A STUDY ON OOGENESIS AND OVARIAN MATURATION IN *PUNTIUS POOKODENSIS* ANNA MERCY AND EAPEN JACOB, 2007

By

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THESIS

Submitted in partial fulfillment of the requirement for the degree.

MASTER OF FISHERIES SCIENCE

Faculty of Fisheries

Kerala Agricultural University

2009

DEPARTMENT OF FISHERY BIOLOGY

COLLEGE OF FISHERIES

PANANGAD, COCHIN

DEDICATED TO

MY FAMILY AND MY TEACHERS

DECLARATION

I hereby declare that this thesis entitled "**A STUDY ON OOGENESIS AND OVARIAN MATURATION IN PUNTIUS POOKODENSIS ANNA MERCY AND EAPEN JACOB, 2007**" is a bonafied record of research work done by me during the course of research and that the thesis has not formed the basis of award to me for any other degree, diploma, association or other similar title of any other University or society.

Panangad,
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CERTIFICATE

Certified that this thesis entitled **"A STUDY ON OOGENESIS AND OVARIAN MATURATION IN *PUNTIUS POOKODENSIS* ANNA MERCY AND EAPEN JACOB, 2007"** is a record of research work done independently by **Mrs. Seena Augustine P. A. (2005-14-104)** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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ACKNOWLEDGEMENT

I wish to express my deepest sense of gratitude to my major advisor, Dr.T.M. Jose, Professor, Department of Fishery Biology for his keen interest, guidance, encouragement, support and valuable suggestions during every step of course work and my research, and also for his help and guidance in carrying out the histological studies, without whose help I would not have finished my thesis.

The help, guidance, support and facilities given by Dr. T.V. Anna Mercy, Professor, Department of Fishery Biology, in collecting and rearing *Puntius pookodensis* have enabled me to complete my thesis work.

. My sincere thanks to her for all encouragement, which helped me a lot in preparing the thesis.

I am very much thankful to Dr. J. Rajasekharan Nair, Professor, Department of Fishery Biology, for his help, constant support, critical suggestions, in carrying out the studies in reproductive biology of *Puntius pookodensis*.

My sincere thanks to Dr.K.V. Jayachandran, Professor and Head, Department of Fishery Biology, for his encouragement and wise counseling for completion of my thesis.

My sincere thanks to Smt. Alphi Korath, Assistant Professor (Sr.grade), Department of Management Studies for her help in the statistical analysis of data, which has enabled me to arrive at meaningful conclusions.

. Faculty members of the College of Fisheries have provided a great learning experience and I sincerely thank them.

I wish to extend my thanks to all the staff members of Department of Fishery Biology, Management studies, Fish Processing Technology, Fishing technology, Aquaculture, Fishery Hydrography and Library who directly or indirectly helped me in completing the research work.

I am also thankful to Dr. D. D. Nambudiri, former Dean i/c and Dr. Mohana Kumaran Nair, Dean i/c, College of Fisheries, Panangad. I wish to extend my thanks to Mrs. Shyla G. PhD. student, Aquaculture Department for providing facilities for microphotography.

I am also very much grateful to Mr. Eapen Jacob for all his help, wise advises and other information that helped me a lot in completion of my thesis work.

My sincere thanks to Mrs. Tessy. K. Thomas, Programmer for helping in computerizing data processing.

I would also like to thank Mr. Krishnapriyan, Mrs. Amitha, Mr. Saji, Ms. Jayasree, Mr. Sajan and Ms. Anu for their help and expertise during the work. I would like to thank my friends Indira, Jayaraj, Jayasree, Maya, Parvathy, Jibina, Manjusha, Gomathy and Tessa for their support, cooperation and help during my research work.

Finally I express my deep sense of gratitude to my God and then to my family members especially my Husband, my Parents, my sisters and my kids for their constant love, faith, prayer and inspiration without whom I could not have reached this far.

SEENA AUGUSTINE. P.A.

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INTRODUCTION

1. INTRODUCTION

1.1 ICHTHYOBIODIVERSITY IN INDIA

South East Asia is generally considered to have become the centre of cyprinid evolution because the cyprinids are extra ordinarily numerous and diverse there as well as on the Indian subcontinent (Cech and Moyle, 2000). Of the 34 biodiversity hotspots in the world, India is endowed with a rich biodiversity of fresh water fishes in the Western Ghats and the North Eastern Hills (Kurup, 2002). The Western Ghats, the range of hills running along India's west coast is one of the richest regions in terms of its biological diversity. The forest hills, the narrow coastal plain to their west, and the Arabian Sea, harbor a vast range of life forms from lowly bacteria and fungi to plants, fishes, birds, reptiles and mammals. Biodiversity studies show that the Western Ghats is a gold mine of ornamental fishes. Of the 300 fishes so far assessed from the rivers of Kerala, 155 have ornamental value and of them 53 species are endemic to the Western Ghats (Anna Mercy, 2004 c). Captive studies have been made on the desirable qualities of 90 species of fishes and captive breeding technology has been developed for 12 species (Anna Mercy, 2003).

The vastness of India's ichthyobiodiversity can be seen from the range of habitats they occupy. The fish diversity in the major river systems of India has been divided into four types based on the habitat preference. Out of 2118 species inhabiting Indian waters, 154 are cold water species, 433 are warm water species, 171 are brackish water species and about 1360 are marine species (NFBGR, 2007). Of these 34 species are commonly found both in cold and warm waters. About 67 species are commonly seen in warm and brackish water, 73 species are common in warm water, brackish water and sea, 16 species found only in brackish water and 82 species commonly seen in cold water, brackish water and the sea.

An understanding of the biology and reproductive strategies of these fishes is essential for the preservation of the stock and its habitat. It is also

essential for carrying out ranching programme in the case of endangered species, conducting other aspects of conservation and management of fish germplasm like declaration of parts of the rivers as aquatic sanctuaries, protection and preservation of endangered species and mitigation of anthropogenic maladies (Ranjeet and Kurup, 2002).

1.2 BIODIVERSITY THREATS TO THE FRESH WATER FISHES

Fishes exhibit diversity in their morphology, habitat and biology. The habitat always has a direct impact on the diversity in morphology and physiology of the fish inhabiting therein. Habitat destruction for the betterment of human life leads to considerable reduction of many valuable species of fishes (Jameela Beevi and Ramanchandran, 2002). Unsustainable and unethical fishing by using fish poisons, dynamiting and a wide array of prohibited fishing methods are rampant in the uplands and lowlands of most rivers. Habitat destruction of natural spawning and breeding grounds of the fishes through sand extraction and construction of physical obstructions across rivers have contributed to population decline and endangerment of fresh water fishes. Out of 98 species evaluated as endangered, (based on IUCN criteria) all of them were 'threatened' and 15 species were on the verge of extinction. Many of the species reported as 'endangered' are now found only in areas protected under forest and wild life jurisdiction, which clearly indicates the reasons for their endangerment (Kurup and Ranjeet, 2002).

Furthermore, the escape of imported exotic species and genetically modified organisms (GMO) into natural waters, can lead to irreparable damage to the ecosystem, resulting in degraded environment, disease transmission, changes in biodiversity and possible genetic contamination. Overexploitation of the wild stocks would lead to stock changes and depletion unless the fisheries are managed following sustainable practices (Kutty, 2003). The various types of destructive fishing activities practiced along the river systems are summarized below.

1.2.1 Causes for declining freshwater fish biodiversity

1.2.1.1 Use of small meshed fishing gears:

The use of small meshed fishing gears is prevalent in downstream sections of most of the rivers. Such practices, which are adopted for short-term profit, kill the eggs, embryos, larvae and juveniles of the fishes, thus ultimately leading to reduction in population.

1.2.1.2 Wanton destruction:

Diverse types of chemicals such as copper sulphate and fish poisons of plant origin are widely used in upstream, middle and down stream parts of most rivers. Plant based poisons coming from various plant parts are used in shallow, low velocity waters. Pesticides and insecticides are used for catching fishes that are either nocturnal or dwelling in small caves or crevices. Dynamiting is a major method for catching food fishes but is less commonly used to catch ornamental varieties since it kills fishes instantaneously. Electro-fishing is increasing in popularity in the down streams of the rivers. It is mainly targeted at larger fishes; however, smaller ornamental fishes are also killed by this method. These methods have affected a number of food and sport fishes of upland waters, especially in rivers and streams originating in Assam, Nepal, Bhutan, Uttaranchal and Himachal Pradesh (Dehadrai et al., 1994; Shrestha, 1997; Ponniah et al., 1998).

1.2.1.3 Destruction and modification of habitats:

Destruction of fish habitat is another major cause of the decline in the ornamental fish population. Dams, bunds and levees act as barriers for free migration of fish in the rivers. Deforestation accelerated the decline of fish populations due to excessive siltation and soil erosion. These activities destroy the feeding and breeding grounds of many fishes (Sehgal, 1994; Kirchoffer and Hefti, 1996).

1.2.1.4 Introduction of new species

The introduction of exotic and alien species to the natural waters has resulted in competition for food and space and ultimately in the decline of indigenous species. In Periyar Lake, which is well known as one of the biodiversity hotspots of Kerala, exotic species such as *Cyprinus carpio,* have already established breeding populations and contributed more than 70% of the exploited stock. A high percentage of diet overlap exists between the native fish species like *Tor khudree, Gonoproktopterus curmuca, Lepidopygopsis typus* and the exotic species like *Oreochromis mossambicus* and *Cyprinus carpio*. Percentage contribution of exotics in the landing showed their clear-cut preponderance over indigenous fish species by weight. Tilapia has established its population in almost all rivers of Kerala. The exotic high yielding African catfish *(Clarius gariepinus)* is another danger to the indigenous species. Alien species such as Catla (*Catla catla*), Rohu *(Labeo rohita)* and Mrigal *(Cirrhinus mrigala)* have been cultured in most of the reservoirs and ponds of Kerala, which has caused a gradual reduction of the endemic populations in these water bodies (Kurup and Ranjeet, 2002).

1.2.1.5. Over fishing:

Over fishing of potential ornamental species like *Puntius denisoni, Tetraodon travancoricus* and loaches without assessing their population size could lead to their extinction in the near future. Unfortunately, with the targeting of half a dozen fishes for the domestic and international trade, the stock size of these fishes has declined drastically and, as a result, most of them are now endangered. The decline in population of fishes such as *Tor* species and *Schizothorax* species in upland waters (Nautiyal, 1994 ; Mahanta et al., 1998); *Notopterus chitala, Ompok pabda, Pangassius pangasius* etc in warm waters (Menon, 1989) and *Mugil cephalus, Liza tade, Nematolosa nasus* etc in brackish waters (Pandit and Mandal, 1994) are due to overexploitation of their stocks.

1.2.1.6. Aquatic pollution:

Aquatic pollution is probably the most significant factor causing major decline in populations of many fish species (Dehadrai *et al.,* 1994). Industrial sewage (municipal) and pesticide pollution have been detrimental to fish life in many water bodies. These are causing permanent damage to genetic resources in addition to their direct toxic effects. (Jhingran, 1991).

1.2.1.7. Genetic problems in threatened species:

Overexploitation of the fish resources coupled with the habitat destruction results in the shrinkage of fish population and severe genetic drifts and accumulation of homozygosity threatens many fish populations (Meffe, 1986; Das, 1989; Mishra *et al*., 2000; Narain, 2000).

1.3 NEED FOR ICHTHYOBIODIVERSITY

For a better tomorrow we must keep a strong monitoring on the changing environment. Sustainable fishery is not about fishing for economic purposes only but it has a great concern for saving the fish habitat and the aquatic environment. It is essential to save fresh water resources and the whole aquatic environment along with fishes and other aquatic organisms to keep the ecosystem undisturbed as far as possible. These freshwater resources are, in fact our life supporting system that cannot be exploited any more for economic purposes only. Maximum sustainable yield should be changed according to the changing environment and it must be commensurate with fish population of a particular species. Any deviation would lead to further erosion of biodiversity that would be detrimental to fisheries and environment as a whole. Right information at right time can save this biodiversity. In order to alter the trends of biodiversity to a positive direction, the role of right information input and information technology as a tool is quite inevitable.

1.4 MANAGEMENT MEASURES RELEVANT FOR CONSERVATION OF THE FRESHWATER FISHES

Management measures aimed at conserving freshwater fish biodiversity should be included in the fishery policies. In addition, the information available is utilized by central and state government agencies and all those who are deeply involved in implementing various measures for the protection of the fish biodiversity of these ghats.

1.4.1 Strategies for conservation of ichthyobiodiversity

1.4.1.1 In-situ conservation

In-situ conservation of fish is useful where genetic diversity exists and where wild forms are present. This is done through their maintenance within natural or manmade ecosystems in which they occur.

1.4.1.2. Ex-situ conservation

In *ex-situ* conservation, the threatened species are conserved outside their natural habitats. The two main pillars of *ex-situ* conservation programme are (i) Live Gene Bank and (ii) Gamete/Embryo Bank. In a Live Gene bank, the endangered species are reared in captivity, bred therein and genetically managed avoiding inbreeding depression, domestication and unintended selection (Minckley and Deacon, 1991; Jensen, 1994).

1.4.1.3. Cryopreservation

In gamete/embryo bank, adequate sample representative of the natural genetic variations of endangered species are kept in suspended state of animation under extra low temperature (-196˚C) in liquid nitrogen (LN_2). Establishment of Gene Bank by cryopreserved milt, eggs and embryos assures further availability of genetic materials of threatened categories and for extensive breeding programmes of economically important species. (Chao and Liao, 2001).

1.4.1.4. Habitat Restoration

Most of the damage done to the various fish habitats so far is irreversible, at least in short term, where the fish species or communities are severely threatened (Wang and Xie, 1997). In many cases unique stocks have completely disappeared. Even where habitat restoration is contemplated stock transfer could be an important interim measure. How ever there are a number of important examples of habitat restoration in temperate areas and it should be emphasized that habitat protection and restoration are the principal long term means through which successful conservation will be achieved (Surtida, 1998).

1.4.1.5. Captive Breeding

In the world conservation strategy, captive propagation is considered to be an integral part of the global strategy to conserve genetic diversity and is recommended wherever 'on site' conservation becomes untenable. Despite these recommendations and the strong advocation of captive propagation as conservation option by the conservationists, captive propagation is viewed doubtfully by numerous biologists (Franklin, 1980). How ever now a days the science of captive propagation has a firm and respectable foundation. It is growing rapidly and benefiting from numerous successes.

1.4.1.6. National Conservational Strategies.

The Fisheries Act of 1897, modified in 1956, is a land mark in the conservation of fishes in India, whereby among other items, the use of explosives or poisons too indiscriminately kill fish in any water is prohibited (Menon, 1989).

In a huge country like India with diverse ecosystems, where enforcement of law is not an easy task, the most effective way of tackling the problem would be to arouse mass consciousness on the subject so that people themselves could protect the fish genetic resources (Das,1989). Zoo Outreach Organization conducted a workshop on Conservation, Assessments and Management Plan (CAMP) for freshwater fishes of India in 1997, hosted by

the National Bureau of Fish Genetic Resources, ICAR. The recommendations of this workshop became the base line data for a large number of research projects on the conservation of the fresh water fishes (NBFGR, 1998).

Ornamental fishes are the most popular pets of the world and aquarium keeping is the second largest hobby next to photography. Ornamental fish keeping and its propagation has been an interesting activity for many, which provide not only aesthetic pleasure but also financial openings. Tropical fishes have always attracted the attention of ornamental fish hobbyists. About 600 ornamental fish species have been reported worldwide. India being a tropical country possesses a rich diversity of ornamental fish, with over 100 varieties of indigenous ornamental fishes. The Western Ghats of India is in fact a gold mine of ornamental fishes (Anna Mercy, 2004 a,b).

1.5.OBJECTIVES OF STUDY

Puntius pookodensis Anna Mercy and Eapen Jacob, 2007 is a newly identified species of *Puntius* and an indigenous ornamental fish of the family Cyprinidae (Anna Mercy and Eapen Jacob, 2007). It is endemic to the Western Ghats of Kerala and inhabits only the Pookode Lake, which is a fresh water lake at Wayanad district, Kerala. *Puntius pookodensis* has all the desirable qualities of being an ornamental fish. Being small it is not considered as a food fish.

Knowledge about the basic reproductive biology of this fish will definitely help in the commercial production under captivity. Production of the fish in large quantities under hatchery conditions will enable their supply in the domestic and also in the export market as an ornamental fish. The standardization of the captive breeding of *Puntius pookodensis* has been prioritized under NBFGR – NATP programme entitled "Germplasm inventory evaluation and gene banking of fresh water fishes of India". Anna Mercy *et al.* (2005 a) developed captive breeding technology for *Puntius pookodensis.* Present study is conducted with the following objectives:

(i) To study the quantification of maturity stages and size at first maturity.

(ii) To describe sexual dimorphism.

(iii) To estimate fecundity.

(iv) To determine the spawning frequency.

(v) To observe the cyclic changes of the oocyte maturation and their histological differentiation during oogenesis.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 REPRODUCTIVE BIOLOGY

With many species fast becoming endangered, it becomes inevitable to pinpoint the cause of their endangerment so as to take up a programme for their conservation. The first step in the understanding of the needs and requirements of any organism is the study of its biology. The study of reproductive biology comes first and foremost in biological studies and involves the study of (i) oogenesis (ii) spawning frequency, (iv) fecundity and (v) sexual dimorphism, Therefore, a detailed study on the biological features of the threatened species will be very valuable in implementing any programme on conservation of the fish genetic resources (Virjenhosk *et al.,* 1998).

2.2 REPRODUCTIVE STRATEGY IN FISHES

A complete knowledge of the reproductive system and the reproductive biology of fishes are essential to understand the reproductive strategy of any given species. Quite some work has been done on the fish reproductive system namely the gonads and their development. The notable of these early works are those of Yamamoto (1956 a), Yamamoto and Yamazaki (1961). The literature on the reproductive organs and reproduction was reviewed by Raven (1961), Hoar (1969), Nagahama (1983), West (1990) and Jalabert (2005). Recent works on the same aspects involve those of Dasgupta (2002), Kurian and Inasu (2002), Goswami and Dasgupta (2004), Nisha Raj (2005) and Divipala (2008).

2.3 OOGENESIS IN FISHES

The most important aspect of reproductive biology is gametogenesis. Oogenesis comprises formation, development and growth of ova. Despite a great diversity in their reproductive strategies, the ovaries of numerous teleost species show a similar general structure. In

most cases they are elongated organs oriented longitudinally within the abdominal cavity. Ovaries of some species such as trout and salmon are not completely surrounded by the mesovarium and the ovigerous lamellae are open in the body cavity where mature oocytes are directly released at ovulation, and where they can remain for some time before being laid (Jalabert, 2005).

The ovaries are compartmentalized by numerous septa formed by folds of the germinal epithelium, usually called the ovigerous lamellae; projecting into the lumen. These lamellae are the sites of nests of oogonia, and oocytes at early stages of entry into the meiotic prophase. The posterior part of each ovary is prolonged by an oviduct connected to the genital papilla. At ovulation, mature oocytes are released from their follicles into the ovarian cavity, before being laid outside through the oviduct and the genital papilla. Immature oocytes bud off from the ovigerous lamellae. This immature oocyte is characterized by a large nucleus, containing one large nucleolus. At the end of this stage; multiple nucleoli are mostly arranged towards the periphery of nuclear membrane. This is followed by vacuolization of cytoplasm, yolk deposition (vitellogenesis) migratory nucleus and mature ova. These mature oocytes with micropile are ready for ovulation (Jalabert, 2005).

Vitellogenesis has often been used to designate more or less restricted phases of a whole of complex processes, comprising the synthesis of organic compounds within the oocyte (endogenous vitellogenesis) and the incorporation of macromolecules synthesized in the liver and brought by the blood and absorbed through the follicular layer into the oocyte (exogenous vitellogenesis) (Mellinger, 2002). In endogenous vitellogenesis, the process of vitellogenesis starts from around the nucleus. In such case the nucleus is believed to be the source of vitelline. However, the major part of the vitelline is exogenous.

Oocyte developmental stages have been extensively described in numerous species with some differences depending on the species and the

classification criteria. some of the notable works are those of Yamamoto (1956 b) on *Liopsetta obscura*; Yamamoto and Yamazaki (1961) on *Carassius auratus*; Selman *et al*. (1993) on *Brachydanio rerio*; Glasser *et al*. (2003) on *Ctenopharyngodon idella*; Gui *et al.* (2003) on *Carassius auratus gibelio*; Cek *et al*. (2001) on *Puntius conchonius* and Selman and Wallace (2005) on *Fundulus heteroclitus*. Some of the recent reviews are those of Wallace and Selman (1981); Guraya (1986); Patino and Sullivan (2002) and Jalabert (2005).

Histological studies provide very precise information on oocyte developmental stages, but their interpretation is confusing because different authors use different terms for the same structures. Males are in general more difficult to stage than females and may give a less defined estimate of the spawning season and spawning frequency (Fairbridge, 1951; June, 1953; Crossland, 1977 and Forberg, 1982).

Oocyte development in teleosts has been extensively reviewed by Yamamoto (1956a), Wallace and Selman (1981), De Vlaming (1983), Wallace et al. (1987) and Jalabert (2005).

2.4 SIZE AT FIRST MATURITY

It is the length at which 50% of the fish population is regarded to have attained maturity (Kagwade, 1968). This varies not only from species to species but also with in the species, which are subjected to varied internal and external stimuli. This variation in the length at first maturity may be related to the ecological factors, food supply and assimilation (Keshava *et al.,* 1988). Usually in the fishes, it is the males, which mature earlier and so remain smaller than the females as greater part of their energy reserves are diverted towards gonadal growth and development. However, in species exhibiting territoriality and parental care it is the males, which are larger as they are involved with the care of the eggs and young ones (Nickolsky, 1963). The females usually mature later so that they can somatically grow larger and produce more number

of viable eggs.

Besides the above factors, population density and quantity and quality of food available also have a significant role to play in determining the size at first maturity of the given species. In expanding populations the females tend to mature at an earlier age. Their maturing earlier owes to the fact that expanding population is usually found in favorable environment and so attained larger size at a younger age. On the other hand where the environment is favorable for growth and adult survival, fishes tend to delay reproduction. The advantage in such cases is the increase in fecundity, which is proportionate to the somatic growth of the fish. The size at first reproduction has an important role in understanding the life history of a species during its evolution and gives a rough estimation of the ultimate ($L_{\acute{a}}$) size of the species.

3.5 SPAWNING FREQUENCY IN FISHES

The success of any species lies in its ability to reproduce in most appropriate time for the higher rate of survival of their offspring. The frequency of spawning depends on the environment and the reproductive strategies evolved by the given species over the time. The two basic strategies observed in nature are semelparity and iteroparity. Semelparity otherwise known as the “big bang” reproduction involves species which spawn only once in their lifetime and then die. The Pacific salmons are a very good example of this type of reproduction where all the adults take up reproductive migration and die by the end of spawning. On the other hand we have the iteroparity in which the species spawn repeatedly in a season or in their life. This is so in case of most of the fishes, even more so in the tropical ones.

The frequency of reproduction forms the index of the predictability of the environment and can be elucidated by the ova diameter studies. It has been demonstrated that by studying the intraovarian egg dimensions of fishes in the ripe condition or penultimate

stage of maturity, it is possible to elucidate the duration of spawning periods and individual spawning frequency (Clark, 1934; Hickling and Rutenberg, 1936; De Jong, 1939; June, 1953; Prabhu, 1956; Quasim and Qayyum, 1961; Grimes and Huntsman, 1980).

Based on oocyte size distribution, Wallace ad Selman (1981) classified ovaries into three basic types:

(i) 'Synchronous ovaries' in which all oocytes develop and ovulate in unison and there is no replenishment from the earlier stages. Such ovaries are found in species that spawn once and then die. The oocyte size distribution consists of a single mode (semelparous fishes.)

(ii) 'Group synchronous ovaries', in which at least two size groups of oocytes are present at some time, the larger group or clutch usually being more homogenous than the smaller.

(iii) 'Asynchronous ovaries', in which oocytes at all stages of development are present at the same time. The oocyte size frequency distribution is continuous except in the ripe stage where there may be a clear separation of the yolked oocytes.

However, a protracted breeding season in itself does not imply multiple spawning for each female, as it might simply reflect a lack of population synchrony in gonad development showing asynchronous breeding populations (De Jong, 1939; De Vlaming, 1983). Also the type of oocyte development is not species specific.

'Isochronal' or 'total' spawners is the other name given to the group synchronous spawners while it is 'partial', 'heterochronal', 'multiple' or 'serial' spawners for the asynchronous spawners. According to Holden and Raitt (1974) the oocytes will be shed within a short period- a week or so in the group synchronous spawners and only a compliment of the yolked oocytes is spawned in case of the asynchronous spawners.

De Vlaming (1983) suggested that the multiple spawning generally refers to more than one spawning in a season, and 'fractional spawning' is used for species that spawn part of an ovulated clutch.

Based on spawning frequency (Prabhu, 1956; Karekar and Bal, 1960), the fishes are categorized into four groups. This classification is based upon the works of Hickling and Rutenbnerg (1936).

(i) Category A: Spawning takes place once in a season during a short duration, the individual spawning once. Ovary contains a ripe stock distinctly and clearly separated from immature stock.

(ii) Category B: Spawning takes place once in a season but with longer duration. Range in size of the ripe ova nearly one half of the total range of intra-ovarian eggs.

(iii) Category C: Spawning more than once during a protracted spawning season. Ovary with a batch of ripe stock, an immature stock and intermediate ripening stock in between the ripe and immature.

(iv) Category D: Spawning extended over a very long period or almost round the year but intermittently, the individual spawning many times in the spawning season. Batches of eggs in the ovary are not well differentiated from one another, usually shown by fishes in tropical structured communities.

3.6 FECUNDITY IN FISHES

3.6.1 Absolute fecundity

Though it is easy procedure for estimation of the reproductive potential of any given fish species, it does not give an accurate picture of the egg release as the fishes tend to be multiple spawners or even batch

spawners. Hence a precise estimation of fecundity is almost impossible. Absolute fecundity is defined as the number of ripe eggs found in the female prior to spawning (Bagenal and Braun, 1968). On the other hand fertility may be defined as the actual number of young produced rather than the number of eggs. Both endogenous and exogenous factors have a profound effect on the fecundity. The variation in fecundity is dependent on season, climatic condition, environmental habitat, nutritional status and genetic potential (Bromage *et al.,* 1992). If suitable conditions are not found then the eggs might become atretic, degenerate and ultimately get resorbed into the body. Grimes and Huntsman (1980) used gravimetric sub sampling to estimate fecundity in vermillion snapper, *Rhomboplites aurorubens*. The same was followed in *Priacanthus hamrur* (Sivakami *et al.,* 2001), *Mystus gulio* (Dasgupta, 2002) and *Horabagrus brachysoma* (Kurian and Inasu, 2002).

Estimation of the absolute fecundity of multiple spawners is difficult. Usually all the yolked ripe/ripening eggs are counted based on the classic work of Hickling and Rutenberg (1936) for multiple spawners. The estimation of fecundity in open substrate spawners is also difficult owing to the extremely high fecundities and protracted spawning periods. On the other hand fecundity estimation of the brood hiders and nest spawners and all the fishes showing parental care is easy due to low fecundity. Here eggs are larger with high survival rate. In case of mouth brooders the fecundity depends more on the brooding capacity of the parent than on the fertility. The fecundity is the least in live bearers where the newly born are stronger than those that hatch out of an egg. The fecundity is inversely related to egg size, yolk content and incubation time.

2.6.2 Relative fecundity

Relative fecundity is the expression of absolute fecundity in terms of numbers per unit length and weight of the body or ovary of the given

fish. The relationships are linear and are expressed as, $F=a\ X^b$ where X=length, weight or age and 'a' and 'b' are constants (Bagenal and Braum, 1968)

3.7 SEXUAL DIMORPHISM

The reproduction strategies of fishes are often clearly reflected in the anatomical differences between the sexes. Sexual dimorphism and sexual dichromatism are secondary sexual characters coming under the external anatomical adaptations of reproduction in fishes (Cech and Moyle, 2000). The secondary sexual characters assume importance in connections with the external identification of the sexes under captive conditions.

The relative growth of body parts of a fish may vary with sex at different stages of its life history and this was exemplified by earlier workers (Godsil, 1948; Pitcher and Hart, 1990). They stated that there were different ratios between different morphometric characters and total length of males and females in the fish species studied by them. Sexual dimorphism in the Western Ghats indigenous ornamental fishes like *Puntius amphibius, P. fasciatus, P. pookodensis, P. melanostigma, P. filamentosus, Chela dadiborjori, Chela fasciata and Danio malanbaricus* was recorded by Anna Mercy *et al.* (2007).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 SYSTEMATICS

Specimens of *Puntius pookodensis* collected from the Pookode Lake at Wayanad District, Northern Kerala were used for the present study and the distinguishing characters based on external morphology and colour were studied.

3.2 BREEDING BIOLOGY

A total number of 112 specimens (77 females and 35 males) collected during 2007 were used for the study of different aspects of breeding biology. The females ranged from 25mm to 60mm and the males ranged from 20mm to 55mm total length. The different aspects of breeding biology namely size at first maturity, spawning frequency based on ova-diameter studies and fecundity were studied using the methods followed by Nair and Nair (1984).

3.2.1 Quantification of maturity stages.

The maturity can be defined as the cyclic morphological and histological changes undergone by the gonads to grow and ripen. For study of spawning biology quantification was done based on external evaluation of the gonads. Characters used for macroscopic quantification were color and shape of gonad, space it occupied in the body cavity, size of ova, texture of ovary and blood supply.

3.2.2 Maturation of ovary and oogenesis

3.2.2.1 Oocyte distribution

The study was based on the progression of the oocyte stocks in the different maturity stages. Ova diameter measurements were made from the different maturity stages using an ocular micrometer. During the initial quantification of maturity stages itself a few ovaries belonging to all the stages

of maturity were preserved in Gilson's fluid for ova diameter measurements. The ova diameter measurements were done on a monocular microscope with
4 ×10 magnification, after calibrating the ocular micrometer using a stage micrometer. A mixed sub-sample was taken from different parts of the ovary to eliminate the error due to differential distribution of ova stocks in the different parts of the ovary.

3.2.2.2 Histology

For histological studies fresh material was used. Small pieces of the ovary at different stages of maturity were fixed in Bouin's fluid. Paraffin sections at 6-8 microns thickness was stained with Harris's haematoxylin and counter stained with eosin (Weesner, 1960). The sequence of histological changes during the origin, maturation and liberation of the germ cells were studied from these sections.

3.2.3 Size at first maturity

Size at first maturity was computed with a total of 112 fishes of which 77 were females (ranging from 25 mm to 60 mm TL) and 35 fishes were males (ranging from 20 mm to 55 mm TL). The length at first maturity is the size at which 50% of the population is mature.

The total lengths of all the fishes collected for the study were grouped according to different length groups. The percentage occurrences of mature fishes (early ripening, late ripening, ripe and partially spent) for the females and males have been taken. By plotting the percentage occurrence of mature fish (males and females) against respective length classes (5mm), the length at which 50% of the fishes were mature was demarcated.

3.2.4. Spawning frequency

Ova diameter measurements of ripe ova were made using an ocular micrometer. A mixed sub-sample from different parts (anterior, middle and posterior) of a ripe ovary was taken, to eliminate any error due to differential

distribution of ova stocks in different parts of the ovary. Ova diameter classes (64µm interval) and their respective percentage frequencies were plotted to study the spawning frequency.

3.2.5. Fecundity

The ripe ovaries were preserved in 5% formalin. These were later weighed after removal of the excess water and also their length and width were taken. The ovaries, being small, were kept whole in Gilson's fluid with appropriate labels showing standard lengths, ovary length, ovary weights, etc. Then they were shaken vigorously and left to stand for about 24 hours. The Gilson's fluid hardened ova were liberated easily as the ovarian tissues break down. The ovary can also be preserved in Gilson's fluid without any disadvantage. Composition of the Gilson's fluid:

60% alcohol-100 ml;
80% alcohol-15ml;
Saturated formalin-20ml;
Water-880ml;
Glacial acetic acid-18ml.

Fecundity count: For the absolute fecundity all the ripe/ripening eggs (yolked eggs) in the ovary were counted as per Hickling and Rutenberg (1936). If the ovaries were large, sub samples of almost the same weight were taken from different parts of the ovary and fixed in Gilson's fluid before being counted. Based on the weight of the ovary, average weight of the sub sample and average number of eggs in the sub sample, the absolute fecundity was computed. In case of a smaller ovary it was fixed as a whole and all the yolked eggs were counted.

$$\text{Fecundity} = \frac{\text{Weight of the ovary} \times \text{Average number of eggs per sub sample}}{\text{Average weight of the sub sample}}$$

Relative fecundity was expressed in terms of eggs per unit length and weight of the fish and ovary. The linear relationship between absolute fecundity and (i) Total length, (ii) Body weight, and (iii) Weight of ovary were computed by regression analysis after $\log_{10}$ transformation of the respective x and y values.

$$\text{Log } Y = \log a + b \log X$$

The linear equation was fitted by the method of least squares, allowing the use of standard statistical procedures for subsequent analysis.

The gonado somatic index (GSI) was estimated for ripe fishes using the formula

$$\text{GSI} = \frac{\text{Weight of the ovary} \times 100}{\text{Weight of the fish}}$$

3.2.6 Sexual dimorphism

The specimens, both males and females were studied for differences in finnage, tubercles and coloration.

RESULTS

4. RESULTS

4.1 SYSTEMATICS

4.1.1 Systematic position

The latest systematic position of *Puntius pookodensis* Anna Mercy and Eapen Jacob, 2007 based on Nelson (2006)

Classification is as follows:

Super order	:	Ostariophysi
Order	:	Cypriniformes
Suborder	:	Cyprinoidei
Family	:	Cyprinidae
Sub family	:	Barbinae
Genus	:	*Puntius*
Species	:	*pookodensis* Anna Mercy and Eapen Jacob, 2007

4.1.2 Description of the species

The photographs of the male and female fish are given in Plate 1a and Plate 1b.

4.1.2.1 Distinguishing characters

D.iii/6(2),7(2),8(2);P.1/12-13;V.1/7/1;A.iii/5;C.19.

Puntius pookodensis is described as a newly identified species of *Puntius* from Pookode Lake, Wayanad, Kerala. It is characterized by the combination of the following characters: Scales large, incomplete lateral line, 22-23 scales in lateral series, lateral transverse rows 4/3 ½, 18 circumferential scales, a shoulder spot and one or two spots on the caudal peduncle. The species is compared with its closest relatives, *Puntius ticto* and *P. punctatus* found in southern India, and with a look-alike *P. shalynius* from north-east India.

4.1.2.2 Colour

The fish possesses an iridescent silver body and yellowish fins with one or two broad black blotches at the caudal peduncle and a shoulder spot on the third to fourth scale along lateral line. There is a streak of reddish orange color more prominent towards the posterior part of the body, which is more intensified in mature male fishes during breeding season whereas mature females do not have this colour

4.1.2.3 Geographical Distribution (as per Anna Mercy & Eapen Jacob, 2007).

India: Western Ghats of Kerala. They inhabit the Pookode Lake, Wayanad District in Kerala.

Plate 1a: *Puntius pookodensis* - Male

Plate 1b*: Puntius pookodensis* - Female

Plate 1

4.2 BREEDING BIOLOGY

4.2.1 Classification of the maturity stages in *Puntius pookodensis* (female)

(i) Stage- I: Immature virgin

These are the young individuals that have not yet spawned. Differentiation of the gonads had just taken place. Ovaries in this stage were very small, triangular and translucent; slightly pinkish occupying less than one-fourth of the body cavity. Ova were not visible to naked eye. A cursory examination under the microscope was required to differentiate ovary from the testis. Microscopically, the oocytes were oval/spherical in shape and completely transparent with a large nucleus. Maximum size of the ova was 160µ. Under microscope the testes appeared as undifferentiated tissue.

(ii) Stage- II: Maturing virgin

In maturing virgins ovaries were usually slight creamy, translucent occupying about one third of the body cavity. The length of both the lobes was equal. Ova were still microscopic oval/spherical in shape with large nucleus and with slight yolk deposition up to yolk vesicle stage. Ova size had gone up to 352µ. The testes occupied less than 1/4 of the body cavity. They were thin and transparent.

(iii) Stage- III: Early Ripening (Spent recovering)

Ovaries were usually off white /creamy in colour occupying half to less than three fourth of the body cavity (process of vitellogenesis). They were usually turgid with a transparent ovarian wall and ova quite visible to the naked eye. Ovaries increased in weight rapidly. The maximum size of ova observed in this stage was about 640µ. This stage may also be called as spent recovering stage for fishes that have at least spawned once. In this stage the testes were transparent occupying about ¼ of the body cavity and they were thin and transparent.

(iv) Stage- IV: Late Ripening

Ovaries were pale yellow in colour, occupying three – fourth or more of the body cavity. The ovary was turgid and the ovarian wall was thin, the ova being visible through the extremely thin ovarian wall. The maximum size of ova seen in ovaries of this stage was about 800µ. The testes occupied less than ½ of the body cavity.

(v) Stage- V: Ripe (Reproduction)

Ovaries were yellow in colour and occupied more than ¾ of the body cavity. Ovarian wall thin and ova quite distinct. Ovary was very turgid. Ovarian lobes were quite stout and equal in length. The maximum size of ova seen in this stage was round 896µ. The testes occupied about more than half of the boy cavity and the lobes were translucent.

(vi) Stage VI: Partially Spent

Ovaries at this stage were slightly flaccid, pale yellowish in hue and not as stout as the ripe ovaries but still retaining a number of residual ripe ova/atretic ova after the spawning. The largest ova that could be found in this stage were 704µ. This stage enters the maturation cycle at early ripening stage. Here the testes looked flabby and occupied about ¼ of the body cavity.

4.2.2 Maturation of ovary and oogenesis in *Puntius pookodensis*

4.2.2.1 Oocyte distribution

Based on the studies on distribution and percentage frequency of the ova stocks (based on ova diameter in different stages of ovarian development), the following results have been obtained and are given in table1 and fig.1 to 6.

i. Immature virgin: (Fig:1)

All the transparent ova in this stage have been found to be below the size

of 224µ. The mode of the immature stock is at the ova diameter class group of 32-96µ. Fig.1

ii. Maturing virgin: (Fig:2)

The ova diameter ranged from 32 to 352µ by this stage. The percentage contribution of the immature stock (up to 224µ) is 93.2% with the mode at 32-96µ. Up to an average ova diameter of 224µ the oocytes are transparent without any yolk material and represent the immature stock. The remaining 6.9% was from the ripening stock, the early yolk vesicle eggs appeared at an average ova diameter of 256µ and on reaching an average diameter of 352µ. The ripening stock is thus represented by the ova diameter range of 256-352µ.

iii. Early ripening: (Fig:3)

The ova diameter size had gone up to 704µ by this stage. The percentage contribution of the immature stock had further gone down to 78.1% with a mode at 32-96µ. The ripening stock contribution had gone up to 21.1 % which was divided into many batches with no distinct mode (288-608µ). Above an average diameter of 640µ, the ova constitute the ripe stock. There was about 0.8 %of the ripe stock with a size range of 640-704µ.

iv. Late ripening: (Fig:4)

The range of the ova had been extended to 832µ. Of this 75.5% was by the immature stock and had a mode at 32-96µ. The percentage contribution by the ripening stock was 18.2 % with its batches showing no mode. The remaining was from the ripe stock which had gone up to 6.3 %.

v. Ripe: (Fig:5)

The range of ova size was the highest with a maximum size of 896µ. The contribution of the immature stock had further dropped down to 67.25% with a mode of 32-96µ. The percentage of the ripening stock was 15.42 % with its batches showing no definite mode. The ripe stock had gone up to 17.34 % with a

distinct mode of 704-768µ.

vi. Partially spent: (Fig:6)

The ova diameter had come down to 704µ. The percentage contribution of the immature stock had come down to 59.15 % with a mode at 32-96µ. The ripening stock constituted only about 39.03 % distributed with no clear mode. There was about 1.85 % of ripe stock, which may be the unspawned residual ova.

Table 1: Oocyte distribution in different maturity stages of ovary of *Puntius pookodensis*

ova diameter class (um)	Immature virgin		Maturing virgin		Early ripening		Late ripening		Ripe		Partially spent	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
32-96	455	70	470	60.7	402	50.2	374	44	338	36.7	261	31.5
96-160	169	26	192	24.8	187	23.4	225	26.5	208	22.5	169	20.4
160-224	26	4	60	7.7	36	4.5	43	5	74	8.05	61	7.3
224-288			42	5.4	15	1.9	16	1.9	32	3.47	42	5.06
288-352			12	1.5	42	5.3	11	1.3	27	2.93	71	8.5
352-416					27	3.4	17	2	23	2.5	64	7.7
416-512					38	4.77	37	4.3	23	2.49	51	6.17
512-608					24	3	26	3	15	1.63	45	5.4
608-640					22	2.7	48	5.6	22	2.39	51	6.17
640-704					6	0.8	11	1.3	28	3.04	15	1.85
704-768							26	3	92	10		
768-832							17	2	28	3		
832-896									12	1.3		
896-960												

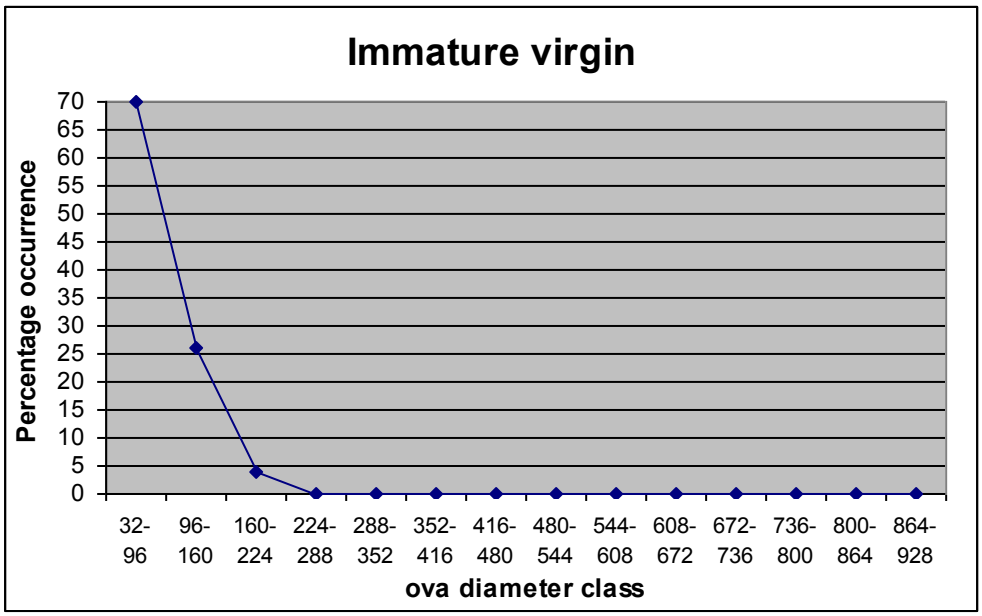

Figure 1: Graph showing percentage frequency of ova diameter classes in an immature ovary of *Puntius pookodensis*

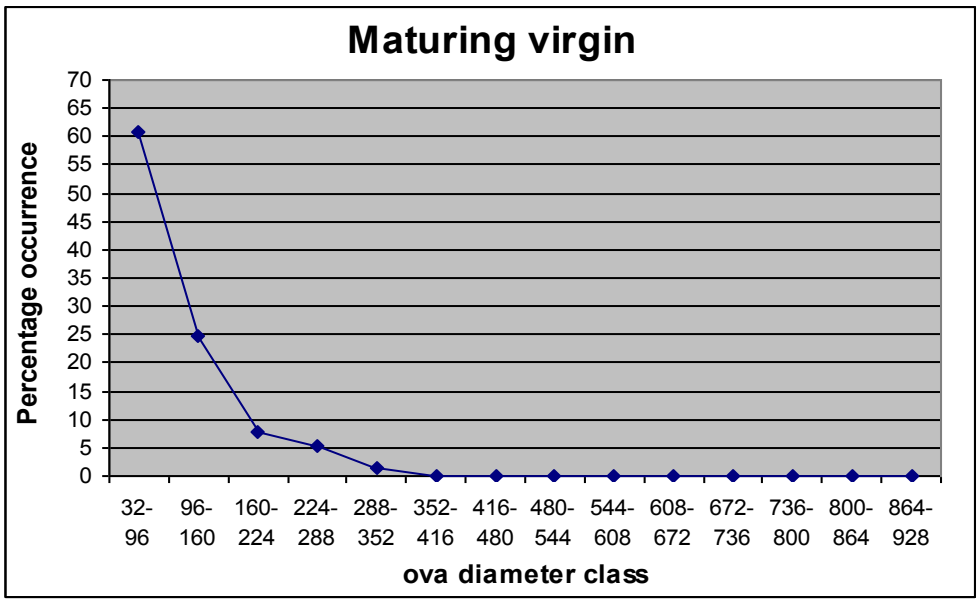

Figure 2: Graph showing percentage frequency of ova diameter classes in a maturing virgin ovary of *Puntius pookodensis*

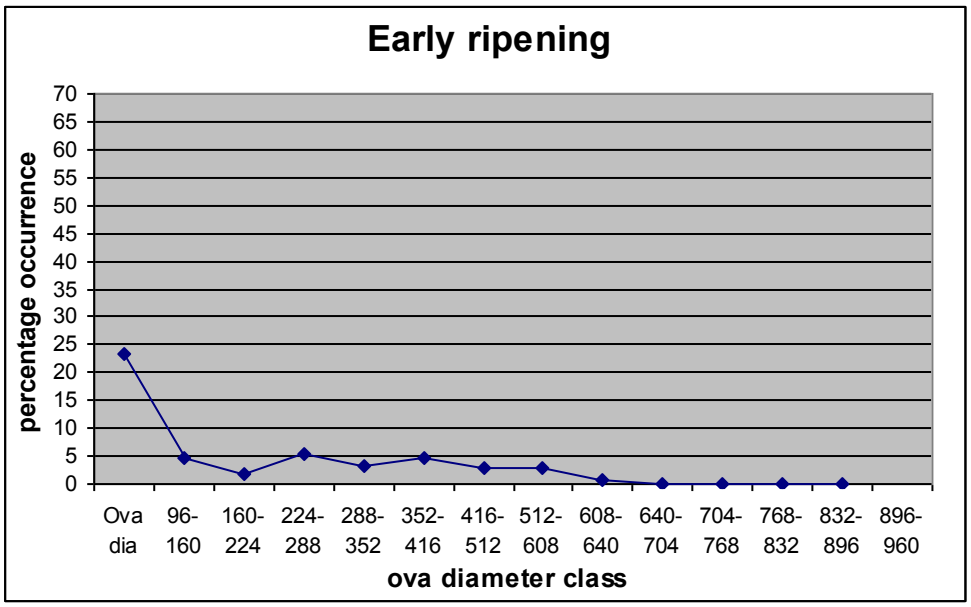

Figure 3: Graph showing percentage frequency of ova diameter classes in an early ripening ovary of *Puntius pookodensis*

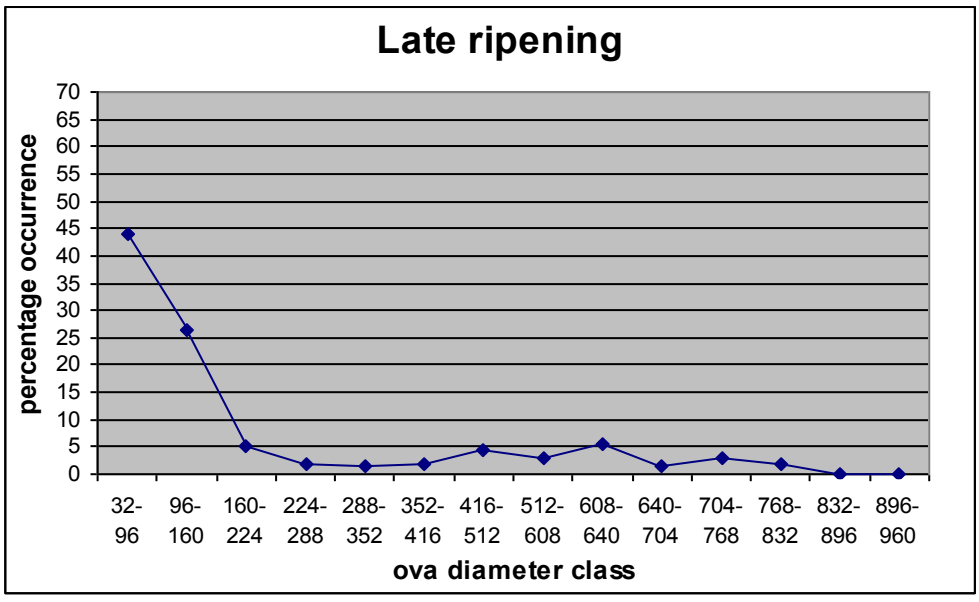

Figure 4: Graph showing percentage frequency of ova diameter classes in a late ripening ovary of *Puntius pookodensis*

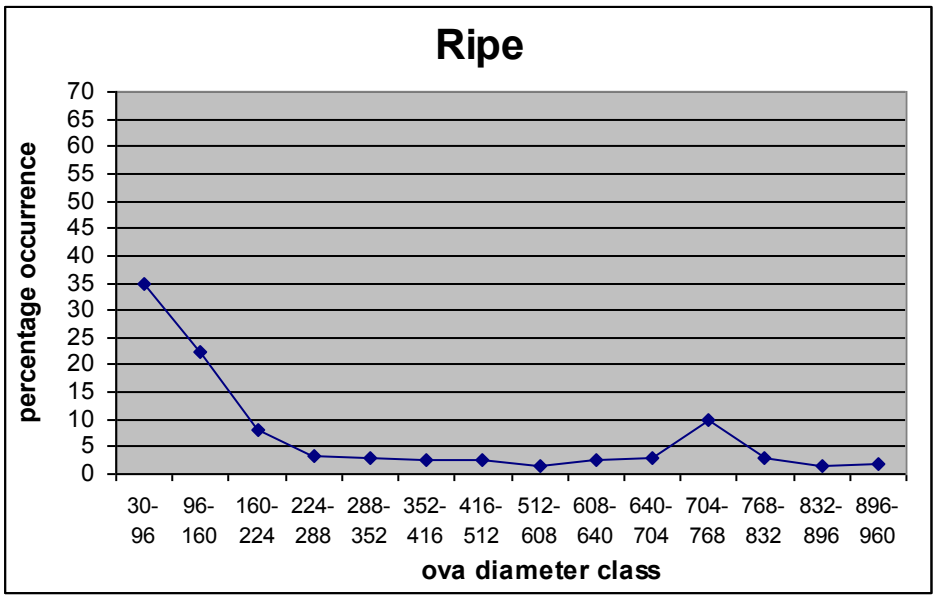

Figure 5: Graph showing percentage frequency of ova diameter classes in a ripe ovary of *Puntius pookodensis*

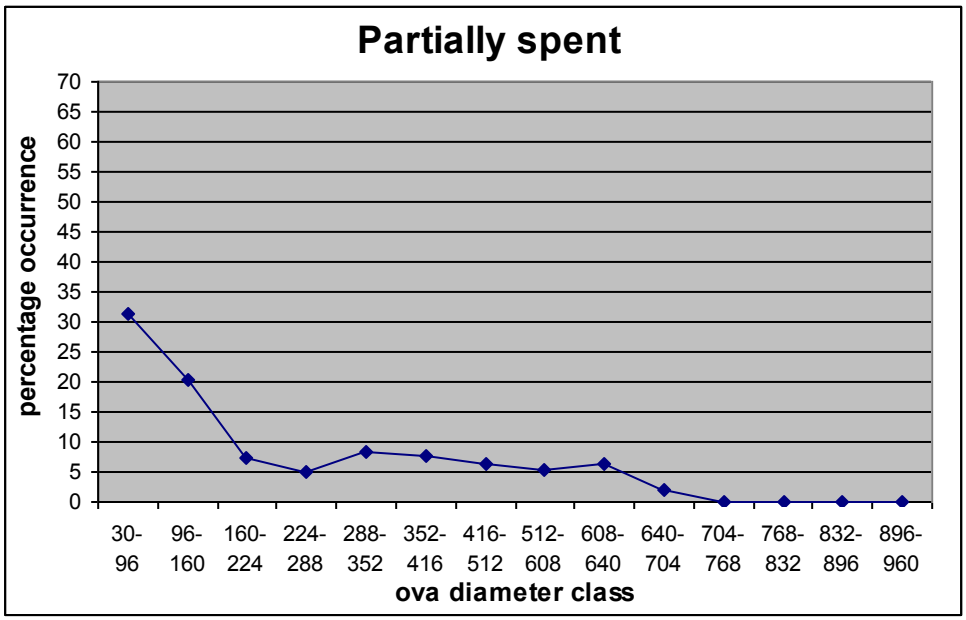

Figure 6: Graph showing percentage frequency of ova diameter classes in a partially spent ovary of *Puntius pookodensis*

4.2.2.2 Histology

Based on the morphological changes taking place in the ovum during the course of maturation, the following stages were discernible. These were usually based on the size, amount and distribution of various cell inclusions like nucleus, nucleolus, and other cytoplasmic inclusions like yolk nucleus, yolk vesicles, yolk granules and lipid globules.

The histological study of immature ovary shows ovigerous lamellae, having nests of oogonia, germinal epithelium and immature oocytes in the chromatin nucleolus stage (Plate 2a and 2b). Plate 3a shows section of ripe ovary with oocyte at all stages of development.

The oocyte development was classified into 9 different oogenic stages. The basic stages were:

1. Chromatin nucleolus stage:

Measuring about 32μ in diameter, this stage was the youngest encountered in the different maturity stages of the ovary. With an indistinct cell membrane, yet with a distinguishable outer limit, the cells of these stages showed a thin collar of deeply basophilic, granular cytoplasm with a centrally placed large nucleus. The spherical nucleus was with a distinct nuclear membrane enclosing centrally placed large nucleolus and the chromatin material scattered in the nucleoplasm. These immature oogonia could be seen as clumps and occurred deep within the germinal epithelium. (Plate 3b)

2. The early perinucleolus stage:

The oocytes appear bigger, more spherical due to accumulation of more cytoplasm. The thus thickened collar of cytoplasm was strongly basophilic. The centrally placed nucleus had also grown into germinal vesicle acquiring a spherical shape and gaining a vesicular interior. The chromatin material was found to show less staining affinity and were seen to fuse together leading to the formation of the nucleoli, while the existing nucleolus, has moved to periphery almost retaining earlier size and structure.(Plate 3b)

3. The perinucleolus stage:

The oogonium remained spherical in shape but had further increased in size. The cytoplasm continued to be granulose but changes have occurred in staining affinity. The chromatin material was less basophilic than in the previous stage and took up a light pinkish hue with haematoxylin. The chromatin nucleolar and perinucleolar stages are sometimes referred to as 'primary growth phase' (Wallace and Selman, 1981) or first growth phase (Forberg, 1982). (Plate 3b)

4. The yolk vesicles stage:

The follicular layer got closely pressed on to the outer surface of the oocyte, getting squeezed between the enlarged oocytes and thus was not as distinct as in the previous stage. The oocytes were much enlarged. The vitelline membrane had become distinct in this stage as a compact membrane of dense, homogenous cytoplasm surrounding the oocyte. The cytoplasm remained granular but a number of yolk vesicles have appeared peripherally. The vesicles were of similar shape, empty, with an outer cytoplasmic shell. The cytoplasm was faintly basophilic and turns reddish with eosin. While the nucleolar bits were deeply basophilic, the chromatin material remains poorly basophilic and turns pinkish with hematoxylin. . (Plate 3b).

5. The primary yolk stage:

The appearance of clusters of minute granules, the yolk granules on the periphery of the cytoplasm and the complete proliferation of yolk vesicles were distinguishing characters of this stage. The oocyte had further increased in size. Now, owing to mutual pressure, the oocytes began to loose their characteristic shape but were mostly oval or spherical. The follicular layer was thin. The germinal vesicle was oval or elliptical in shape and central in position with an irregular, wavy nuclear membrane. All the nucleolar bits have migrated to the nuclear periphery and were closely aligned to the inner surface of the nuclear membrane and showed a clumping tendency. The nucleoli and the nuclear

membrane were deeply basophilic while the chromatin material was faintly basophilic. (Plate 4a).

6. The secondary yolk stage:

The yolk vesicles clump together, fusing to form larger vesicles, may be due to the appearance of globules. The nuclear membrane remained highly irregular and wavy as in the previous stage, but the membrane itself had become hazy and quite indistinct. The yolk formation in this stage began with the accumulation of yolk globules in the periphery of the oocytes below the vitelline membrane. (Plate 4a,).

7. The tertiary yolk stage:

The follicular layer and vitelline membrane remained prominent as before and were almost equally thick. The oocytes were still irregular in shape on account of the pressure from the adjacent oocytes. They have further grown in size. The yolk vesicles too have further grown in size and were less in number. The yolk globules have greatly increased in number and size and almost completely fill the cytoplasm while the yolk granules lie interspersed with the yolk globules.(Plate 4a).

8. Migratory nucleus stage: (Sub peripheral nucleus stage).

The distinguishing features of this stage in the teleosts are the peripheral migration of the germinal vesicle and the liquefaction of the nucleolar material, which exude into the cytoplasm. The follicular layer is also very conspicuous, the individual cells being more distinguishable. The yolk globules almost completely fill the cytoplasm. (Plate 4b)

9. The mature oocyte:

The oocyte is now mostly spherical/oval in shape and covered by a fine outer sheath. The vitelline membrane had by now developed into a well recognizable membrane. The yolk vesicles are few and scattered and the

interspaces were filled with yolk globules and granules. Yet another development at this stage in teleosts is the formation of the micropyle. (Plate 5a).

The fate of the unextruded ova and empty follicles:

In the partially spent and spent recovering ovary a few unspawned eggs undergo degeneration. The atresia started with the liquefaction of the yolk, consequently, numerous minute, deeply staining ovoid bodies appears in the oocytes. The follicular layer gradually lost its compactness and strength, thinning down considerably and becoming pliable, convoluted and discontinuous at places. Through these the liquefied yolk partially extrudes. The follicular layer becomes much vascularized and the blood cells freely migrated into it. In spent ovaries, in addition to the unspawned oocytes, which were degenerating, there were the empty follicles of the spawned ova. These empty pockets easily got pushed out of existence by the fast developing immature oocytes. (Plate 5b)

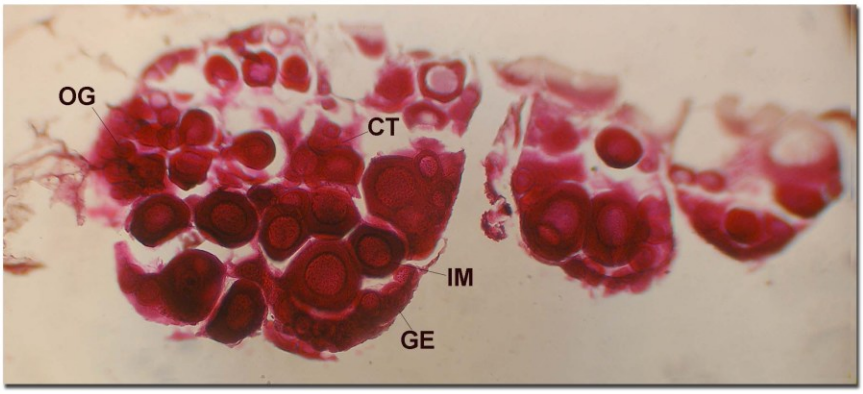

Plate 2a: Transverse section of immature ovary showing - IM:immature oocyte; GE:germinal epithelium; CT:connective tissue and OG:ovigerous lamellae with oogonia (X80)

Plate 2b: Part of immature ovary showing OG: ovigerous lamellae with oogonia and OL: ovarian lumen. (X80)

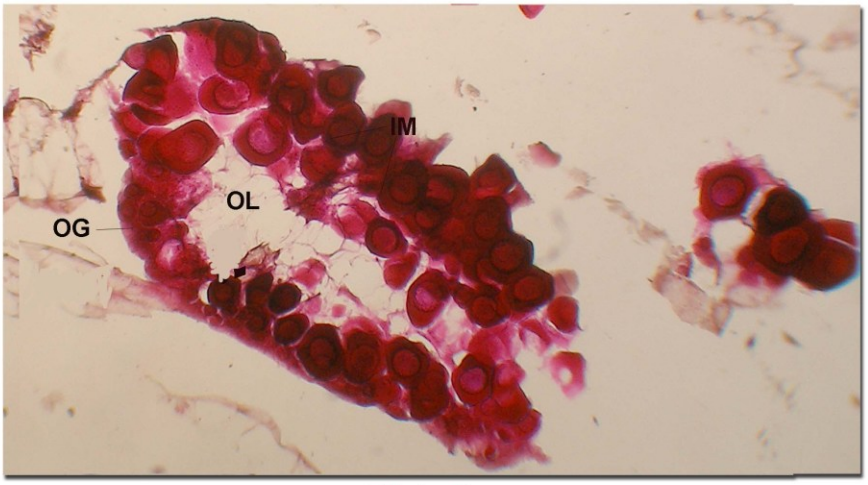

Plate 2

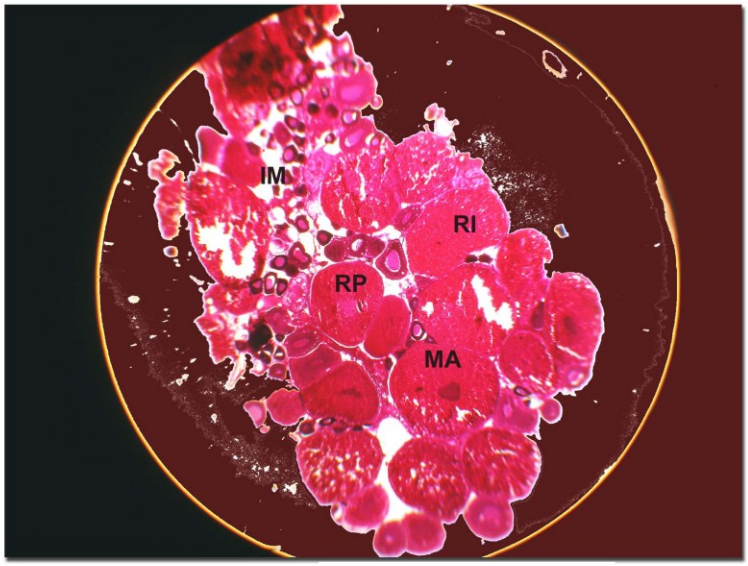

Plate 3a: Transverse section of a ripe ovary showing - IM:Immature ova; RI : Ripening ova; RP:Ripe ova and MA: mature oocyte. (X 80)

Plate 3b: Part of ovary showing-EP: Early perinucleolar stage; LP:Late perinucleolar stage; CN : chromatin nucleus stage; YV: Yolk vesicle stage; FE:follicular envelope; NU: Nucleoli and CY: cytoplasm. (X 400)

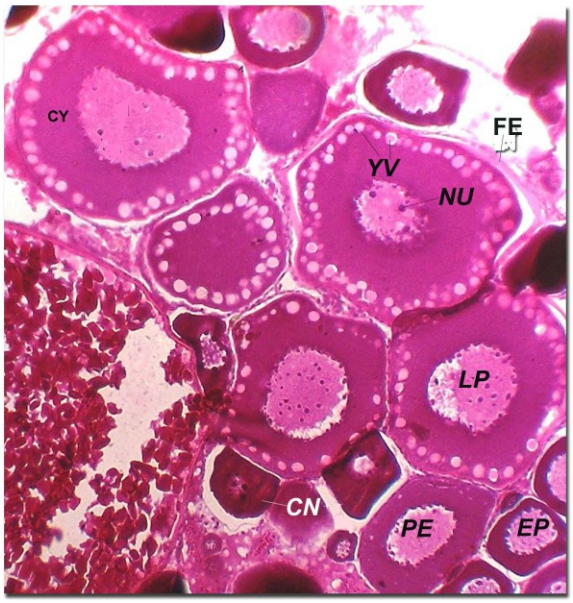

Plate 3

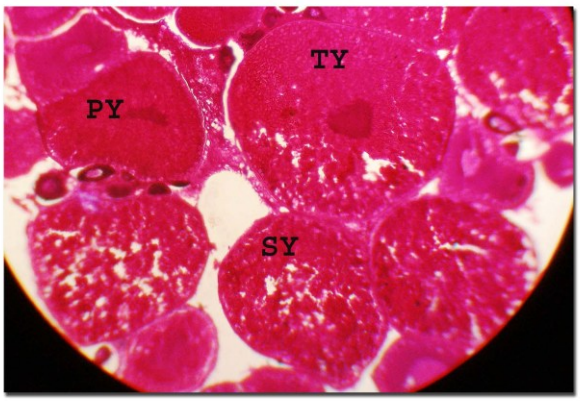

Plate 4a: ovary section showing
PY:primary yolk stage; SY:secondary yolk stage;
TY:tertiary yolk stage. (X200)

Plate 4b: section of ovary showing
MNS:migratory nucleus stage. (X400)

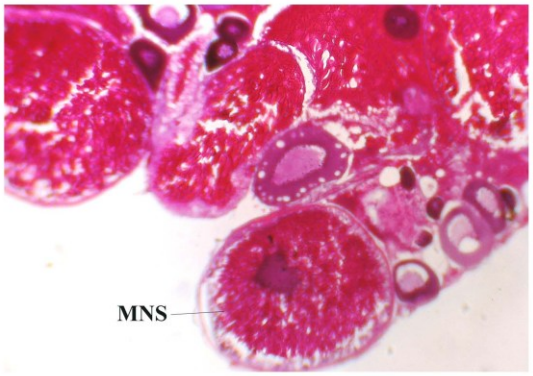

Plate 4

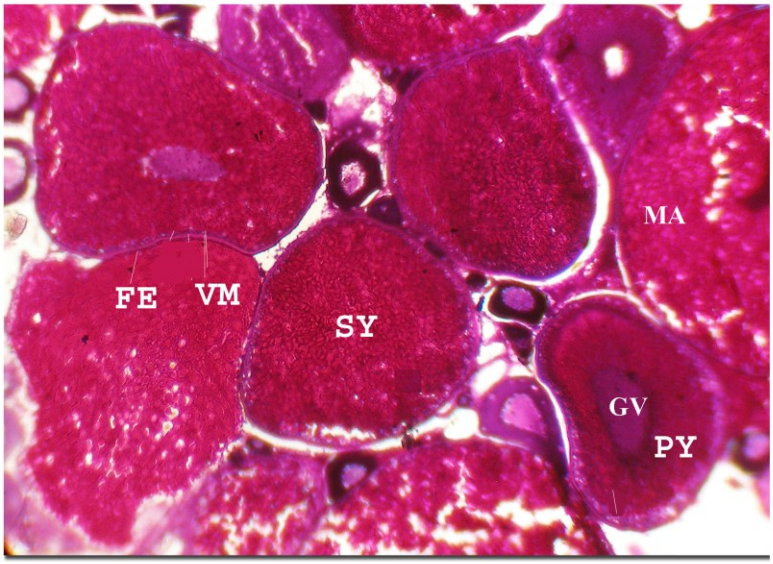

Plate 5a: section of ovary showing MA:mature oocyte; FE:follicular envelope; VM:vitelline membrane and GE: germinal vesicle. X400

Plate 5b: section of ovary showing AO:atretic oocyte with rupturing follicular envelope. X400

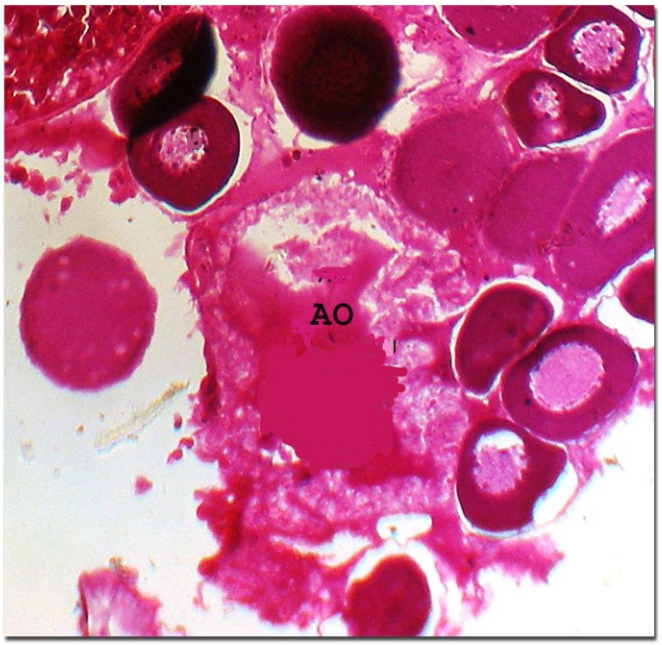

Plate 5

4.2.3 Size at first maturity

The length (total length) at first maturity was determined by analyzing the data relevant to all mature fishes (stage III and above) examined. The percentage occurrence of mature fishes (early ripening, late ripening, ripe and partially spent) was plotted against different length classes of 5 mm for both the female and male fishes. The results are given in Table 2 and 3 for the females and males of *Puntius pookodensis* respectively.

While the first mature male fishes appeared in the 25-30 mm (TL) group (16.66%), the first mature females appeared only in the group of 30-35 mm (13.33%). All male fishes were mature on reaching a total length of 40 mm and all female fishes on reaching a length of 45 mm total length. The size at first maturity for males was 32.5 mm TL (30-35 mm) and 38.5 mm TL for females (35-40 mm). (Fig: 7 and 8)

Table 2: Table showing the total length and percentage occurrence of mature female of *Puntius pookodensis*

Sl. No.	Length class (TL) mm	Total No.	No.Mature	% Mature
1	25-30	4	0	0
2	30-35	15	3	13.33
3	35-40	17	7	41.18
4	40-45	23	18	78.26
5	45-50	10	10	100
6	50-55	4	4	100
7	55-60	4	4	100

Table 3: Table showing the total length and percentage occurrence of mature male of *Puntius pookodensis*

Sl. No.	Length class (TL) mm	Total No.	No.Mature	% Mature
1	20-25	2	0	0
2	25-30	6	1	16.66
3	30-35	10	5	50
4	35-40	11	8	72.73
5	40-45	2	2	100
6	45-50	2	2	100
7	50-55	2	2	100

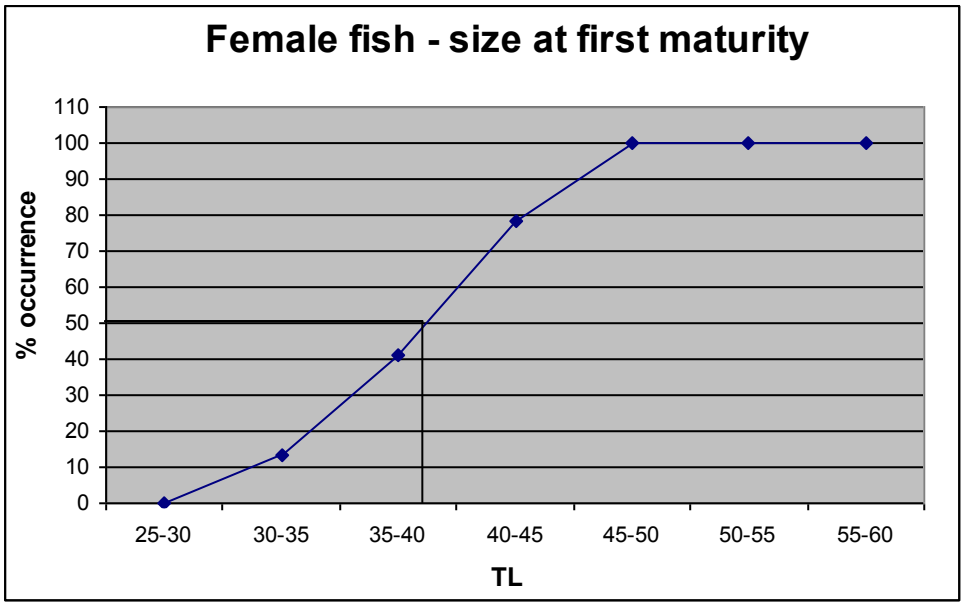

Figure 7: Graph plotted for the size at first maturity in females of *Puntius pookodensis*

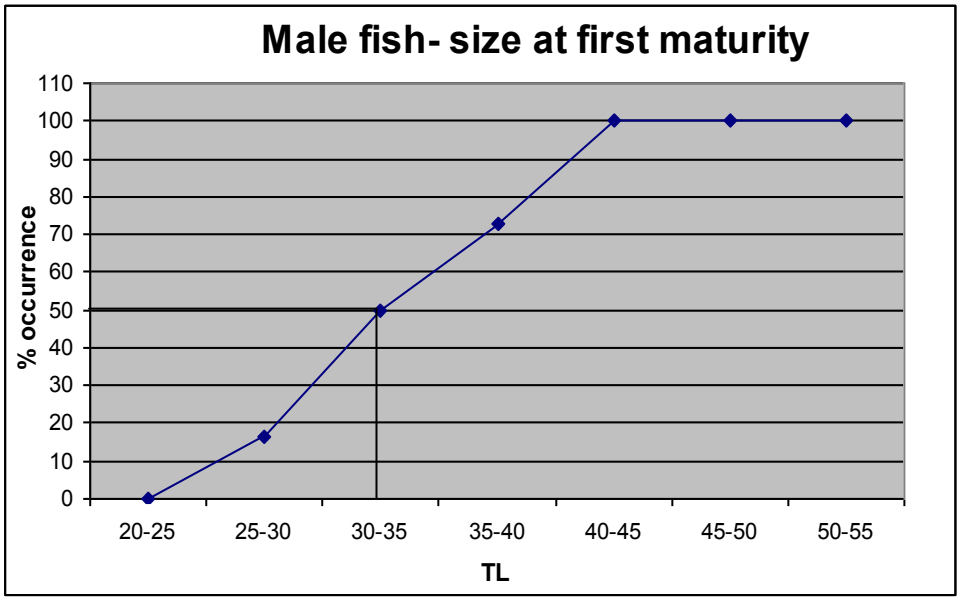

Figure 8: Graph plotted for the size at first maturity in males of *Puntius pookodensis*

4.2.4 Spawning frequency in *Puntius pookodensis*

From the ova diameter frequency distribution of a ripe ovary, it is observed that there are three batches of eggs representing the immature, ripening and ripe ova stocks. The ova diameter size ranged from 32- 224µm for the immature stocks. The ova were transparent without any yolk material. The ripening stock had an ova diameter range of 244-640µm. The first yolk vesicle eggs appeared at an ova diameter of 244µm and on reaching a diameter of 640µm the eggs had become ripe and ready to ovulate. Above an ova diameter of 640µm, the eggs constitute the ripe stock.

Ova diameter frequency showed that the immature and ripe stocks have single batch of ova, but the ripening stock showed many batches which were not well differentiated from each other. There was a large stock of immature ova constituting to about 67.25% of the total ova count and ranged over a diameter of 32-224µm with maximum value at 32-96µm range. The ripe stock was about 17.34% with ova size going up from 640µm and with a mode at 704-768µm diameter class. The maximum size of ova diameter recorded was 896µm. There was always the presence of large percentage of immature stock in any developmental stage of the ovary and a distinguishable stock of ripe ova in the ripe ovary.

The ripening stock contributed to the remaining 15.42% ranging from 224-640µm. This stock consisted of many batches with different stages of maturity. This indicates that the spawning may be extended over a very long period of almost round the year with the individual spawning intermittently.

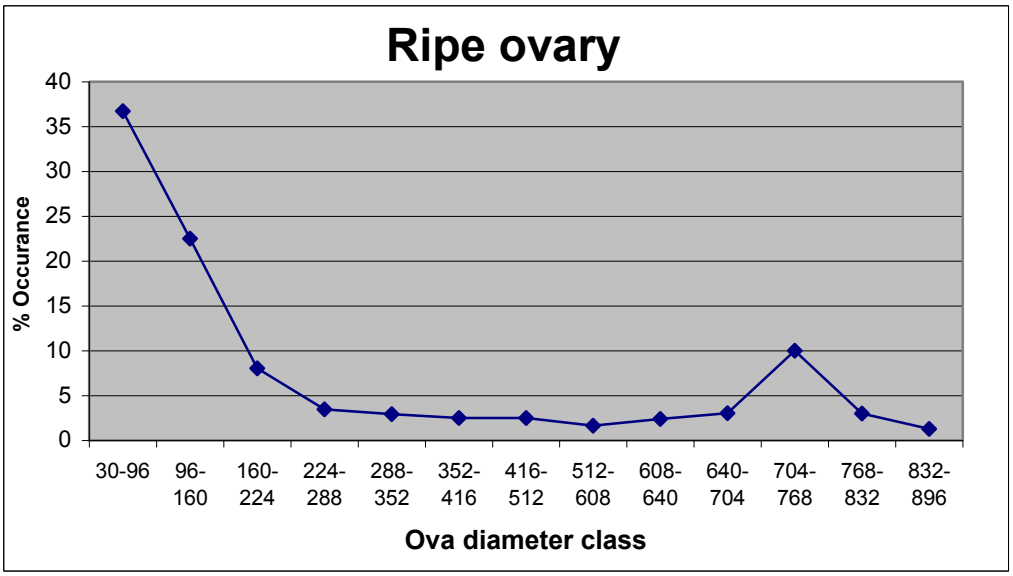

Figure 9: Graph showing percentage frequency of ova diameter classes in a ripe ovary of *Puntius pookodensis*

4.2.5 Fecundity in *Puntius pookodensis*

Results for the fecundity counts are given in the table 4 and 5. Absolute fecundity ranged from 426-823 (35 -57mm TL and 0.555-2.267 gm body weight). The gonado somatic index (GSI) values ranged from 5.52-15.7. The relative fecundity values ranged from 121.71-164.6 per cm body length and from 330.6-767.57 per gm body weight of fish. The ovary weight ranged from 70-310mg. The relative fecundity values ranged from 2.55-7.05 per mg ovary weight.

The relationship between Absolute fecundity and i) Total length, ii) Body weight and iii) Weight of ovary are given in Table 6 and figures 10-12). Fecundity increases with increase in the length and weight parameters. All the linear relationships are significant at 1% level.

Table 4: Absolute fecundity observed in *Puntius pookodensis*

Sl.No.	Total body length (mm)	Body weight (gm)	Ovary weight (mg)	GSI %	Fecundity
1	35	0.555	70	12.61	426
2	41	1.032	155	15.02	745
3	42	1.029	160	15.55	602
4	48	1.171	112	9.56	790
5	49	1.95	285	14.61	802
6	50	1.65	259	15.7	823
7	51	1.83	132	8	791
8	55	1.95	182	9.33	730
9	57	2.267	310	13.67	792

Table 5: Relative fecundity observed in *Puntius pookodensis*

Sl. No.	Fecundity	Per cm body length	Per gm body weight	Per mg ovary weight
1	426	121.71	767.57	6.09
2	745	181.71	721.9	4.81
3	602	143.33	585.03	3.76
4	790	164.58	674.64	7.05
5	802	163.67	411.28	2.81
6	823	164.6	498.8	3.2
7	791	155.1	432.24	5.99
8	730	132.73	374.36	4.01
9	792	138.95	349.36	2.55

Table 6: Relationship between fecundity and length, weight of fish and ovary weight.

Sl.No.	Variant (x)	Equation Log Y = Log a + b Log X	Correlation coefficient ®
1	Total length (cm)	Log F = 2.1066 + 1.106 Log TL	0.807*
2	Body weight (gm)	Log F = 2.789 + 0.350 Log BW	0.84*
3	Ovary weight (mg)	Log F = 2.101 + 0.333 Log OW	0.975*

* Significant at 1% level

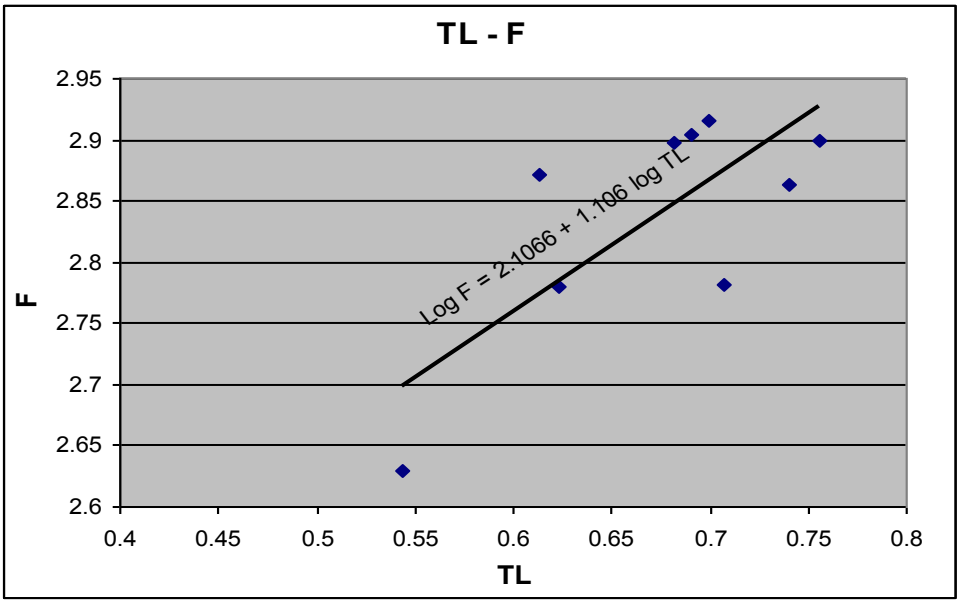

Figure 10: Relationship between total length and fecundity of *Puntius pookodensis*

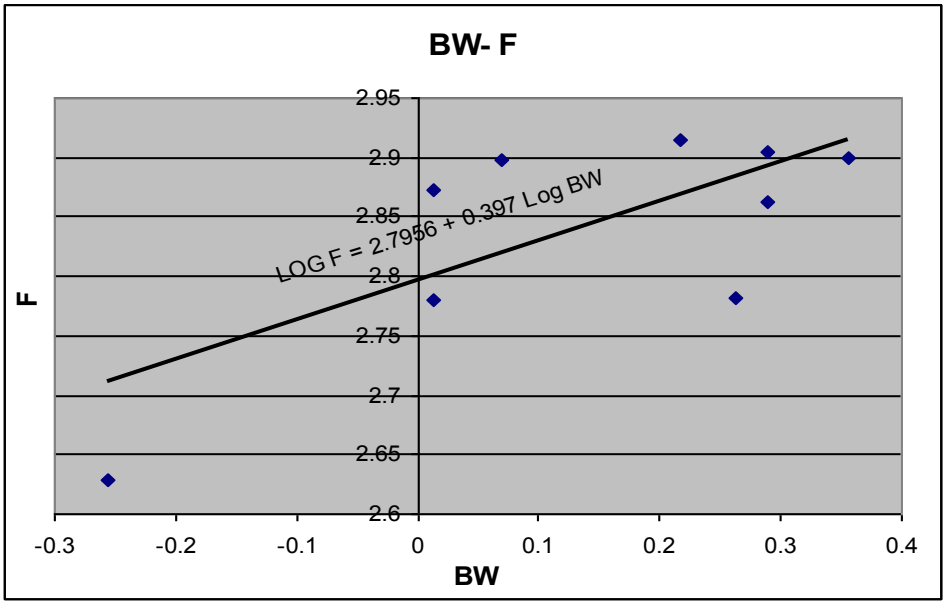

Figure 11: Relationship between body weight and fecundity of *Puntius pookodensi*

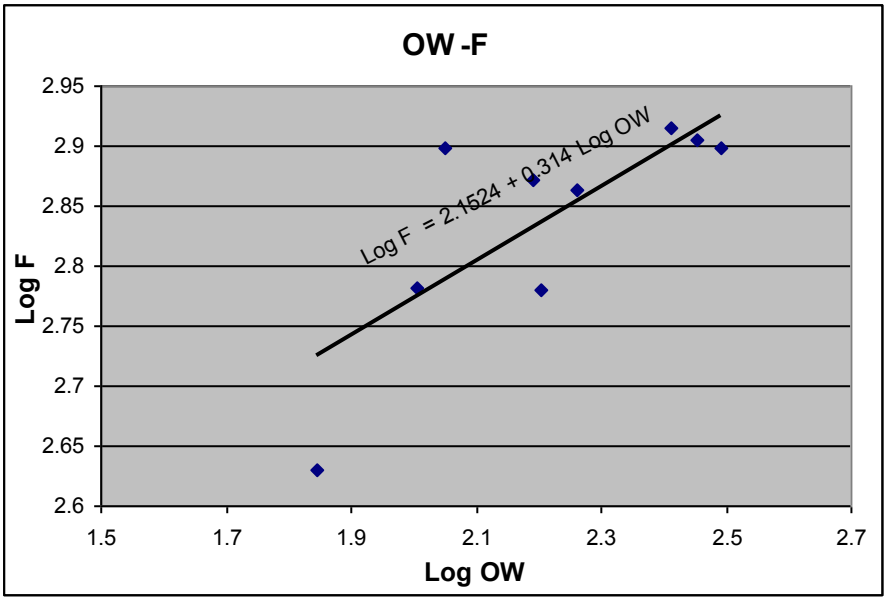

Figure 12: Relationship between ovary weight and fecundity of *Puntius pookodensis*

4.2.6 Sexual dimorphism in *Puntius pookodensis*

Puntius pookodensis exhibits clear cut sexual dimorphism. Sexual dimorphism is more prominent during breeding season. Males have reddish orange colour more prominent towards the posterior part of the body. Females do not have this colour. It was observed that in the males, colour was intensified during the spawning season. Usually it is the females which are larger as they mature late. The males are rather smaller and slender, while the females have a broader abdomen due to the presence of ripe ovary within. During the captive breeding experiments the females were identified by checking the soft, bulged belly during the ripe condition.

DISCUSSION

5. DISCUSSION

5.1 BREEDING BIOLOGY

The success of any fish species is ultimately determined by the ability of its members to reproduce successfully. One of the most defining features of a species is therefore its reproductive strategy which will be the summation of many adaptive traits that enables individual fishes to leave the maximum number of offspring. These traits comprise of size at first maturity, size and age specific schedules, reproductive effort or gonado somatic index, and the manner and timing of spawning (Mills, 1981). Hitherto, no study on the biology of the *Puntius pookodensis* has been conducted. Hence in the present study an attempt was made to understand the various aspects of the breeding biology of this species.

5.1.1 Quantification of maturity stages

A series of scales of ripeness have been worked out for each group of fishes for delimiting the different stages of the sexual cycle, based on a universal scale of six stages (Nickolsky, 1963) mainly for temperate species. In the present study a modified six- stage key is used based on the reproductive strategy of tropical fishes.

5.1.2 Maturation of ovary and oogenesis

5.1.2.1 Oocyte distribution

The quantification of maturity stages is based on the distribution of oocyte stocks in the different maturity stages. In the present study, it can be seen that the ovaries of *Puntius pookodensis* show asynchronous development. 'Asynchronous ovaries' show sufficient number of oocytes at various stages of development within the ovary (De Vlaming, 1983). The oocyte size frequency distribution is continuous; however in a ripe ovary a clear cut mode for the ripe stock is obtained.

5.1.2.2 Oogenesis in Puntius pookodensis

Development of oocyte in teleosts has been reviewed by Wallace and

Selman (1981), De Vlaming, (1983), Nagahama (1983), Guraya (1986), Wallace *et al.,* (1987) and Jalabart (2005).

According to Bromage and Cumaratunga (1988) and Selman *et al.* (1993), the yolk vesicles are not yolk in a strict since they do not serve as a nutrient source for the embryo. Selman and Wallace (1989) recommended that the term yolk vesicles can be replaced by the term 'cortical alveoli' in future studies. Wallace and Selman (1981) stated that in most teleost species the yolk vesicles appear just before the appearance lipid droplets and yolk granules. In *Puntius pookodensis* the yolk vesicles appear at the end of perinucleolar stage. The yolk granules appear first at the primary yolk stage and the yolk globules at the secondary yolk stage. The presence of yolk spheres, granules and globules are characteristic of vitellogenic oocytes (primary, secondary and tertiary yolk stages) as per Yamamoto (1956b), Yamazaki (1965) and Davis (1977). According to Wallace and Selman (1981) the fusion of yolk granules begin soon after their initial formation or as late as final maturation. In *Puntius pookodensis* the yolk granules and yolk globules retain their shape and structure even at the migratory nucleus stage. So the fusion of yolk materials may be during final maturation. Atretic oocyte is indicated by an irregular shape, due to the rupture of outer membrane and oozing out of yolk material (Forberg, 1982) as is also seen in the present study

5.1.3 Size at first maturity

In *Puntius pookodensis*, the size at first maturity is found to be 37mm TL (40-45 mm) for females and 33mm (30-34 mm) for males. The largest female obtained during the present study is 57 mm TL while the largest male is 50 mm TL. It is the female that is larger or at least achieves a larger size. This is a kind of reproductive strategy to enhance the existence of the race by increasing the fecundity, directly related to the size of the female fish (Keenleyside, 1991). The females spent more energy first for somatic growth and from there on, gonadal growth and maturation. However, the early maturation of the males diverts all the energy reserves towards the gonadal development and its maturation at a relatively younger age. In *Puntius pookodensis* the males attained sexual maturity at a smaller length than females. Similar observations had been reported in many freshwater fishes like *Labeo boggut* (Selvaraj *et al.,* 1972), *Barbus sarana* (Murthy, 1975), *Nemacheilus triangularis* (Ritakumari and Nair, 1979)*, Puntius*

denisonii, P. filamentosus, Nemacheilus triangularis and N. semiarmatus (Anna Mercy *et al.,* 2005 b), *Prestolepis marginata* (Nisha Raj, 2005) and *Chela fasciata* (Divipala, 2008*).*

Anna Mercy *et al.* (2005a) reported the size at first maturity of *Puntius melanostigma* as 50 mm for males and 55 mm for females. In the case of the African minnow, *Barbus paludinosus* sexual maturity was reached within a year at 50.0 mm TL (Cambray and Burton, 1985). In the case of European minnow, *Phoxinus phoxinus* the short lived populations of river Frome in England contained two spawning age groups and the largest fish caught was only 78.0 mm long. The size at first maturity ranged from 50-55 mm as two year olds (Mills, 1987). Six *Barbus* species studied in Sri Lanka had maximum total length of between 42.0 and 101.0 mm and a short life span (De Silva *et al.,* 1985). In the freshwaters of South Africa out of the 52 *Barbus* species studied 43 attained maximum fork lengths of less than 150.0 mm (Cambray and Burton, 1985).

5.1.4 Spawning frequency

Ova diameter studies reveal that this species comes under the category D of Karekar and Bal's classification (1960), characterized by spawning that is extended over a very long period of almost round the year with the individual spawning intermittently. But according to De Silva *et al.* (1985), the species with a distinguishable stock of ripe ova in the ripe ovary will have peak spawning periods. In the present study *Puntius pookodensis* may be designated as a multiple spawner with an asynchronous ovarian development. The oocyte size frequency distribution is continuous except in ripe ovaries, where there may be a clear separation between the ripe and yolked oocyte (Wallace and Selman, 1981), a pattern very clearly exhibited in *Puntius pookodensis.* De Vlaming (1983) also found that most species with asynchronous development have protracted spawning season with multiple spawning. Multiple spawners are also termed partial, heterochronal, serial spawners (Holden and Reitt, 1974; Macer, 1974), implying that only part of the complement of yolked oocytes is spawned and those individuals spawn over a protracted period, a pattern similar in *Puntius pookodensis.* The classic examples for this strategy among cyprinids are the minnows and barbs largely restricted to the lake and river margins and to very shallow riffle areas (Mills, 1991). *Puntius pookodensis* is a tropical barb distributed in the lake of Western Ghats. Early maturation and multiple spawning

is an adaptation in unstable environments where adult mortality may be high (Cambray and Burton, 1985). Sterba (1953) described the release of clutches of ovulated eggs in batches in *Chela labuca*, indicating fractional spawning activity in the tropical small cyprinids. Fractional spawning describes the batch release of ovulated eggs (De Vlaming, 1983). According to Gale and Buynak, 1978 satinfin shiner, *Notropis analostanus* and fathead minnow, *Pimephales promelas* (Gale and Buynak, 1982) showed batch release of eggs under captivity.

Burt *et al.* (1988) suggested that as multiple spawning within years can result in a much higher annual reproductive effort, early and continuous reproduction should be considered the base line condition. This condition would be associated with less seasonal environment, early maturation, smaller body size and smaller relative ovary size presumably at the cost of a short life span, typically shown by the tropical cyprinids.

5.1.5 Fecundity

In the present study, the absolute fecundity values in *Puntius pookodensis* ranged from 426 to 823 in fishes whose size ranged from 35 to 57 mm TL. In European minnow, *Phoxinus phoxinus* with a maximum size of 78.0 mm, Mills (1987) estimated maximum egg production value as 3172 eggs.

Studies show that the large repeat spawners have larger fecundity than recruit/first time spawners. The number of eggs released increased with age and size ranging from 426 for 35 mm fish to 823 for a fish of size 50 mm. Similar studies have been done on *Danio malabaricus* (Anna Mercy *et al.,* 2005 c).

With increase in body size, the relative fecundity per mg ovary weight ranged from 2.55 to 7.05 in *Puntius pookodensis*. This indicates an increase in size and weight of eggs from the small recruit spawners to the large repeat spawners. Hempel and Blaxter (1967) reported similar findings for the Atlantic herring (*Clupea harengus*) and Hislop (1975) for the haddock (*Merlangus aeglifinnus).* The fecundity is most significantly correlated with gonadal weight. The correlation coefficient of fecundity with total length is almost similar to that of fecundity with body weight. Similar findings were found in six *Barbus* species studied in SriLanka (De Silva *et al.,* 1985). In general, the correlations are highly significant, but with much variation, which is far from being fully explained by the factors total length, body weight and gonad weight. It is likely that other factors such as age and/or environmental factors contribute

considerably to the variation in fecundity, independently from body weight and gonad weight (De Silva *et al*., 1985).

The GSI values ranging from 8.0 - 15.55 % were recorded in ripe individuals of *P. pookodensis*. Wootton (1979) suggested that sub-tropical and tropical species which have an extended breeding season with females spawning many times show smaller changes in the amplitude of the GSI than those with a short season.

5.1.6 Sexual dimorphism.

In the present study *Puntius pookodensis* exhibits clear cut sexual dimorphism. Usually females are larger than the males, as they mature late. Males are rather slender, while the females have a broader bulged abdomen due to the presence of ripe ovary within. In *Puntius pookodensis* an orange streak is present at the base of caudal fin. It was observed that in the males this colour was intensified during the spawning period. In the males of 17 species of barbs sharing common features with *Puntius pookodensis*, Anna Mercy *et al.* (2007) reported the absence of minute wart-like protuberances on the opercle during the breeding season which is one of the characteristic features of genus *Puntius* Hamilton. Similar observation was found in the present study in *Puntius pookodensis*. Identification of the sexes has to be done mainly by checking the soft, bulged belly during the ripe condition of the female as well as the presence of intensified reddish orange colour towards the posterior part of the body in males which is absent in females.

Anna Mercy *et al.* (2007) recorded sexual dimorphism in some of the indigenous ornamental fishes of Western Ghats. Some of these fishes having sexual dichromatic dimorphism were *Puntius amphibius, P. fasciatus, P. pookodensis, P. melanostigma, P. conconius and P. melanampis*. Structural sexual difference was found in *P. filamentosus*. In the present study, *Puntius pookodensis* do not show any structural sexual dimorphism.

SUMMARY

6. SUMMARY

The present study was made to understand the oogenesis and ovarian maturation, breeding and other aspects of reproductive biology of *Puntius pookodensis*. The methodology, results and conclusion are as follows:

1. Specimens of *Puntius pookodensis*, collected from the Pookode Lake, Wayanad were used for the present study.

2. A total of 112 fishes (77 females and 35 males of size ranging from 20 to 57 mm TL) were used for the study of reproductive biology.

3. For the study of the external morphology of gonads, both fresh and preserved specimens were used.

4. The gonads were quantified into 6 maturity stages based on external morphology. (Immature virgin, maturing virgin, early ripening, late ripening, ripe and partially spent.)

5. The ova diameter distributions of the 6 stages of maturity were studied to understand the maturation of ova in the ovary.

6. The ovaries of *Puntius pookodensis* showed asynchronous development, in which oocytes at all stage of development were present in the same ovary at the same time.

7. Oogenesis was studied using the standard histological procedures. Harris's Haematoxylin and eosin were used for the histological differentiation.

8. The oocyte development was classified into 9 different oogenic stages

(Chromatin nucleolus stage, early perinucleolus stage, perinucleolus stage, yolk vesicles stage, primary yolk stage, secondary yolk stage, tertiary yolk stage, Migratory nucleus stages, and mature oocyte stage).

9. Size at first maturity for females and males were found to be 38.5mm TL and 32.5mm TL respectively.

10. Based on spawning frequency study of the ripe ovary, *Puntius pookodensis* was found to be a multiple spawner, with a protracted spawning season, the individual spawning intermittently.

11. Absolute fecundity of the fish ranged from 426 to 823 in fishes of size range 35 to 57mm TL and 0.555 to 2.267 gm body weight. The number and size of eggs were found to be directly proportional to t size and age of the fish. Fecundity showed a positive linear relationship at 1% level of significance, with the length of fish, weight of fish and ovary weight

12. The sexual dimorphism was very slight and is more prominent during spawning season. It involved intensification of colour in mature males. During breeding season the abdomen of female is broader and bulged due to the presence of ripe ovary.

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A STUDY ON OOGENESIS AND OVARIAN MATURATION IN *PUNTIUS POOKODENSIS* ANNA MERCY AND EAPEN JACOB, 2007

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ABSTRACT OF THE THESIS

Submitted in partial fulfillment of the requirement for the degree.

MASTER OF FISHERIES SCIENCE

Faculty of Fisheries

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2009

DEPARTMENT OF FISHERY BIOLOGY

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ABSTRACT

8. ABSTRACT

Puntius pookodensis is a newly identified species of Puntius from Pookode Lake, Wayanad, Kerala, which is an indigenous ornamental fish endemic to the Western Ghats of India.

A total number of 112 specimens (77 females and 35 males of size ranging from 20mm to 60mm TL) collected during the period of 2007 were used for the present study of different aspects of breeding biology. They were collected from the Pookode Lake at Wayanad and the distinguishing characters based on external morphology and colour were studied.

The gonads were quantified into 6 maturity stages based on external morphology. (Immature virgin, maturing virgin, early ripening, late ripening, ripe and partially spent.).

Studies on oocyte distribution of the 6 stages of maturity were done to understand the ova maturation in the ovary. Ovaries show asynchronous development.

The oocyte development was classified into 9 oogenic stages. The first mature male fishes appeared in the 25-30 mm (TL) group and the first mature females appeared in the group of 30-35 mm. All male fishes were mature on reaching a total length of 40 mm and all female fishes on reaching a length of 45 mm total length. The size at first maturity for males was 32.5 mm TL (30-35 mm) and 38.5 mm TL for females (35-40 mm).

From the ova diameter frequency distribution of a ripe ovary *Puntius pookodensis* was found to be a multiple spawner with a protracted

spawning with the individual spawning intermittently. Absolute fecundity of the fish ranged from 426 to 823 in fishes of size range 35 to 57mm TL and 0.555 to 2.267 gm body weight. The number and size of eggs were found to be directly proportional to t size and age of the fish. Fecundity showed a positive linear relationship with the length of fish, weight of fish and ovary weight.

A complete knowledge on reproductive biology will definitely help in the commercial production and selective breeding under captive condition for the domestic and export market of the indigenous ornamental fish and also conserve the germplasm.