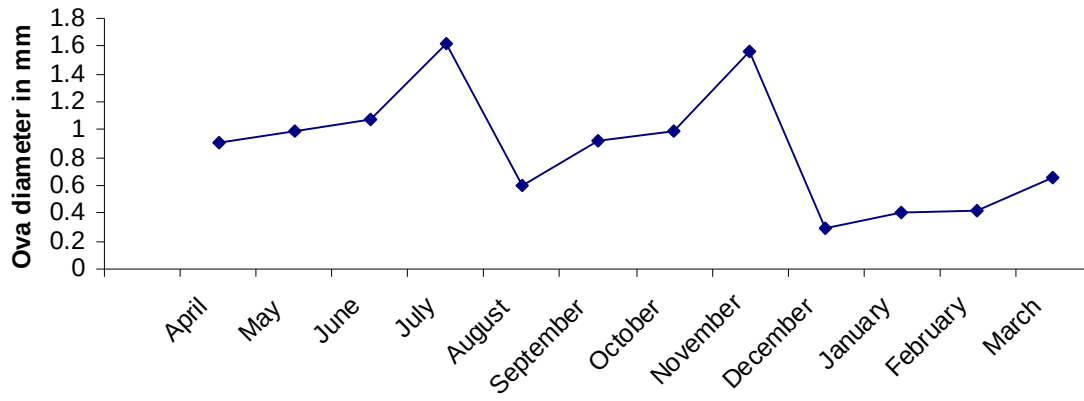


Fig.10.4 Percentage occurrence of female *Puntius amphibius* in different stages of maturity

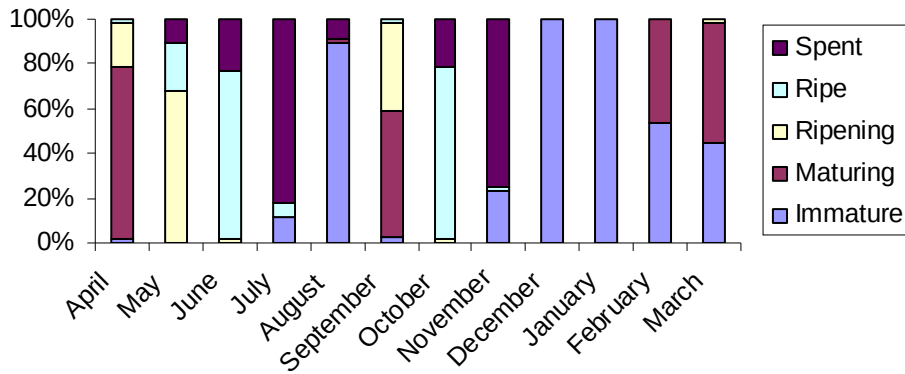


Fig10.5 Percentage occurrence of female *Puntius parrah* in different stages of maturity

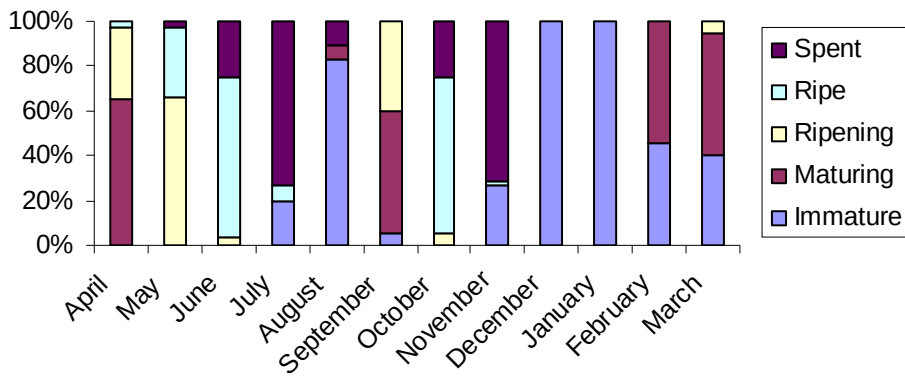
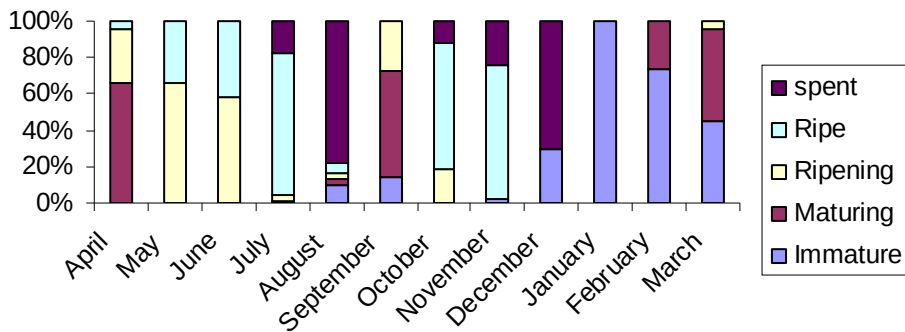


Fig 10.6 Percentage occurrence of female *Puntius sarana subnasutus* in different maturity stages



SUMMARY

INTRODUCTION

Water is the essential and basic resource of fish life on earth .The state of Kerala, situated on the south west coast of India, abounds with extensive inland water spreads which are highly rich in fishery potentialities suitable for fish culture. With the decline in production from many capture fisheries, people are looking for other ways of utilizing aquatic biodiversity. One useful option is the sustainable harvest and culture of ornamental fishes. With judicious tapping of the freshwater fishery resources, Kerala could become one of the leading states in India in ornamental fish trade, thereby generating employment opportunities and increasing export earning considerably. An ecological approach to the management of the aquatic resources is a pre requisite for the effective utilization of the available waters suited for fish culture. A scientific database on resource characteristics and bionomics of fish is indispensable for any program designed for the management and conservation of fish species of commercial importance. The primary need for selecting a fish for culture is the availability of knowledge on different aspects of life cycle of the species that are of major significance for successful culture operations. A study on reproductive characters is essential in the determination of population stock, size from egg numbers, periodicity of strength of broods (year class recruitment), spawning time and place and sex composition of exploited stock. Reproductive parameters are of great value in fishery predictions and formations of management resources.

The three freshwater species undertaken for the present study were *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.) which have, all the desirable qualities of ornamental fishes. They are smaller, daintier and more colourful. All these three species are more active inside the aquarium and they react very well to the

artificial feeds. No detailed studies on the breeding habits and reproductive cycle of species such *Puntius amphibius* (Val.), *Puntius parrah* (Day) and *Puntius sarana subnasutus* (Val.) have not been attempted except for some general observations.

The present study was carried out at the lowest zone of Chalakkudy River. The fish specimens were collected from Mambra, Annamanada, Kundoor, and Kanakkankadavu regions of the river during the periods of study from April 2003 to March 2005. In this area the river is flowing as a single channel and do not have any major tributaries and obstructions. This zone has a length of 43 km along the main river and the altitude difference is 50m. Slope is very less when compared to other zones of the river. The riverbed is sandy in the down stream area. Large deposits of sand were noticed in this zone. In order to unravel the reproductive characteristics of the species during the periods of study, the following aspects were dealt with:-

1. Length-weight relationships and condition factor
2. Biochemical variations during reproductive cycle
3. Sexual dimorphism
4. Sex ratio
5. Length at first maturity
6. Gonado somatic index
7. Fecundity
8. Spawning season

1. LENGTH WEIGHT RELATIONSHIP AND CONDITION FACTOR

Growth of an organism means a change in length or weight or both with the increasing age. The growth in fish is considered in terms of increase in volume. Increment in size is due to conversion of the food matter into building matter of the body by the process of nutrition. The significant departure of regression coefficients from the isometric growth value in male and female of *Puntius amphibius*, *Puntius parrah* and *Puntius sarana subnasutus* indicates that the general parabolic equation $W = a L^b$ express the length weight relationship in adults better than cubic law, $W = c L^3$.

The comparison of elevations disclosed significant difference among two sexes ($P < 0.01$) hence pooling of data to provide single equation expressing the length-weight relationships of these three will not be justifiable, thus necessitating fitting up of separate equation for males and females.

Length weight relationship of *Puntius amphibius* can be expressed as follows,

For males,

$$\text{Log } W = \log 0.000365 + 2.20013 \log L \quad (r = 0.7987)$$

For females,

$$\text{Log } W = \log 0.000003121 + 3.15598 \log L \quad (r = 0.912215)$$

The Kn values showed two peaks (Oct-June) and two troughs (July August and December and January). The values of Ponderal index (k) were found in conformity to the Kn values.

Length weight relationship of *Puntius parrah* can be expressed as follows,

For males,

$$\text{Log W} = \log 0.0002988 + 2.2307 \log L \text{ (r} = 0.7907\text{)}$$

For females,

$$\text{Log W} = \log 0.000003234 + 3.08569 \log L \text{ (r} = 0.9712\text{)}$$

The length weight regression was found to be highly significant in both sexes. ($P < 0.001$). The comparison between males and females as carried out using students 't' test. The results show that 'b' values are significantly different. The Kn values showed two peaks (Oct - June) and two troughs (July August and December and January). The values of Ponderal index (k) were found in conformity to the Kn values during this period.

Length weight relationship of *Puntius sarana subnasutus* can be expressed as follows,

For males,

$$\text{Log W} = \log 0.000022322 + 2.861298 \log L \text{ (r} = 0.983157\text{)}$$

For females,

$$\text{Log W} = \log 0.000005016 + 3.119967 \log L \text{ (r} = 0.9596\text{)}$$

The Kn values showed two peaks (November, July) and two troughs (August and January February). The values of Ponderal index (k) were found in conformity to the Kn values during this period.

In the present study the highest 'b' value was arrived in females of *Puntius amphibius* followed by *Puntius sarana subnasutus* and *Puntius parrah*. The exponential value of 3.15598 in females of *Puntius amphibius* implies that the females gain weight at a faster rate in relation to its length. This is also true in the case of *Puntius sarana subnasutus* and *Puntius parrah*. Based on the results of the length-weight relationship and condition factor, of *Puntius amphibius*, *Puntius sarana subnasutus* and *Puntius parrah* it can be concluded that the growth of females is quite satisfactory and the overall growth performance of females is significantly higher than that of males. The higher regression coefficients in the females may be attributed to their more robust appearance and deep body as against the more slender males.

2. BIOCHEMICAL VARIATIONS DURING REPRODUCTIVE CYCLE

Most of the biochemical constituents of fishes are subject to marked seasonal changes which have been attributed to factors such as maturation, spawning, age, growth and feeding. Attainment of full maturity almost always marks a change in the growth pattern resulting from the reproductive drain due to diversion of materials meant for somatic growth to the gonads. The biochemical composition of three tissues namely muscle, liver, and gonad were studied. Two parameters viz. protein, fat are estimated in relation to the different maturation stages of the gonads.

The monthly variations of protein in muscles, liver and ovary of *Puntius amphibius* significantly correspond with the maturity stage of that month. The protein level in muscle was high during January, February, March, August which denotes the presence of

immature ovaries or in other words during these months there was minimum translocation of protein towards ovary. While ovary begins to mature, the muscle protein content decreases gradually. This denotes the movement of protein towards ovary. The minimum protein level was during the month of June which was corresponded to the full ripe ovaries. The liver protein content shows a little different pathway. The liver protein content shows an increase during the months of April and August which denotes a conduction and storing effect of protein from the somatic tissue for maturation of ovary where the following months call for heavy need of protein for the ovaries. The months of May, June, September and October show significant thinning of liver protein corresponds with the proceeding of ovary maturation. The liver protein content showed minimum values during the months of June and October which denote the full ripening of the ovary and thus the spawning period. The ovary protein content showed a gradual increase and attained a peak value during the breeding months.

In *Puntius parrah* (Day) the values of protein contents were little higher and the mobilization pattern was resembles that of *Puntius amphibius* (Val.) .The month wise analysis of protein content of muscle, liver and ovary of *Puntius parrah* (Day) more or less follows that of *Puntius amphibius* since the pattern of ovary maturation and breeding seasons were coincides . In *Puntius sarana subnasutus* (Val.) along with the ovary maturation proceeds there was gradual thinning of muscle protein occurred. The liver protein utilization followed the same pattern of the other two species. The month wise variations of protein content in muscle, liver and ovary of *Puntius sarana subnasutus* (Val.) showed significant difference from the other two species *Puntius amphibius* (Val.) and *Puntius parrah* (Day) since the two peak spawning months are different .The

muscle protein content was maximum during the months of January, February and March .The diminution of muscle protein from the months of April to July and October to November corresponds with the ovary maturation of two breeding seasons. During the months of August and December there was an increase in muscle corresponds with the two spent seasons. The liver protein content showed diminishing from May to July and September to November corresponded with the two breeding seasons .The ovary protein content showed a maximum value at July and November when the peak breeding season appeared.

In *Puntius amphibius* (Val.) the monthly variations of lipid content percentage in muscle, liver and ovary exactly follows the maturation pattern of ovary .The muscle lipid content was maximum during the months of January, February, March, August and December, which corresponds with the sexually rest phase. The draining of muscle lipids starts from April and September corresponds with the two breeding seasons. The muscle lipid show maximum decline during the months of June and October when the ovaries maximum maturation ,the ripe stage .The liver lipid content showed a inclination during the months of April and August .This rise may be corresponded with the storing phase of lipid for ovary maturation procedures. During the months of April to June and September to October the liver lipid content showed a declining pattern since the there was translocation of lipid to ovary. The minimum lipid content was seen in the months of June and October when the ovary attain ripe stage and spawning began.The ovary showed a continuous increase in its lipid content from the month of March to June and August to October which corresponded with the maturation phase and attained maximum value during June and October, the breeding seasons.

In *Puntius parrah* (Day) the lipid content was slightly higher in muscle and liver but ovary showed a slight depression at all the maturing stages when compared to *Puntius amphibius*. The month wise analysis of lipid percentage follows the same pattern as found in *Puntius amphibius* (Val.) since the breeding months coincides (June and October). *Puntius sarana subnasutus* topped in lipid content among the three. The translocation of lipids into ovary was in greater percentage when compared to *Puntius amphibius* and *Puntius parrah*. The monthly analysis of lipid content of muscle, liver and ovary showed slight variation since the peak breeding occurred at the months of July and November.

3. SEXUAL DIMORPHISM

Sexual dimorphism is a common characteristic of many fishes worldwide. The patterns of sexual dimorphism in fish are often a reversal of that are seen in other taxa, where males being larger than females, with female fecundity in fish often co-varying with body size, selecting for larger females, which often out grow males.

In *Puntius amphibius* the male are noticeably shorter and leaner than females which are comparatively stout. Small sized males were produced in the population either they attain maturity earlier and also have shorter life span or the sperm competition is minimum. Since within population, the female male ratio is near 1: 1.85, so naturally minimum competition may exist between the males within the population. In *Puntius parrah* the males are comparatively shorter and slender than females. The female to male ratio found to be near 1:1.78. The small sizes of males may be due to the lack of sperm competition because females are more in number within in the population. In *Puntius*

sarana subnasutus male female length difference was not so prominent even though males are slightly shorter than females. But the male to female ratio is significantly high, lies in the range of 1:1.9.

In *Puntius amphibius* the main differences in nuptial colouration can be listed as following., the opercular bone in the male has a dark red colouration, but opercular bone in female has no prominent red colour, the male eyes were brightly orange-red and about eight out of ten part of orbital margin , the caudal black spot was more bright in males than females the scarlet coloured band is more sharp and bright in males than females and the central part of caudal fin is more coloured in males than females . . In *Puntius parrah* in males the pelvic fins are more yellowish in males than females. The tip of pelvic fins are bright yellow in males where they are little pale in females. The anal fins are a yellowish shade in males where they are pale in females. In females the scarlet colour is extended up to the black spot at the caudal region where in males the colour spread more around the black spot. Caudal black spot is brighter in males than females. The central part of caudal fin is more coloured in males than females. In *Puntius sarana sabnasutus* in male there is a special golden orange mark present on the gill opening of both sides. In male the pectoral fins are with Orange colour in their throughout length where in female the pectoral fins are very pale yellow in colour and. Male ventral fins and anal fins are deep orange in colour throughout the length, where female ventrals and the anals are orange at the tip only. The nuptial colouration in these species well marked during the breeding season.

Morphological differences especially intense nuptial colouration, between the sexes may be attributed to over activity of male hormone. Sexual dimorphism and colouration are

two important aspects of orientation and selection of mates and therefore, both play important roles in courtship behavior in fishes.

4. SEX RATIO

Sex - ratio indicates the comparison of number of males and females in a population. In natural environment the optimum sex -ratio is usually close to 1:1, however, it is some times shifted towards predominance of one or the other sex. Considerable variation from the ideal sex-ratio 1:1 was observed in the cases of *Puntius amphibius* , *Puntius parrah* and *Puntius sarana subnasutus*. The month wise distribution of the two sexes revealed that the sexes were disproportionate on the sample populations. In *Puntius amphibius* male female sex ratio was found to be 1:1.85 which was significantly different from the ideal ratio 1:1 where the male female ratio was 1:1.79 and 1:1.9 in *Puntius parrah* and *Puntius sarana subnasutus* respectively. In *Puntius amphibius* a preponderance of females over the males was observed throughout the year except in August, January and February when the ratio was nearly 1:1. In *Puntius parrah* a dominance of females over the males was observed throughout the year except in January when the ratio was nearly 1: 0.85. In *Puntius sarana subnasutus* a preponderance of females over the males was observed throughout the year.

In *Puntius amphibius* and *Puntius parrah* during the months of May, June, October and November which are the peak breeding periods the sex ratio highly skewed towards female and the corresponding chi square values were very high and significant considerably. In *Puntius sarana subnasutus* the months of July and November showed high female male ratio and the corresponding chi-square values were also high. During

the spawning season, the females of these three species perhaps become heavy, due to the bulkier ovaries and get trapped easily, when a medium sized cast net is operated. Further it is said that during the spawning season female become lethargic and hence get caught more easily than the males which are more agile and comparatively slender. The higher occurrence of males in lower length groups and females in higher size groups was observed in *Puntius amphibius*, *Puntius parrah* and *Puntius sarana subnasutus* .

5. LENGTH AT FIRST MATURITY

Mature fish are defined as those which have had a chance to spawn at least once. . Usually fishes attain maturity at a particular length of the individuals. .Length at first maturity is defined as the length at which 50 % of the fish of a given stock become sexually mature. In *Puntius amphibius* the females and males were found to be mature at 83 mm and 63 mm respectively. In *Puntius parrah* the females and males were found to be mature at 88 mm and 68 mm where in *Puntius sarana subnasutus* the length at first maturity of the females and males were found to be 165 mm and 125 mm respectively. Thus males attain sexual maturity at a smaller length than the female. In *Puntius amphibius* *Puntius parrah* and *Puntius sarana subnasutus* the females are larger in size. The maximum size of the males and females encountered in *Puntius amphibius* during the present investigation was 85 mm and 120 mm respectively. The corresponding values of *Puntius parrah* were 90 mm and 125 mm, where the maximum size attained by the males and females of *Puntius sarana subnasutus* were 200 mm and 230 mm respectively. The difference in size at first maturity and the maximum size attained in the

two sex group may be due to the fact that body-size dimorphism was very prominent among these three species. Males were comparatively slender and shorter than females. So naturally these characteristics were also reflected in the length attained at first maturity.

6. GONADO SOMATIC INDEX

Gonads undergo regular seasonal and cyclical changes in weight in relation to the total weight of the fish. Such changes are indicative of the spawning season of the fish and the relationship is termed as gonado somatic index. During the peak periods of G. S. I., the percentage of mature and ripe fishes was very high when compared to other groups. These peaks represented the peaks of spawning in an year. Lowest value of G. S. I. noted in the months of August, November, December, January and February in both the species of *Puntius amphibius* and *Puntius parrah*. In *Puntius sarana subnasutus* low G. S. I values were encountered during the months of August, December, January and February. After the first peak G. S. I. came down and then again G. S. I. was increased to the second peak. From these observations it was clear that the increase of ovary weight is associated with the progress of maturity of the ovary.

The maximum average gonadosomatic index observed in *Puntius amphibius* and *Puntius parrah* were during June and October, while it was during the months of July and November in *Puntius sarana subnasutus*. It might be due to the completion of maturity and subsequent steep fall in the index values clearly indicated spent condition of the fish. In these fishes mean values of G. S. I increased with increasing mean body weight and length during gametogenesis. Hence larger fish developed proportionately larger ovaries

during gameto genesis. The peak G. S. I values coincided with the monsoon season as the rainfall diminishes during post monsoon season the value attained its lowest position. Later the second peak in G. S. I values coincided with the north east monsoon during October and November. The above observations revealed that the peak period of spawning were in June and October in *Puntius amphibius* and *Puntius parrah* and during July and November in *Puntius sarana subnasutus*. Since these two spawning periods were connected with southwest monsoon and north east monsoon ,the first ovulation period points out the early arrival of southwest monsoon which can be considered as a factor for the prediction of the south west monsoon in kerala.

7. FECUNDITY

Fecundity is one of the most common measure of reproductive potential in fishes, because it is a relatively easy measurement to make. It is the number of eggs in the ovaries of a female fish. Fecundity varies from one species to another, depending on the environmental conditions, length, age etc. In *Puntius amphibius* the fecundity ranged from 2322 to 13710 where in *Puntius parrah* and *Puntius sarana subnasutus* it was between 2499 and 16303 and 24970 and 87894 respectively. The present study revealed that the average relative fecundity of *Puntius amphibius* was 454 and that of *Puntius parrah* was 531 where, it was 384 in *Puntius sarana subnasutus*. The average number of ova per gram weight of ovary was found to be 2338 in *Puntius amphibius*, 2370 in *Puntius sarana subnasutus* where in *Puntius parrah* it was 3483. Fecundity is correlated with length, body weight and weight of ovary.

In *Puntius amphibius*, *Puntius sarana subnasutus* and *Puntius parrah* the highest degree of correlation was seen between fecundity and ovary weight. Fecundity in *Puntius amphibius*, *Puntius sarana subnasutus* and *Puntius parrah* were found to be almost close to the cube of length and directly proportional to the fish weight and these results would be valuable in enumerating the fecundity without sacrificing the specimens.

8. SPAWNING SEASON

Under natural conditions, reproduction in fish is timed by changes in the external environment. Seasonal reproduction is of considerable adaptive significance to fish stocks, and were typically linked with the hydrological cycle. The activity of all the internal system is cued by environmental variable to ensure that reproductive development, ovulation and spawning occur at the most appropriate time. In tropical fish reproductive seasonality is in relation to day length and short term changes in temperature stimulate spawning episodes. Water flow or level also be important. Spawning season was ascertained by applying indirect methods such as quantification of maturity stages, monthly occurrence of gonads in different stages of maturity, monthly progression of ova towards maturity and seasonal variations in the gonadosomatic index.

During August all the specimens of *Puntius amphibius* (Val.) and *Puntius parrah* (Day) belonged to immature stage. Thenceforth majority of the fish underwent ripening rapidly and interestingly by the end of September, most of them were in advanced degree of maturing and ripening and a small number had even reached the ripe condition. During October a majority part of the population had spawned as manifested by the appearance

of spent fish in the population in November. Majority of the fish belongs to the maturing and ripening stages from March onwards and their number showed a rapid increase in the subsequent months to attain peak in June. The presence of spent fish was observed only from the month of May which would suggest that actual spawning might have commenced in May. Both species might have completed their spawning by the end of July as manifested by the total absence of spent fish during August. Two distinct spawning seasons could be delineated, which were found preceding and succeeding the months of recrudescence. Based on the results of the present study it can well be concluded that *Puntius amphibius* (Val.) and *Puntius parrah* (Day) inhabiting the Chalakudy river spawn twice in an year the first spawning take place during May -June, with intense spawning June and while the second spawning occurs during October. November with intense spawning activity during October.

In *Puntius sarana subnasutus* by the end of October, most of them reached the ripe condition. From October to December the spent females were seen. Majority of the fish belongs to the maturing and ripening stages from March onwards and their number showed a rapid increase in the subsequent months to attain peak in July. The presence of spent fish was observed only from the month of July which would suggest that actual spawning might have commenced in July. It might have completed the spawning by the end of August as manifested by the total absence of spent fish during September. Based on the results of the present study it can well be concluded that *Puntius sarana subnasutus* (Val.) inhabiting the Chalakudy river spawn twice in an year the first spawning take place during June-July, with intense spawning in July and while the

second spawning occurs during October –December with intense spawning activity during November.

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