STUDIES ON THE MATURATION AND REPRODUCTION OF *PRISTOLEPIS MARGINATA* JERDON UNDER CAPTIVE CONDITIONS

By

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Dedicated To
My Beloved Son, Keshav

DECLARATION

I hereby declare that this thesis entitled " **STUDIES ON THE MATURATION AND REPRODUCTION OF *PRISTOLEPIS MARGINATA* JERDON UNDER CAPTIVE CONDITIONS** " is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis for the award to me of any degree, diploma, associateship, or other similar title, of any other University or Society.

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1. INTRODUCTION

1.1 ICHTHYOBIODIVERSITY IN INDIA

India is blessed with vast and varied fish germplasm resources distributed widely in vivid aquatic ecosystems. There are about 28,500 fish species reported throughout the world (Nelson, 1994) representing more than half of the vertebrate biodiversity (Stiassny, 1996). Nearly 2,200 species of finfishes have been recorded from different ecosystems of India. Out of these, about 400 species are commercially important, which include cultured, cultivable and wild caught species. These fishes have acquired a wide variety of forms and habitats, which have been reflected in their adaptation to live in markedly varying biotypes, ranging from cold torrential mountain streams to the dark abyssal depth of the seas. The approximate ecosystem-wise distribution of the fish germplasm resources of India is cold water (73; 3.32%), warm waters of plain (544; 24.73%), coastal brackishwater (143; 6.50%) and marine (1440; 65.45%) (Das and Pandey, 1998).

A few countries mostly located in the tropical belt contain a high percentage of biodiversity and high degree of endemism and these are considered as "megadiversity" countries. A dozen of countries are identified as megadiversity countries and India is one among them. It has two major biodiversity "hotspot" areas, *viz.*, the Himalayas, the North Eastern Hills and the Western Ghats (Myers, 1990).

1.2 FISH BIODIVERSITY DETERIORATION: CAUSES

With the rapid development and population explosion, there is an ever-increasing demand for fish as protein rich food as well as ornamental additions. Due to these anthropogenic stresses, aquatic ecosystems of India are under constant pressure which detriment aquatic

flora and fauna (Jhingran, 1991). The various causes of imperilment of fishes in the aquatic ecosystems have been identified as:

1. Habitat destruction – Construction of dams, siltation from the catchment areas havedestroyed the feeding and breeding grounds of many fishes (Sehgal, 1994).

2. Over-exploitation – Over-exploitation of fishery resources, due to its extraordinary economic value, has been a causative factor, which increases the vulnerability of different ecosystems.

3.Wanton destruction: Wanton killing by dynamiting, electric fishing and poisoning of brood fishes in spawning season and juveniles during the post monsoon periods have affected a number of food, game and ornamental fishes, especially in the upland rivers and streams of North East India.

4. Aquatic pollution: Pollution is probably the single most significant factor causing major decline of many fish species (Dehadrai, 1996). Industrial, sewage (municipal) and pesticides pollution have been creating detrimental environment to fish life in many water bodies (Jhingran, 1991; Pandey *et al.*, 1999).

5. Uncontrolled introductions of exotic fishes: Introduction of exotic fast growing fishes is causing threat to our indigenous fish diversity (Menon, 1994).

6. Diseases: Fishes are easily susceptible to diseases caused by various agents like bacteria, fungi, and viruses, the most virulent and menacing being the Epizootic Ulcerative Syndrome (EUS). The erosion of genetic variability and biodiversity is a serious threat from such diseases (Das and Pandey, 2002).

1.3 GENETIC PROBLEMS IN THREATENED SPECIES

Overexploitation of the fish resources coupled with the habitat destruction result in the shrinkage of fish population. The associated

severe genetic problems in effective population take the form of genetic bottlenecks, genetic drifts and accumulation of homozygosity (inbreeding depression) (Mishra *et al.*, 2000; Narain, 2000).

(i) Genetic bottleneck: Genetic bottlenecks effectively sample, although not necessarily random, a few individuals from a gene pool, resulting in a remnant population with less overall genetic variability. Losses in adaptation of the species in the changing environment ultimately making it unfit to survive (Schonewald–Cox *et al.*, 1983).

(ii) Genetic drift: It is a prolonged bottleneck leading to the repeated loss of variance (Das, 1989).

(iii) Inbreeding depression: It is probably the most serious problem of the endangered fishes with small population sizes (Mishra *et al.*, 2000). Inbreeding is defined as the mating of the individuals of common ancestry that share more genes due to descent than those individuals randomly selected from the population. Inbreeding results in predictable increase in homozygous genotypes differentially affecting different traits.

1.4 NEED FOR CONSERVATION OF FISH BIODIVERSITY

Maintenance of fish biodiversity along with other biotic resources can be viewed as prerequisite for the well being of the human being (Narain, 2000). There are four basic reasons ascribed for the preservation of the biotic resources.

(i) Diversity or variability seems aesthetically pleasing in most environment (Smith and Chesser, 1981).

(ii) There is often local pride in population or species that are characteristic of an area. People often become distributed when local form of the animal is threatened by the extinction and this concern is an important reason for the conservation of at least some species (Smith and Chesser, 1981)

(iii) It is generally agreed upon by the ecologists and evolutionary biologists that species diversity and genetic variability are necessary for the long-term maintenance of stable, complex ecosystems and species (Smith and Chesser, 1981). Some genetic traits from the diverse germplasm resources may be useful to increase the aquaculture production through hybridization and genetic engineering (Brown *et al.*, 1989; Das, 1996).

(iv) All the living beings in an ecosystem co-evolve for their mutual benefits during the evolutionary process. Any species getting extinct upsets the ecological balance to the detriment of each species and also to the community as a whole (Das, 1996)

1.5 STRATEGIES FOR FISH BIODIVERSITY CONSERVATION

It is essential to prevent further decline of the germplasm resources by devising all the possible measures of conservation and rehabilitation (Das, 1996; Das and Pandey, 2002). The conservation policy should promote the management practices that maintain integrity of ecosystems, prevent endangerment and enhance recovery of the threatened species. The irreparable harm caused to the fish and the habitats need to be compensated through aforestation, eco- restoration, soil conservation, complete ban on deforestation particularly in the fragile mountains and strict implementation of the Indian Fisheries Act 1897 (modified in 1956) along with these following measures would positively help in restoration of the threatened fish fauna.

1.5.1 *In-Situ* Conservation

In-situ conservation of the fish is useful where genetic diversity exists and where wild forms are present. It requires the establishment of a network of aquatic reserve (fish sanctuaries/marine parks) of the sufficient size so that the population would be sustaining (Padhi, 1987).

The network of reserves should encompass the bulk of biodiversity, which is to be protected. Strategically located such reserves may act as source of recruits for the area that are exploited (Das and Pandey, 2002). Mass awareness programmes and ranching of the depleted stocks for the fish's rehabilitation seems to be cost effective and pragmatic approach for *in situ* ichthyobiodiversity conservation. *In situ* conservation of aquatic germplasm resources is a new concept for the developing countries. This would require large investment

1.5.1.1 Habitat Restoration

Obviously, enormous damage has been done to many fish habitat and the situation is often not easy to reverse especially in the 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. However, 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.

1.5.1.2 Stock Transfer

Valuable stock transfer to create new 'safeguard' populations can be done without any threat to the existing stocks, but it is important that certain criteria are taken into account in relation to any translocation proposal. Most of the fish stocks are concerned to obtain substantial number of fertilized eggs by catching and stripping adults during their spawning period (Maitland, 1990).

1.5.2*Ex Situ* Conservation

Cryopreservation of sperms, eggs or embryos and storage of cell cultures represent alternate methods of maintaining genetic variants. However, the maintenance of such collection is cumbersome and expensive. 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.

1.5.2.1 Cryopreservation

In gamete/ embryo bank, adequate samples of the natural genetic variants are kept in suspended state of animation under extra low temperature (-196 C) in liquid nitrogen. Storage of fish milt, eggs and embryos without loss of viability is of considerable value in conservation of fish genetic resources as well as in aquaculture. Long term cryopreservation technique was developed and standardized for sperms by the National Bureau of Fish Genetic Resources, Lucknow. This has primarily helped in the development of gene bank for conservation of endangered fish species. However, the technique is successful only for sperm and not much research is being carried out on eggs (Chao and Liao, 2001). At the moment the technique is only of limited value in relation to the conservation of fish species.

1.5.2.2 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

the conservationists, numerous biologists view captive propagation dubiously. It has also been pointed out that captive breeding of animals for conservation purposes had a shaky start which caused skepticism (Warland, 1975) as too many projects seemed to be consumers rather than producers of wildlife (Perry *et al.*, 1975). Now a days, the science of captive propagation has a firm and respectable foundation.

1.6 OBJECTIVES OF THE STUDY

Pristolepis marginata Jerdon commonly called "Malabar catopra" or "Malabar sunfish" or "Chutichi" in the vernacular is an attractive ornamental fish belonging to the family Nandidae. This species which is endemic to the Kerala part of Western Ghats inhabits clear and rapid streams. The species has been enlisted as vulnerable by the CAMP, 1998–Conservation Assessment and Mangement Plan Workshop for fresh water fishes of India held at NBFGR, Lucknow. "A taxon is vulnerable when it is not critical or endangered but is facing a high risk of extinction in the wild" (defined by the IUCN Red List criteria of population decline, habitat deterioration and other factors).

Conservation of the endangered/vulnerable fishes cannot become successful if it does not provide protection to the resources, which support the diversity and abundance. Hence *P. marginata* was one of the prioritized species for the captive breeding under NBFGR–NATP (National Bureau of Fish Genetic Resources – National Agricultural Technology Project) programme entitled "Germplasm inventory evaluation and gene banking of fresh water fishes of India". Anna Mercy *et al.* (2003) developed the captive breeding technology for *P. marginata* Jerdon. The present study is based on this paper. This study was conducted with the following objectives:

(i) To study the size at first maturity, spawning frequency and fecundity in captivity

(ii) To observe the cyclic changes of gonadal maturation and their histological differentiation (Oogenesis and spermatogenesis).

(iii) To study some of the life history traits like length-weight relationship and relative condition factor.

Review of Literature

2.REVIEW OF LITERATURE

2.1 REPRODUCTIVE SYSTEM

A thorough knowledge of the reproductive system is essential for a proper understanding of their biology. The morphology of the fish gonad was studied as early as 1792 by Cavolini. Since then a volume of literature has accumulated on the structure and function of the fish gonads. Among the early works, notable are those of Hickling (1936), Yamamoto (1956). Later works include those of Grimes and Huntsman (1980) on *Rhomboplites aurorubens*, Nair and Nair (1982) on *Ambassis commersonii and* Sherly (1993) on *Pristolepis malabaricus.* Recent works include those of Dasgupta (2002) on *Mystus gulio*, Kurian and Inasu (2002) on *Horabagrus brachysoma*, Raj (2002) on *Mystus montanus*, Goswami and Dasgupta (2004) on *Nandus nandus*,

The literature on the reproductive organs and reproduction was reviewed by Marshall (1956), Hoar (1957 & 1969), Raven (1969) and Nagahama (1983).

2.2 SPAWNING BIOLOGY

'Spawning' means the emission of male and female gametes from the body to the exterior, where fertilization occurs. Very often, these terms have been found confusing in the literature. 'Breeding' for instance, has been frequently used as a synonym to spawning, although breeding includes a sequence of events related to both pre-spawning and spawning phases. Thus 'breeding season' signifies the time of peak maturity and the period during which spawning occurs in a population. The phenomenon of breeding is frequently accompanied with a complex behavioural patterns involving nest building, pairing, courtship, parental care, migration and shoaling.

Thus the specialization in reproduction, which the teleosts have undergone as a group, is almost unique in the entire animal kingdom. The study of the spawning biology is very important from the point of view of capture and culture fisheries.

(i) For proper exploitation, management and conservation of the natural fish populations.

(ii) Protection and conservation of food and ornamental fish species.

(iii) Collection of natural seed resources for culture purpose.

(iv) Seed production under captive conditions.

(v) Proper management practices in culture fisheries.

Some important works on the breeding biology of fishes include that of Clarke(1934), Hickling and Rutenberg (1936), Prabhu (1956), James (1967), Grimes and Huntsman (1980), Craig (1987), Sherly (1993) Colombo and Graudi (1996), Sivakami (2001), Raj (2002), Goswami and Dasgupta (2004) and many others.

2.2.1 Quantification of maturity stages

Fishes exhibit periodic or cyclic reproductive behaviour. Maturity can be defined as the cyclic morphological changes the male and the female undergo to attain full growth and ripeness. A thorough knowledge of the maturation cycle and depletion of the gonads will help to understand and predict the annual changes that population undergoes. Reproductive studies of fishes, such as the assessment of the size at first maturity, duration of the spawning season, spawning frequency and fecundity require the knowledge of the stage of gonad development in the individual fish. Quantification of maturity stages is a prerequisite for the study of spawning biology. For the determination of the cycle of maturity of gonads, the most common method is to define the stages of sexual maturity and follow them at monthly, fortnightly or weekly intervals in a sample fairly representative of the population. Routine

assessment of maturity stages is normally done assessing individuals to stages by characters, which can be differentiated with the naked eye. Macroscopic quantification characters mainly used are:

(i) Colour and shape of the gonad

(ii) Space it occupies inside the body cavity

(iii) Texture of the gonad

(iv) Size of ova and blood supply

If needed cursory examination under the microscope is also carried out with regard to gametes.

Gonad staging in a descriptive scale allows a rapid quantitative assessment of the breeding sub-state. (De Vlaming, 1983). However, it is difficult to classify all the fish into exact valid gonad stages. Moreover, development of ovary and testis is often not in the same line; the testicular development is less spectacular than that of ovary and it may not be always possible to differentiate the development of testis into 7 or 8 stages while it is not so difficult in the case of ovary belonging to the same species. Nevertheless, this type of arbitrary classification may still provide a convenient way to interpret the progress of gonadal maturation. However, the morphological examination should always be compared with the periodical histological examination of the gonads to confirm the exact stage of the testis and ovary in a particular month.

According to the convenience of various workers and the peculiarities exhibited by different fishes, the systems of classification of the maturity stages differ especially zoogeographically. A large number of keys for maturity have been reported by various workers (Wood, 1930; Prabhu, 1956; Qasim, 1957). These fish biologists have devised schemes to identify maturity into a number of stages (usually 4 to 5 stages, but sometimes up to 8 to 9 stages). Four stages key was developed by Matsui (1950) for *Katsuwonus pelamis*, Bagenal (1957)

for *Hippoglossoides platysoides* and Nayak (1959) for *Polydactylus indicus* whereas at other extreme Clarke (1934) has drawn up twelve stages for *Saurida caerulae* and Karekar and Bal (1960) as much as fourteen stages for *Polydactylus indicus*. Seven-stage key was developed in *Priacanthus hamrur* (Sivakami *et al.*, 2001) and in *Valamugil seheli* (Moorthy *et al.*, 2002). Sherly (1993) have observed seven maturity stages in the gonad of *Pristolepis malabaricus*. Stages of five, seven, eight or ten have also been used between the above-mentioned extremes by a number of workers. These cover the minor differences between species and those within a single species, giving various degrees of refinement. Because the gross naked eye staging inevitably means subjective judgment, too high a level of refinement is unjustified.

2.2.2 Size at first maturity

The size at first maturity implies the length or age at first maturity. This age of onset of reproduction varies with sex since males typically mature at a smaller size and younger age than females. For both sexes, however the age at the onset of reproduction depends in good part on the nature of environment in which the population of concern lives as well as the nature of population itself. Where the environment is favorable for growth and adult survival, fishes will tend to delay reproduction, while if the conditions are unfavorable, growth and adult survival are low; reproduction tends to take place at a younger age. For e.g., In Europe there are two distinct forms of the Brown trout (*Salmo trutta*) whose life history tactics reflect the environments in which they live (Allen, 1938). The lake dwelling form, inhabiting a fairly stable and productive environment and grows to a larger size, spawns first when five to seven years old. The stream dwelling form, in contrast, lives in a much less stable and productive environment and so grows more slowly and matures in three to five years. On an energetic

basis, it appears that in a more stable environment natural selection favours females that delay reproduction in order to invest their energy in producing large numbers of large eggs, because egg size and number tend to increase with size of the female while in a more unstable environment natural selection favors females that reproduce as quickly as possible, since the probability of survival from one year to the next is low. A similar pattern has been found for the number of young produced in different environments by a live bearing fish (Moyle and Cech, 2000).

The nature of the population to which female belongs also influences the age of reproduction. Females, which belong to an expanding population, tend to reproduce at an earlier age than those in more stable populations. Part of the reason for this is that expanding populations tend to be in favorable environments, so that larger size can be achieved at a younger age. Greenwood (1976) reported that fish becomes sexually mature for the first time at a size, which is a rather constant proportion of their final length. Variations in the lengths at first maturity may be related to ecological factors, food supply and assilmilation (Keshava *et al.*, 1988). The length at which 50% of the fish attain maturity is regarded as the length at first maturity (Kagwade, 1968; Sivakami *et al.*, 2001). 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 ($L\infty$) size of the species (Borah, 2001).

2.2.3 Spawning frequency

The frequency of reproduction is another characteristic of fishes' life history strategies that appears to reflect the predictability of the environment in which the fishes live. The two basic strategies here are Semelparity and Iteroparity. Semelparity is "big bang " reproduction,

where the adults spawn and die (as among Pacific salmon), while iteroparity is repeated reproduction characteristic of most fishes.

Clarke(1934), Hickling and Rutenberg (1936), De Jong (1939), June (1953), Prabhu (1956), Qasim and Qayyum (1961), Grimes and Huntsman (1980) and other workers have 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. Classification of ovaries based on the oocyte size avoids the complex laboratory technique used in histology but may still be time consuming if the size frequency distribution of the most advanced mode is being determined. The technique is much faster with simpler measures such as the size of the largest oocytes, but is of limited value if the corresponding developmental stage of the oocytes is not known.

On the basis of their oocyte size distribution, ovaries are classified into three basic types (Wallace and Selman 1981):

(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 homogeneous 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 ripe ovaries, where there may be a clear separation between the ripe and yolked oocytes.

De Vlaming (1983) considers that most species with asynchronous oocyte development has protracted spawning seasons. However, he also noted that some species with group synchronous development might also have protracted spawning seasons, with the female spawning several times in a breeding season. A protracted breeding season 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, 1940; DeVlaming, 1983). The type of oocyte development is also not necessarily a fixed characteristic of the species.

Fish with a group synchronous development are also referred as 'total' or 'isochronal' spawners, the implication being that the whole clutch of developed oocytes will be shed within a short period – a week or so, according to Holden and Raitt (1974). Fish with asynchronous oocyte development are also referred to as 'partial', 'heterochronal', 'multiple, or 'serial' spawners (Holden and Raitt, 1974), implying that only part of the complement of yolked oocytes is spawned and that individuals spawn over a protracted period. Qasim and Qayumm (1961) has associated multiple spawning within years with less seasonal environments, smaller body sizes, and smaller relative ovary sizes. The term 'partial spawner', in particular, is likely to be confused with a term such as 'fractional spawner', which is used purely to describe the release of eggs. While multiple spawning generally refers to more than one spawning in a season, fractional spawning is used for species that spawn part of an ovulated clutch (De Vlaming, 1983).

Prabhu, 1956; Karekar and Bal, 1960, based on the spawning frequency classified the fishes into four categories. This classification is based upon the works of Hickling and Rutenberg, 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. This is typical of many temperate and polar fishes.

(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 intra-ovarian eggs, the individual spawning once. Mainly seen in tropical and sub tropical fishes.

(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. These fishes show protracted spawning season with individual spawning more than once, mainly seen in tropical fishes.

(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. E.g. perches in the coral reefs and cichlids of Africa.

Category A, B and C is constituted of group synchronous ovaries, whereas Category D is constituted of asynchronous ovary. An evaluation of the progression of the ova in the ripe stage in *Pricanthus hamrur* indicated that there are two batches of ova with mature ova forming a distinct mode followed by a developing batch. (Sivakami *et al.*, 2001). Narejo *et al.*, (2003) places *Monopterus cuchia* under category A where the fish spawns in a single batch during the spawning season, April to June. Sherly (1993) places *Pristolepis malabaricus* under Category B of Karekar and Bal (1960).

2.2.3.1 Histological staging

Histological studies provide very precise information on oocytes developmental stage but their interpretation is sometimes confused because different authors use different terms for the same structures. Males are in general more difficult to stage than females, may give a less defined estimate of the spawning season and spawning frequency (Fair bridge, 1951; June, 1953; Otsu and Uchido, 1959; Crossland, 1977; Forberg, 1982.).

Oocyte development in teleosts have been reviewed by Wallace and Selman (1981), De Vlaming (1983), and Wallace *et al.,* (1987).

2.2.4 Fecundity

Fecundity is the most common measure of reproductive potential in fishes because it is a relatively easy measurement to make. But in most species where the eggs are destined to be released from the ovaries in batches, an accurate determination of fecundity is almost impossible. Fecundity/absolute fecundity is usually defined as the number of ripening/ripe eggs found in the female prior to spawning (Bagenal and Braum, 1968). This contrasts with 'fertility', which is the actual number of young produced than the number of eggs. The fecundity also varied with the seasons, climatic conditions, environmental habitat, nutritional status and genetic potential (Bromage *et al.*, 1992). Hickling and Rutenberg. 1936 suggested that if suitable conditions are not found then the eggs might become atretic, degenerate and ultimately get resorbed in the body. Grimes and Huntsman (1980) used gravimetric subsampling method for fecundity estimation in Vermillion snapper, *Rhomboplites aurorubens.* The same method was followed in *Priacanthus hamrur* (Sivakami *et al.*, 2001), *Mystus gulio* (Dasgupta, 2002), *Horabagrus brachysoma* (Kurian and Inasu, 2002).

Population fecundity is the sum total of the absolute fecundities of all the breeding females in a population. It can be found out by elaborate sampling mechanisms. It is difficult to find out the fecundity of the multiple spawners. Open substratum pelagic spawners have extremely high fecundities, and spawning periods are often protracted since all the eggs can rarely be released by a single spawning act. Brood hiders and nest builders possess relatively larger eggs and are less fecund but they have greater spawning success. In the case of mouth brooders the fertility does not depend on the fecundity but on brooding capacity of the adult. Fecundity and egg size are inversely related. Fecundity and population density are inversely related.

Several reasons have been attributed to the changes in the fecundity of fish. Bagenal (1957) reported that changes in abundance of food items in the Clyde Sea influence the fecundity of the long rough dab. In the case of the plaice, fecundity was found to depend on the density and in turn regulates the population. Fecundity of *Etroplus suratensis* is found to be high in clear waters of Kerala backwaters (Bhaskaran, 1946). Average fecundity of *Nandus nandus* was found to be 40, 595 at Gayeshpur pond (an artificial pond) than at Mogra bheel (8059) due to the better conditions and food availability in pond than at its natural habitat. (Goswami and Dasgupta, 2004).

2.2.4.1 Relative fecundity

The absolute fecundity (F) can usually be related to another variable of the fish by a simple relationship such as $F = a X^b$ where X = length, weight or age and a and b are constants (Bagenal & Braum, 1968). The basic assumption of using relative fecundity is that the number of eggs per gram does not increase or decrease with the length or weight of the fish. In other words, this method of expressing the

results assumes the relation between fecundity and weight is linear. (Bagenal, 1957).

2.3 LENGTH – WEIGHT RELATIONSHIP

In nature, with the increase in length of fish, the weight also increases but in a more rapid way, thereby showing that the weight of fish is a function of length. Since length is a linear measure of volume, $W=aL^3$ where 'W' represents the weight of the fish, 'L' length of the fish and 'a' a constant. But Le Cren (1951) pointed out that it is best to fit a general parabolic equation of the form $W= a L^b$ where 'a' and 'b' are constants to be determined empirically (from the data). For practical purposes the relationship is usually expressed in its logarithmic form or in the linear form as $\log W= \log a + b \log L$.

The fact that weight will be proportional to the cube of any linear dimension has been much discussed. According to Allen, (1938) for an ideal fish, which maintains a constant slope, the value of 'b' will be 3. Hile (1936) and Martin (1949) were of the opinion that it may vary between 2.5 and 4.0. Beverton and Holt (1957) remarked that instances of important deviations from isometric growth in adult fishes are rare. Variation in 'b' value may occur due to different environmental factors.

2.4 CONDITION AND RELATIVE CONDITION FACTOR

Innumerable studies have been conducted on the condition cycle in fishes since the difference in values of condition can frequently yield insight into the circumstances of the fishes' lives e.g. with regard to timing and duration of breeding cycle, with regard to food supply etc., The significance of values of these factors to the fishery science is therefore, considerable.

Variations from the expected weight for length of individual fish or groups of fish as indicative of fatness, general well being or gonad

development may be termed as 'condition' (Le Cren, 1951). Kestevan (1947) has discussed the importance of variations in specific gravity of fish flesh in studies on condition. Usually the density of fish is maintained as same as that of the surrounding medium and hence changes in weight for length are due to changes in form or volume and not specific gravity. Naturally, the value of the condition factor calculated in this way is highly dependent upon the exterior of the fish. The value of Kn depends on physiological factors like maturity and spawning and environmental factors like the availability of food (Brown, 1957). In high-bodied fishes with a broad back the ratio of the weight to length is greater than in elongated ones (Nikolsky, 1963). Such changes are analysed by the condition factor or coefficient of condition factor or ponderal index (Hile, 1936; Thomson, 1943) and is given by the formula $K = 100W/L^3$ where K= condition factor, W= weight of the fish and L = length of the fish. Condition factor formula is based on comparison with an ideal fish, where the cube law relationship holds good. The value of 'K' will be affected if the fish does not follow the cube law. Therefore, by using an empirically calculated length – weight relationship, $W = aL^b$, the relative condition factor (Kn) could be calculated and is given by the formula $Kn = W/ aL^b$ or $W/ \overline{W}$, where W = observed weight of the fish and $\overline{W}$ = calculated weight of the fish (by using the length – weight relationship formula).

Variation in the condition value may be attributed to different factors such as the environmental conditions, food availability and gonadal maturity as has been suggested by many workers (Le Cren, 1951; Keshava *et al.,* 1988, Sivakami *et al.,* 2001; Goswami and Dasgupta, 2004).

Materials and Methods

3. MATERIALS AND METHODS

3.1 REARING THE FISHES IN CAPTIVITY

Captive breeding technology was developed and standardized for *Pristolepis marginata* Jerdon by Anna Mercy *et al.*, 2003. Fishes, which were captively bred, was carefully reared in rounded cement tanks of one metre height and diameter. These tanks were adequately aerated by means of biological filters. Water quality was noted daily. Oxygen was never a limiting factor in these tanks. pH ranged from 7 to 7.5. Fishes were maintained under ambient temperature conditions (26 to 29° C).

3.1.2 Feeding Regime

All the fishes under captivity were fed with a uniform diet, newly hatched larvae were fed with cladocerans preferably *Moina*. The early juveniles were given mosquito larvae till they reached a length of 1.5cm. *P. marginata* were found to accept artificial diet at a very small size. Hence all the late juveniles and adults were maintained under captivity on pelleted feed. Standard pelleted feed obtained in the market under the trade name Higashimaru was used. For juveniles, pellets were crumbled to smaller sized particles.

Feed was given *ad libitum*. Feeding was done twice a day, in the morning and in the evening.

3.2 LENGTH-WEIGHT RELATIONSHIP

A total of 209 fishes (1.2 to 14.9 cm SL) were used for the present study. 75 males (5.8 to 14.9 cm SL) and 85 females (3.4 to 10.4 cm SL) and 49 juveniles (1.2 to 4.7 cm SL) were examined in fresh condition for the calculation of the length – weight relationship. Individuals whose sex could not be distinguished by naked eye or under

the microscope were designated as juveniles. The standard length was measured from the tip of the snout to the base of the caudal fin (± 0.5 mm) and the weight using the electronic balance (± 1.0 mg). Length weight relation was computed separately for the following groups of fishes.

(i) Total population – juveniles, males and females.

(ii) Juveniles

(iii) Males

(iv) Females

(v) Males and females combined.

The data on the length weight were analysed by Le Cren (1951) method. It can be expressed as $W = a.L^b$. Logarithmic transformation of the above formula give a linear equation as $\log W = \log a + b \log L$

Where,

W = weight in grams

L = length in centimeters

a and b = constant values. The regression equation $y = a + bx$ where y = log weight, x = log length, a = log 'a' and b = slope of the linear regression equation, was calculated by the method of least squares. The analysis of covariance was done to test whether the regression (slopes or elevations) of 'y' on 'x' are significantly different for two sexes. (Snedecor, 1961). The significance of the variations in the estimate of 'b' (regression coefficient) for the two sexes from the expected value for ideal fish (3.0) was also tested by't' test to know whether the fish exhibits isometric growth pattern.

3.3 CONDITION AND RELATIVE CONDITION FACTOR

Condition factor and relative condition factor were calculated separately for juveniles (fishes in which sex differentiation has not yet taken place) and adult fishes. Among the adult fishes, condition factor

was found out separately for males and females. Male and female fishes were further divided into repeat and recruit spawners based on the size at first maturity curve. Then, average relative condition factor was calculated for repeat and recruit spawners among both males and females. The fishes, juveniles, males and females which, were used for computing the length weight relationship were used for the calculation of condition factor also. As the fishes do show breeding cycle, with regard to food supply etc, the corresponding 'relative condition factor' was also computed for the above data.

Condition factor,

$$K = 100W/L^b \text{ (Le Cren, 1951)}$$

Relative condition factor,

$$Kn = \frac{\text{Actual weight (W)}}{\text{Expected weight } (\bar{W})}$$

Expected weight is calculated from the equation log W = log a + b log L; where a = a constant, b =slope of the linear regression equation.

3.4 REPRODUCTIVE SYSTEM

For studying the gross morphology, both fresh and preserved specimens were used .The abdomen was opened by a ventral incision and the gonads were studied under a dissection microscope.

3.5 OOGENESIS AND SPERMATOGENESIS

For histological studies fresh material was used. Small pieces of the ovary and testes at different stages of maturity were fixed in alcoholic Bouin's. Paraffin sections were cut at 6 – 8 μ thickness and stained with Delafield's haematoxylin and counterstained with eosin. The sequence of histological changes during the origin, maturation and liberation of the germ cells were studied by sectioning the gonads in the different stages of maturity.

Alcoholic Bouins: Composition	
Picric acid (saturated alcoholic solution)	75ml
Formalin (30 – 40%)	25ml
Glacial acetic acid	5ml

Delafield's hematoxylin	
Hematoxylin	4 g
Absolute ethyl alcohol	25ml
Ammonium alum $NH_4 Al (SO_4)_2$	20g
Glycerine	100ml
Methyl alcohol	100ml

Dissolve 4 g hematoxylin in 25ml absolute ethyl alcohol .Mix gradually into 400ml ammonium alum, (saturated aqueous, approximately 1 part alum to 11 parts distilled water). Leave exposed to light in a flask with a cotton plug for 3-5 days. Filter. To the filtrate add 100 ml glycerine and 100 ml methyl alcohol. Allow ripening for six weeks.

3.6 BREEDING BIOLOGY

3.6.1 Quantification of maturity stages

A total of 160 fishes (75 males and 85 females) were used for the study of breeding biology. After measuring the length and weight of the individual specimen (total length, standard length and weight) of the specimen the body was cut open and the gonad was cleared of peritoneal coverings and fat bodies if any.

Quantification is based on external evaluation of the gonad. Macroscopic quantification characters used are:

(v) Colour and shape of the gonad

(vi) Space it occupies inside the body cavity

(vii) Texture of the gonad

(viii) Size of ova and blood supply

If needed cursory examination under the microscope was also carried out with regard to gametes. A six-stage key was followed for females and males.

3.6.2 Size at first maturity

Size at first maturity was computed separately for males and females. A total of 160 fishes were used for computing the size at first maturity. Size at first maturity was calculated separately for males and females.

The total lengths of all the fishes collected for the study were grouped according to different length groups. The percentage occurrence of four stages (ripening, ripe and partially spent) for females and three stages (ripening, ripe and spent) for males were found out. By plotting the percentage occurrence of mature fish (males and females) against respective length classes (10 mm), maturation curve is obtained. From this maturation curve, the length at which 50% of the fishes become mature can be demarcated.

3.6.3 Spawning frequency

Ovaries belonging to all the stages of maturity were preserved in 5% formalin for ova diameter measurements. The ova diameter measurements were done on a monocular microscope with 10 x X 10 x magnification, after standardizing the calibration of the ocular micrometer using a stage micrometer. A mixed sub sample is taken from different parts of the ovary to eliminate any error due to differential distribution of ova stocks in different parts of the ovary. The diameters so obtained are then represented graphically. The spawning frequency of the fish is obtained by plotting the percentage frequency against different ova diameter classes (ova stocks).

Ova diameter measurements were also taken from ovaries at different stages of maturity to study the progression and development of the ova following the method adopted by Clarke (1934), Hickling and Rutenberg (1936), de Jong (1939), Prabhu (1956), Nair and Nair (1983) and many others.

3.6.4 Fecundity

Ripe ovaries of sixteen fishes of size range 66 to 104 mm SL were used for the present study. During the initial quantification process itself those ovaries (stage V) were preserved in 5% formalin. Later these samples were weighed after wiping with a blotting paper and length and width of each lobe of the ovary was measured. Average of the lengths of the two lobes has been taken as the length of the ovary. From a weighed ovary, a minimum of four sub-samples were taken at random. Weight of the sub-samples ranged between 0.188 to 0.299 g. Weighed sub-samples were then kept in Gilson's fluid (based on modified Simpson, 1959) with appropriate labels showing standard length, ovary weight, sub sample weight etc. It is then shaken vigorously and left for about 24 hours. Gilson's fluid hardens the eggs and helps to liberate them by breaking down the ovarian tissue. The samples can be left for several months without any disadvantages.

Gilson's Fluid: Composition

60% alcohol – 100 ml

80% Nitric acid - 15 ml

Saturated formalin – 20ml

Water - 880 ml

Glacial acetic acid – 18ml

Counting: Fecundity was calculated by gravimetric sub sampling method. Since fecundity is defined as the total number of mature/maturing eggs present in a ripe ovary, all the yolked eggs were

considered for the counts (Hickling and Rutenberg, 1936). Sub samples kept in the Gilson's fluid were counted manually by taking in a watch glass. Average number of eggs was calculated for the subsamples. Then, on the basis of the average count and average weight of the subsample as well as the weight of the ovary, the total or absolute or individual fecundity was computed using the formula

Fecundity

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

3.6.4.1 Relative fecundity

Absolute fecundity calculated was expressed in terms of unit body weight, unit body length, unit ovary weight and unit ovary length.

3.6.4.1.1 Fecundity – length and Fecundity – weight relationships

A logarithmic transformation of absolute fecundity to body length (SL) and ovary length (OL) and absolute fecundity to body weight (W) and ovary weight (OW) can be expressed as follows,

(i) $\text{Log } F = \log a + b \log L$

(ii) $\text{Log } F = \log a + b \log OL$

(iii) $\text{Log } F = \log a + b \log W$

(iv) $\text{Log } F = \log a + b \log OW$

These lines can be fitted by the method of least squares, allowing the use of standard statistical procedures for subsequent analysis.

Results

4. RESULTS

4.1 SYSTEMATICS

4.1.1 Systematic position

The latest systematic position of *Pristolepis marginata* Jerdon based on Talwar and Jhingran (1991) following the classification of Nelson (1994) is as follows

Super-order	:	Acanthopterygii
Order	:	Perciformes
Sub-order	:	Percoidei
Family	:	Nandidae
Subfamily	:	Nandinae
Genus	:	*Pristolepis*
Species	:	*marginata*

4.1.2 Description of the species (as per Talwar and Jhingran, 1991)

4.1.2.1 Common Names

Malabar Catopra	:	English
Chutichi	:	Malayalam

4.1.2.2 Distinguishing Characters

D XIV – XVI II -14; A III (rarely 1V)) 8; P 14 –15 V 15

Body oblong and compressed. Mouth moderate; teeth villiform on jaws, outer row of teeth somewhat enlarged and in some specimens only, two or four enlarged in lower jaw; teeth villiform on vomer. Dorsal spines rather stout; second anal spine strongest but as long as the third spine. Lateral line interrupted (divided) opposite fourth dorsal fin ray on 21st scale, with 25 to 27 scales. (Plate 1.)

Plate 1. ***Pristolepis marginata*** **Jerdon**

4.1.2.2.1 Colour

In life, brownish – green with purplish reflections; often vertically banded. Fins with lighter edges; caudal fin with whitish outer edge.

4.1.2.2.2 Geographical Distribution

India: Western Ghats of Kerala. Inhabits clear and rapid streams.

4.2 BREEDING BIOLOGY

4.2.1 Classification of the maturity stages

4.2.1.1 Female maturity stages

(i) Stage - I: Immature Virgins

Young individuals that have not yet spawned. Differentiation of the gonads has just taken place. Ovaries in this stage are very small, triangular and transluscent. They are usually transluscent; pinkish occupying less than one- fourth of the body cavity. Ova are not visible to the naked eye. Usually a cursory examination under the microscope is required to differentiate ovary from the testis. Microscopically, the oocytes are irregular in shape and completely transparent with a large nucleus. Majority of the ova in this stage range from 33 to 99 μ. Maximum size of the ova being 150 μ.

(ii) Stage - II: Maturing Virgin

Oocytes yet to develop (virgin). Ova are not visible to the naked eye; only transparent immature stock (stage of high mitotic activity). In maturing virgins ovaries are usually pinkish, transluscent occupying about one third of the body cavity. The right lobe is slightly larger than the left lobe. Ova still microscopic, irregular in shape with large nucleus and slight yolk deposition. Ova size up to 350 μ. The majority of the ova being in the 132 to 264 μ.

(iii) Stage - III: Ripening I

Ovary usually yellow or pale yellow occupying half to less than three forth of the body cavity (process of vitellogenesis). Ovary usually turgid and ovarian wall transparent and ova quite visible to the naked eye. Ovary increases in weight rapidly. The majority of the ova ranges between 396 to 595 μ, the maximum size being around 650 μ.

(iv) Stage – IV: Ripening II (Reproduction)

Ovary yellow or pale yellow in colour, occupying $3/4^{th}$ or more of the body cavity. The right lobe of the ovary being larger than the left. The ovarian wall is thin, the ova being visible through the extremely thin ovarian wall. The ovary is turgid but the ripe ova yet to run out when pressure is applied. (vitellogenesis over, yet to ovulate). The ova stocks range between 600 and 1450 μ, majority of the ova being in the size range from 1100 to 1160 μ.

(v) Stage – V: Ripe

Ovary yellowish in colour. Ovarian wall is extremely thin and ova quite obvious. Occupy more than $3/4^{th}$ or whole of the body cavity. Ovary very turgid. Ovulation complete. Slight pressure on the abdomen results in the extrusion of the ripe ova. The size range of the mature ova stock ranges from 990 to 1355 μ, the mode being in the size range 1320 to 1355 μ. Maximum size of the ova is around 1800 μ.

(vi) Stage – VI: Partially Spent

Slightly flaccid, pale yellowish in hue not as stout as the ripe ovaries but still retaining a large number of ripe ova (1100 to 1400 μ) after one or two batches of them have been spawned. Ovaries may contain remnants of disintegrating ripe ova (residual or atretic ova).

4.2.1.2 Male maturity stages

(i) Stage - I: Immature Virgins

Small white triangular bodies similar in shape to immature ovaries, the right lobe slightly bigger than the left. Occupies much less

than 1/4th of the body cavity. Can be distinguished from the immature ovary by the colour and on microscopic examination.

(ii) Stage - II: Maturing Virgin

Occupies less than 1/3rd of the body cavity. White in colour, the lobes are of unequal length.

(iv) Stage - IV: Ripening

Occupies a space in body cavity less than half of the body cavity but more than 1/4th of the body cavity. Fleshy in appearance, whitish lobes are unequal in length.

(vi) Stage – VI: Ripe

Occupies more than half of the body cavity. Lobes are quite fleshy and turgid with a ribbed appearance. Milt oozes out freely from the testis on exertion of a slight pressure on the abdominal walls.

(vii) Stage – VII: Partially spent.

Dull white in colour, flaccid bodies occupying less than half of the body cavity, often thin and elongated.

4.2.2 Size at first maturity

The length (Standard length) at first maturity was determined by analyzing the data relevant to all mature fishes (Stage II and above) examined (Table.1). The percentage occurrence of four stages (ripening I & II, ripe and partially spent) for females and three stages (ripening, ripe and spent) for males were plotted against different length classes – 10 mm for both males and females. (Fig.1 (A & B)).

Table. 1 Percentage occurrence of mature fish with increasing size in *Pristolepis marginata*

Length class (SL) mm	Percentage of mature fishes	
	Males	Females
30 and below	0	0
30 - 40	0	0
40 - 50	0	0
50 - 60	0	14.7
60 - 70	0	58.33
70 - 80	17	70.58
80 - 90	56	88
90 - 100	66.66	94.44
100 - 110	79.1	100
120andabove	86.9	100

While the first mature females appeared in the 30 – 40 mm length group (15%), the first matured males appeared only in the length class 50 – 60 mm (17%). 58% of the females matured before 50 mm where as 56% of the males matured only on reaching a standard length of 70mm. All female fish were mature on reaching a length of 90 mm SL and all male fish on reaching a length of 120 mm SL. The size at first maturity for females is 54 mm SL (50-60 mm length group) and 72 mm (SL) for males (70-80 mm length group).

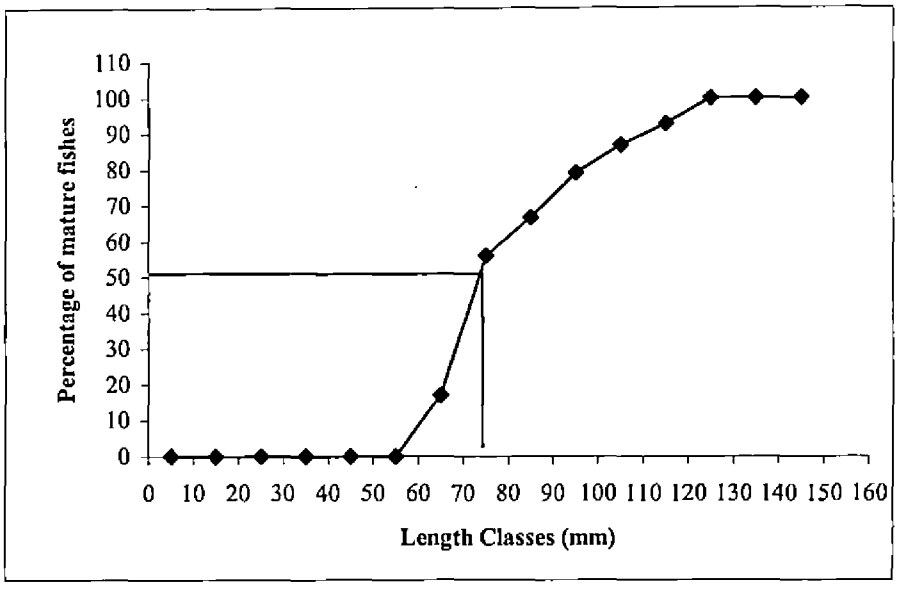


Fig .1 (A) Size at first maturity in males

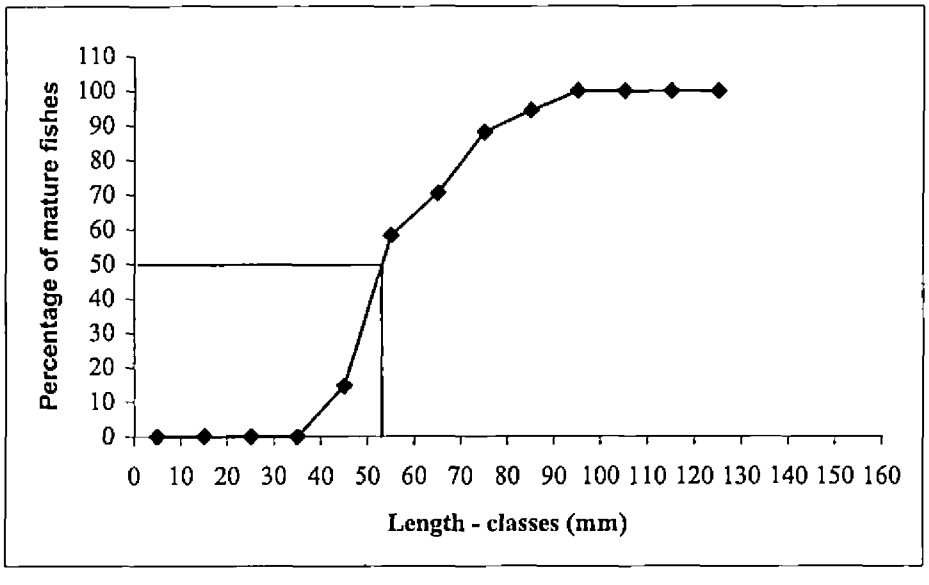


Fig 1 (B) Size at first maturity in females

4.2.3 Distribution of ova in the ovary

Ova diameter measurements were taken from anterior, middle and posterior regions of the ovary for understanding the pattern of

distribution of ova in the different regions of the ovary as well as to eliminate the possible difference in the occurrence of ova in the ovary.

The distribution of ova stocks in the anterior, middle and posterior regions of a ripe ovary are presented in the Table 2. It is evident from the Fig. 2. that the gross picture of the ova stock distribution from the three regions remains almost the same except for the slight variations in the percentage composition of the mode. However, the ripe mode shifts slightly in the posterior region (1300 – 1400 μ) indicating presence of increased number of larger ova in this region as compared to the anterior and middle regions. It was also noted that ova above 200 μ in diameter are yolked generally.

Table 2. Distribution of ova in the ovary

Ova diameter classes (μ)	Percentage frequency in different regions of the ovary		
	Anterior	Middle	Posterior
0 - 100	24	10	9.2
100 - 200	24.2	10	8.7
200 - 300	4.31	4.4	5.1
300 - 400	1.25	2.9	1.3
400 - 500	0.25	0.2	0
500 - 600	2.5	2.5	0.5
600 - 700	3.54	5.2	0.79
700 - 800	0	4.5	5.2
800 - 900	0.76	1.2	1.78
900 - 1000	2.28	4.5	2.6
1000 - 1100	5.34	7.5	4.7
1100 - 1200	14.6	16.5	12.5
1200 - 1300	12.5	18.5	17.9
1300 - 1400	3.55	10.2	21.45
1400 - 1500	1.01	2.3	6.7
1500 - 1600	0	1.2	1.2
1600 - 1700	0.25	0	0.44
1700 - 1800	0	0	0

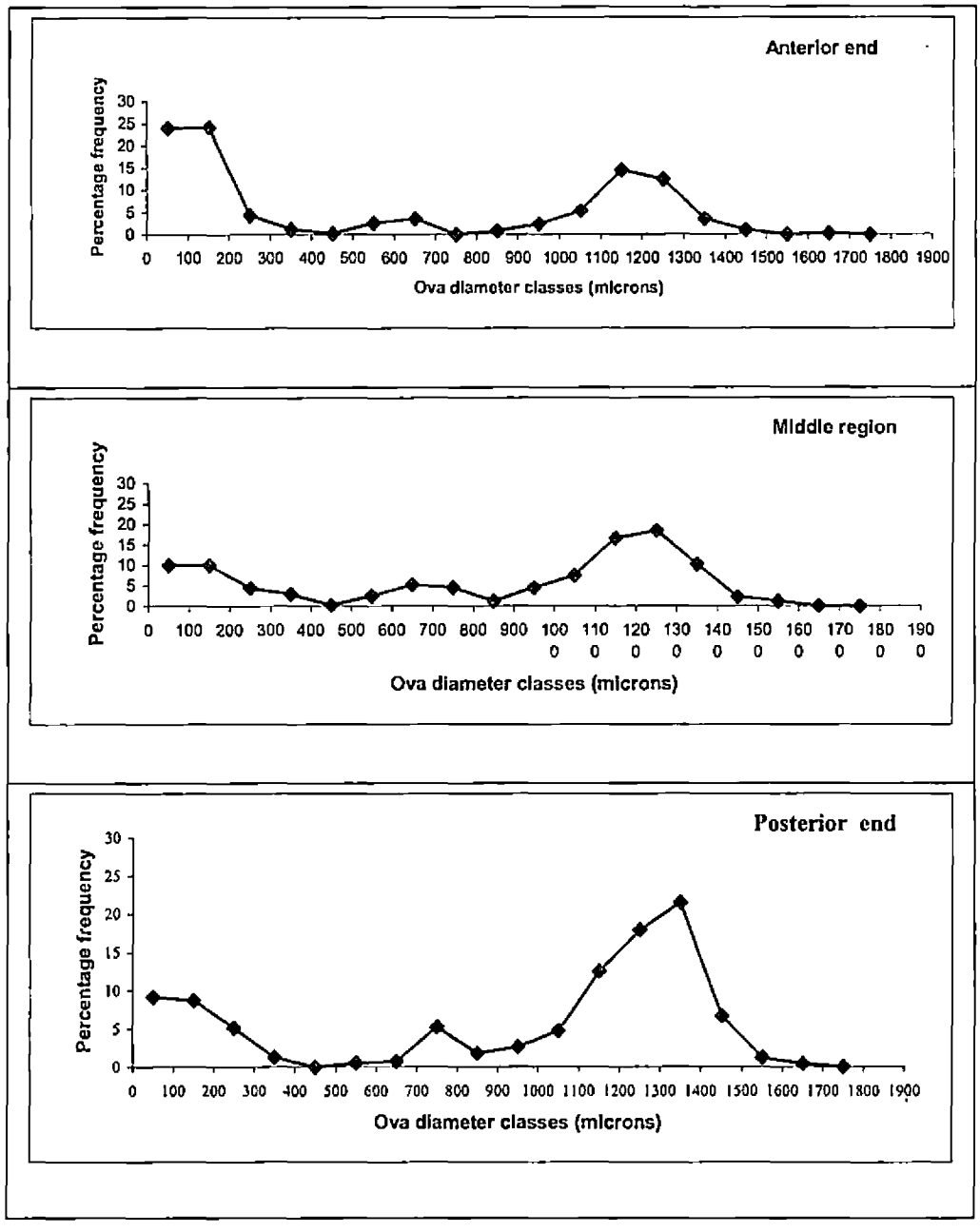


Fig.2. Distribution of ova in the ovary

4.2.4 Growth of ova in the ovary

To eliminate the possibility of slight differences in ova stock distribution between the regions of the ovary, mixed sub samples were taken for the ova diameter measurements to study the growth of ova in the ovary (Table. 3).

Stage I is characterized by immature virgin stock alone. 97.2% of ova are represented in the diameter class of 33 – 100 μ and only 2.8% is represented above 100 μ. But usually this stock falls below 150 μ. The ova stock constituting the virgin stock biomass is generally not yolked. The eggs are transparent each with a large nucleus and generally shapeless.

In the second stage, maximum size of the ova is 400 μ. Yolk deposition is found to occur in ova above 150μ. During the first ripening stage (stage III), there are two modes, one at 100 – 200μ and other at 300 – 500 μ diameter classes. In this stage, leading mode is positioned at 300 – 500 μ constituting about 35% of the total ova stocks. 65% is constituted by the immature stock.

IV th stage is characterized by the presence of two modes. They are positioned at ova diameter classes 300 –400 μ, 600 – 700 μ and the leading mode is positioned at 600 – 700 μ constituting about 58 % of the total ova stocks.

Vth stage is characterized by maximum number of ripe eggs. Leading mode (ripe stock) is positioned at 900 – 1600 μ. Maximum size of the ova present in this stage is 1782 μ. In the secondary mode, ripening stock is seen in diameter class 500 – 900 μ. The immature stocks are presented as two indistinct modes at diameter classes 100-200 μ and 300 – 500 μ respectively. Ripe stock constitutes 70% of the total

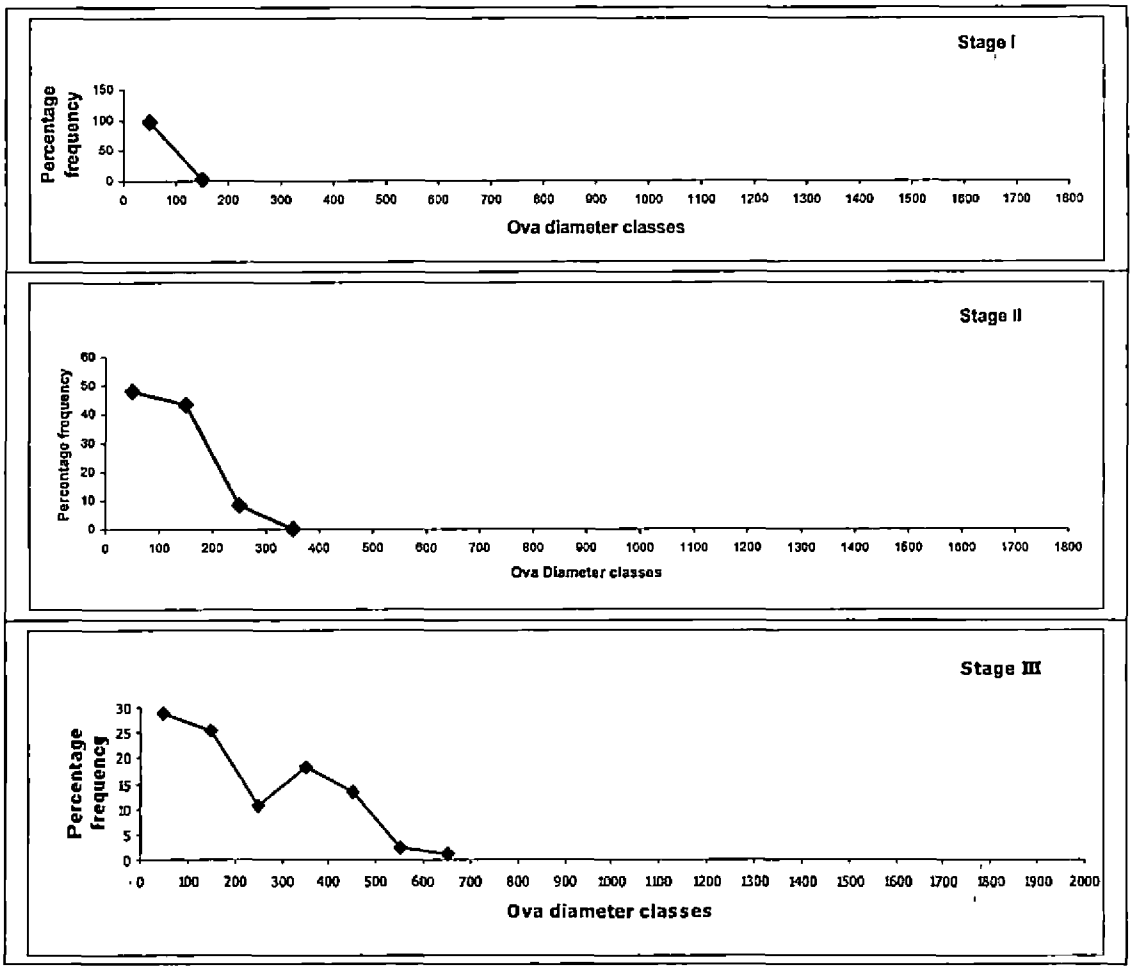
ova stocks and ripening stock constitutes 20% of the total ova stocks. Immature stock forms only 10% of the total stocks.

Table.3 Frequency of ova diameter classes in different maturity stages

Ova diameter class (μ)	Percentage frequency of different maturity stages					
	I	II	III	IV	V	VI
0 - 100	97.2	48	29	11	4.02	10
100 – 200	2.8	43.5	25.3	6.2	4	8
200 - 300		8.3	10.5	3.4	1.5	2.5
300 - 400		0.2	18.32	2.5	2.01	2.01
400 - 500			13.2	0.67	0.4	1.2
500 - 600			2.1	3.25	4.5	4.5
600 - 700			1.23	13.5	8.2	12.5
700 - 800				1.5	5.45	9.3
800 - 900				0.59	1.2	1.17
900 - 1000				6.2	3	8
1000 - 1100				12.5	7.5	16.5
1100 - 1200				19.5	15.8	10.5
1200 - 1300				15	14.8	7.5
1300 - 1400				4.2	14.6	2.1
1400 - 1500				0.25	9.5	2.5
1500 - 1600					2.07	0.5
1600 - 1700					1.7	0.3
1700 - 1800					1.05	1.0

VIth stage is characterized by the backward shift of the leading mode due to the release of fraction of oocytes at the posterior end of the ovary. Ripe stock constitutes about 50% of the total ova stocks. The percentage occurrence of ova diameter class of ripening stock has further strengthened occupying about 30 % of the total ova stocks. The

fall in the ripe stock from the ripe ovary to the partially spent ovary that does not undergo atresia clearly points to the 'batch' or 'fractional' spawning habit of *P. marginata.* Secondary or ripening mode assumes greater importance in partially spent and spent recovering ovaries which help in the recovery of the ovary to the ripe condition in a short period leading to the same individual spawning again during the protracted spawning season of the species.



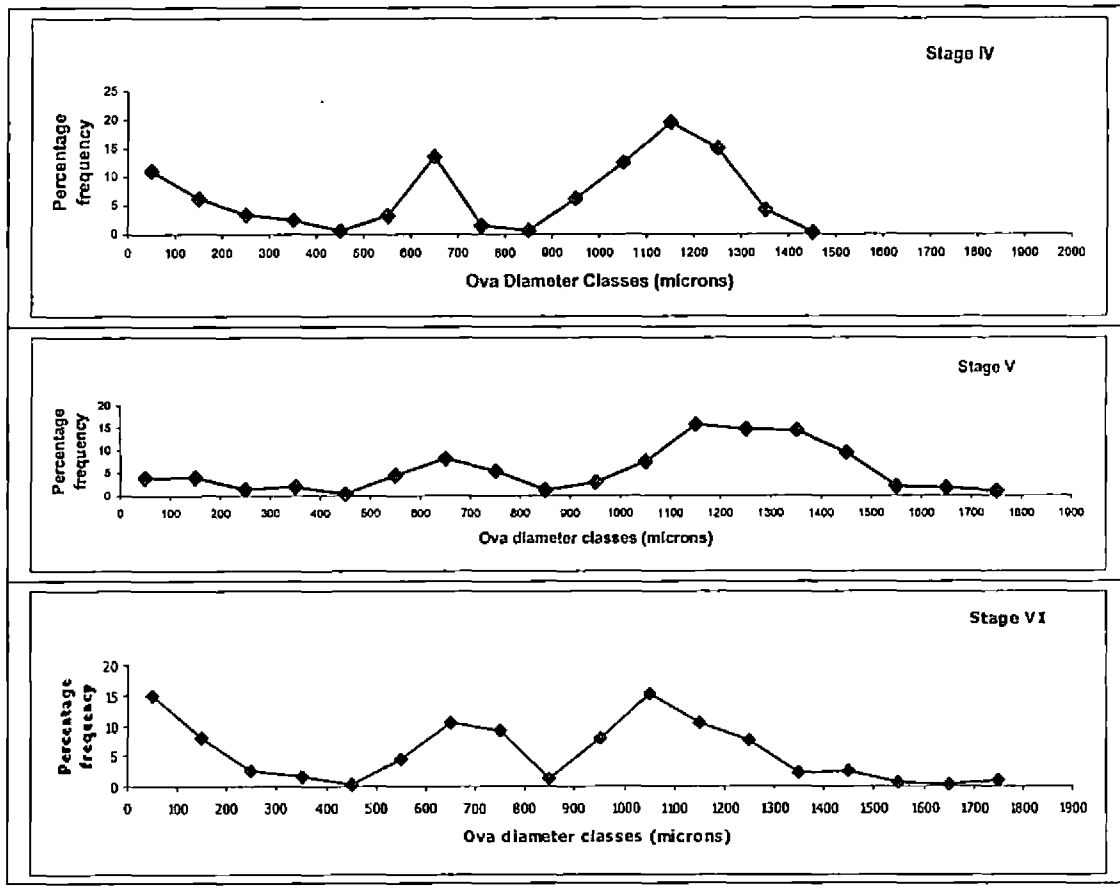


Fig.3. Frequency of ova diameter classes in different maturity stages

4.2.5 Spawning frequency

Ova diameter frequency studies have been carried out and they are illustrated in the Fig. 3. It is apparent from the figures that there are three batches of eggs representing the ripe, ripening and immature stocks. Ripe stock is having a sharp mode quite distinctly separated from the preceeding batches. Ripe stock is destined to spawn immediately. Intermediate ripening stock and immature stock are not as separated from each other as the ripe stock. As the ripe stock of eggs is eliminated by spawning, the batches represented by the ripening and immature stock undergo rapid maturation to replace the ripe and ripening stocks respectively. Simultaneously a new stock of immature stock also gets

budded off from the germinal epithelial layer. This process of maturation of the ripening and maturing stocks occur fast because these batches of eggs have undergone half the process of maturation and hence only take half the time taken by the immature stock to attain full maturity. These newly ripe ova are extruded during the subsequent spawning process, which occur with a very short period thus the same individual spawning more than once during a year. Captivity studies have also shown that *P. marginata* is a protracted spawner spawning in three or four batches. Maturation and spawning of the fish in captivity is highly influenced by the exogenous diet given as well the ambient conditions.

4.2.6 Fecundity

Results of the fecundity counts are given in the Table.4. Absolute fecundity ranged from 1102 to 4965 in fishes of size range 66 – 104 mm SL. The relative fecundity values ranged from 166 to 517 per 'cm' body length and 51 to 150 per 'gm' body weight. Relative fecundity values also range from 622 to 2089 per 'cm' ovary length and 760 to 1365 per 'gm' ovary weight.

The relationship between fecundity and a) standard length of fish (cm). b) Weight of fish (gm), c) Length of ovary (cm) and d) Weight of ovary (gm) were found out and are represented in the Table. 5 and illustrated in Figs. 4 (A) to 4 (D).

Table 4. Absolute fecundity in recruit spawners of *P. marginata*

No	Fish		Ovary		Fecundity						
	Standard length (mm)	Weight (gm)	Weight (gm)	Length (mm)	Avg Wt of the Sub Sample	Avg egg count	Absolute Fecundity	Per 'cm' fish SL	Per 'gm' fish weight	Per 'cm' ovary length	Per 'gm' ovary weight
1	78	19.464	2.058	1.9	287	392	2810	360.2564	144.3691	1478.947	1365.403
2	66	13.15	1.45	1.7	204	240	1102	166.9697	83.80228	648.2353	760
3	93	39.009	2.68	2.2	188	214	3061	329.1398	78.46907	1391.364	1142.164
4	93	29.3	3.92	2.1	201	225	4388	471.828	149.7611	2089.524	1119.388
5	84	23.95	2.27	1.75	204	253	2822	335.9524	117.8288	1612.571	1243.172
6	96	38	4.462	2.7	204	227	4965	517.1875	130.6579	1838.889	1112.73
7	92	37.56	5.02	2	204	174	4271	464.2391	113.7114	2135.5	850.7968
8	104	34	3.46	2.3	219	275	4344	417.6923	127.7647	1888.696	1255.491
9	84	27.6	3.387	1.8	277	291	3558	423.5714	128.913	1976.667	1050.487

Table 4. Continued...

No	Fish		Ovary		Fecundity						
	Standard length (mm)	Weight (gm)	Weight (gm)	Length (mm)	Avg Wt of the Sub Sample	Avg egg count	Absolute Fecundity	Per 'cm' fish SL	Per 'gm' fish weight	Per 'cm' ovary length	Per 'gm' ovary weight
10	80	20.29	2.02	2.25	224	217	1957	244.625	96.45145	869.7778	968.8119
11	64	12.43	1.6	2.05	272	217	1276	199.375	102.6549	622.439	797.5
12	87	33.2	1.8	2.4	184	174	1702	195.6322	51.26506	709.1667	945.5556
13	71	17.18	1.44	1.75	242	222	1320	185.9155	76.83353	754.2857	916.6667
14	68	15	1.2	1.8	245	222	1300	191.1765	86.66667	722.2222	1083.333
15	61	13.65	1.75	1.55	205	152	1301	213.2787	95.31136	839.3548	743.4286
16	80	26	2.12	1.7	215	202	2000	250	76.92308	1176.471	943.3962

Table. 5. Relationship between fecundity and standard length, weight of fish, length and weight of ovary.

Variant (X)	Constant (a)	Regression Coefficient (b)	Correlation Coefficient (r)
Standard length of fish (mm)	0.6977	2.9486	0.7996
Weight of fish (gm)	1.8733	1.093	0.7058
Length of ovary (cm)	2.8256	1.8299	0.2792
Weight of the ovary (gm)	2.9496	1.1415	0.8922

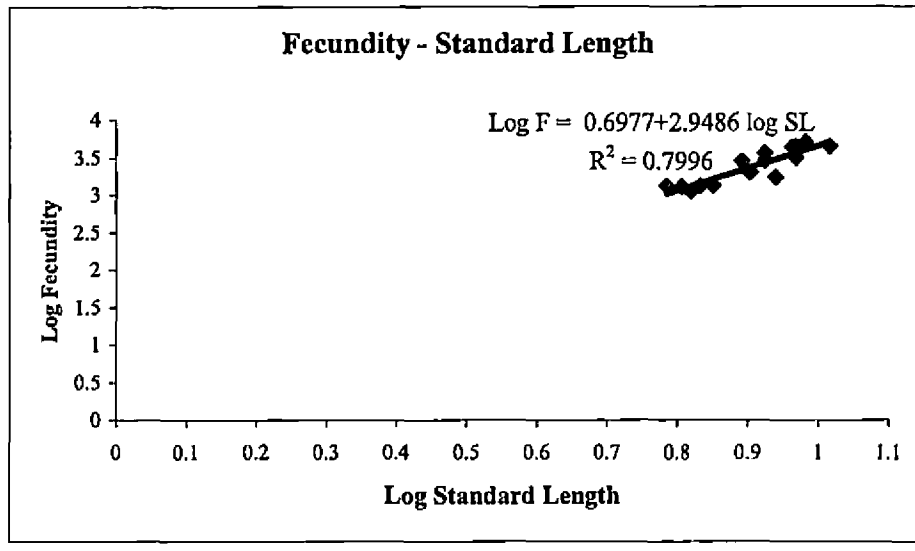


Fig. 4 (A). Relationship between fecundity and standard length

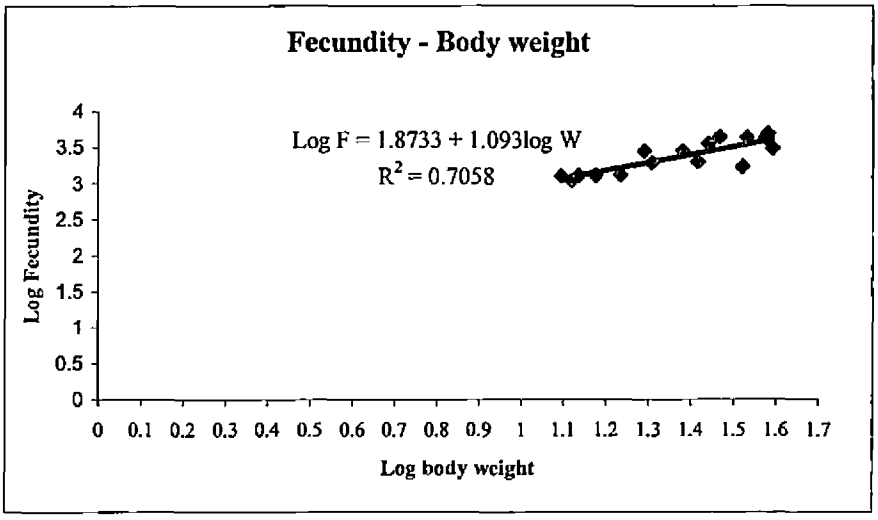


Fig. 4 (B). Relationship between fecundity and body weight

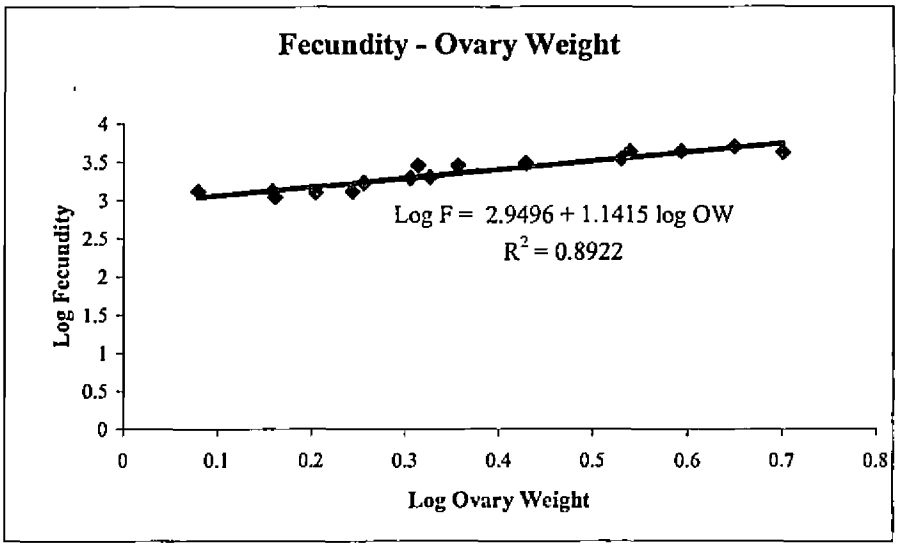


Fig.4 (C). Relationship between fecundity and ovary weight

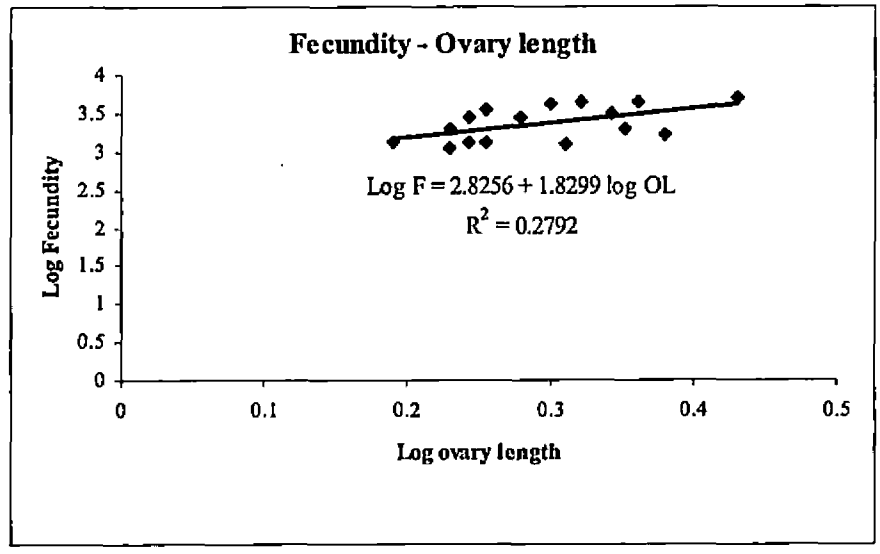


Fig. 4 (D). Relationship between fecundity and ovary length

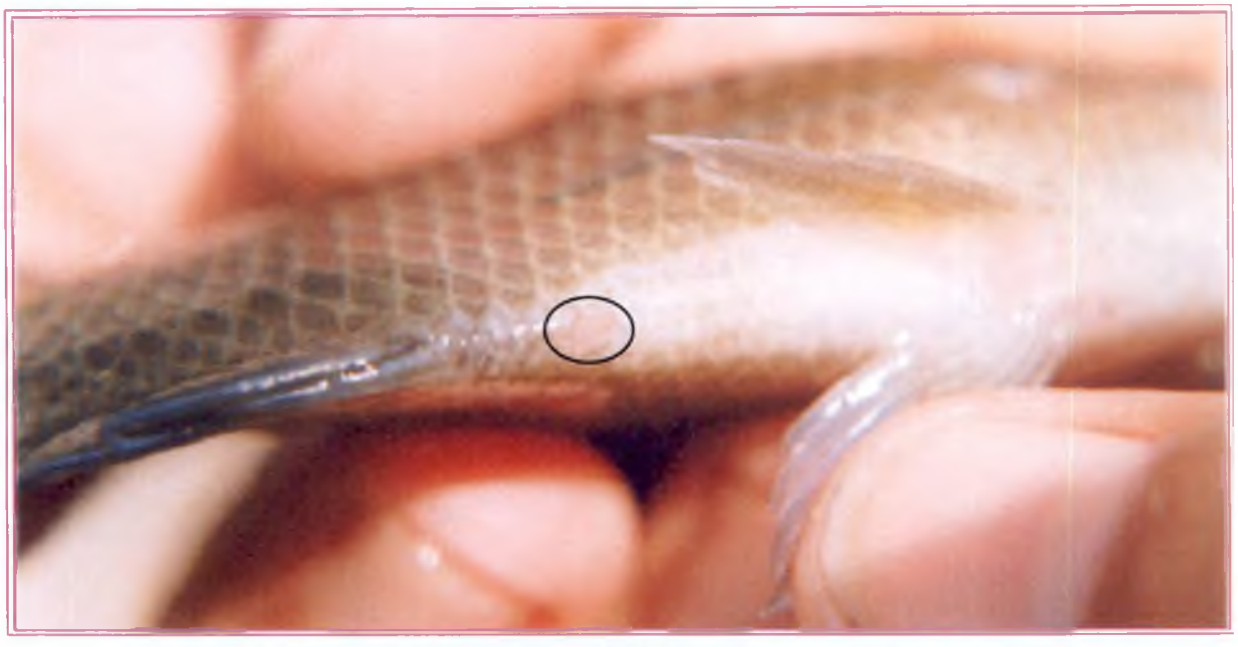
4.3 FEMALE REPRODUCTIVE SYSTEM

4.3.1 External morphology

The female reproductive system of *Pristolepis marginata* is characterized by a pair of ovaries fused medially and lie suspended from the sides of the body by a pair of mesenteries below the air bladder. The ovaries have a broad anterior part, which tapers posteriorly. The immature ovary appeared creamy white in colour and semitransparent whereas the mature ripe ovaries showed varying shades of yellow depending upon the stages of maturity.

The ovary of each side converges posteriorly towards a median line forming a common median oviduct. The oviduct opens into the urinogenital sinus. The urinogenital papilla is seen as a median, oval, slightly raised area just behind the anal opening (Plate 2. (A)).

(A)



(B)

Plate 2. Urinogenital papilla: (A) Female (B) Male

The right lobe of the paired ovaries is conspicuously larger than the left lobe. The overall size of the ovary varies with that of the fish and the state of maturity. The maximum length and weight of the ovary obtained was 3.0cm and 5.02g from a fish of total length 11.5cm and weight 37.56g respectively. In the ripening and ripe fish the ovary is turgid, with the ova visible externally through the thin membranous outer envelope. The lumen of the ovary or ovocoel is almost wholly encroached by the developing oocytes, so the ovary belongs to the closed type.

The oviducts are transparent and indistinct in the immature ovary. When the ovaries mature, the oviducts also get loaded with the ova and become turgid and distended opening to the outside via the urinogenital opening.

4.3.2 Internal anatomy

The ovarian lumen is packed with broods of developing ova, which are covered from the outside by a thin ovarian wall. The ovarian wall is rather thick in many places. It is composed of an outer layer of peritoneum made up of compact cells and is rather thick. Inner to the peritoneum is a thick layer of muscles interspersed with connective tissue. The muscles are mainly longitudinal in arrangement with small strands of connective tissue lying interspersed giving it the appearance of a network of strands enclosing granules and other bodies of various shapes. Inner to the muscular layer is a thin layer of germinal epithelium 3-4 cells in thickness at several places. The layer is made up of spindle shaped or ovate epithelial cells with distinct oval nuclei. This layer, otherwise called as tunica albuginea, dips down at several places towards the longitudinal axis of the ovary giving rise to several septa like ingrowths called 'ovigerous folds' or 'lamellae'. The free surfaces of these lamellae are lined with ovigerous cells. The lamellae from the

opposite sides come together connected by their respective connective tissue strands, the germinal epithelium being arranged on either side of the fused connective tissue septa, thus dividing the lumen of the ovary into several deep transverse grooves.

Transverse sections of the ovary reveal numerous oocytes in varying stages of development adhering to the ovigerous lamella, depending on the stage of maturity of the ovary. The oocytes of early stages of development are placed closer to the germinal layer or closely adhering to it, whereas later stages are placed further away in the crowded lumen. As the ovary matures these ovigerous lamellae assume a zig-zag arrangement owing to the pressure of the developing ova. The lamellae are provided with a rich supply of blood to nourish the developing oocytes.

Since the fish is found to show asynchronism in the development of oocytes, more than one stage of oocytes are always present in the ovary. In the ripe ovaries, besides the groups of mature oocytes, a large number of younger oocytes, both immature and maturing stocks, with and without yolk are also present. The partially spent stage contains empty chambers wherein eggs have probably been spawned. However, the empty chambers are not very distinct because the partially developed oocytes of the preceeding batches grow at a fast rate and fill up the spaces of the spawned eggs and atrophying ova.

4.3.2.1 Intraovarian development of ova (Oogenesis)

Based on the morphological changes taking place in the ovum during the course of maturation, the following stages are discernible. The different stages have been designated using the terminology adopted by Yamamoto (1956) and Yamazaki (1961).

1. The chromatin nucleolus stage (Plate. 3, Fig. C & D)

This is the youngest stage in the ovum, measuring about 100 μ in diameter. These developing ova, also called oogonia, are of varying shapes. Majority are tear drop or comma shaped and a few are spherical or oval in shape. The outer limits are quite distinct but the cell membranes are indistinct. The cytoplasm is fairly granular, deeply basophilic and present as a thin collar around the centrally positioned nucleus. The nucleus is large and spherical. The nuclear membrane is distinct.

2.Perinucleolar stage. (Plate. 4, Fig. E & F)

Concomitant with oocyte growth, the nucleus (germinal vesicle) increases in size and multiple nucleolii appear, generally at its periphery. The cytoplasm stains uniformly. The chromatin nucleolar and perinucleolar stages are sometimes referred to as the primary growth phase.

3. Yolk vesicle (cortical alveoli) stage. (Plate. 4, Fig. G & H)

This stage is characterized by the appearance of yolk vesicles in the cytoplasm. The follicular layer gets closely pressed on to the outer surface of the oocytes, getting squeezed between the enlarged oocytes and thus is not distinct as in the previous stage. The oocytes are much enlarged now and are about 396 μ in diameter. The vitelline membrane has become distinct in this stage as a compact membrane of dense, homogenous cytoplasm surrounding the oocytes. The cytoplasm remains granular but a number of yolk vesicles have appeared in it centripetally. The vesicles are similar in shape, empty, with an outer cytoplasmic shell. The cytoplasm is faintly basophilic and turns reddish with eosin. The germinal vesicle has also grown in size and is now about 100 μ and slightly irregular in outline.

4. Vitellogenic (yolk) stage. (Plate. 5, Fig. J & K)

The appearance of clusters of minute globules on the periphery of the cytoplasm and the complete proliferation of yolk vesicles are

distinguishing characters of this stage. The oocytes have increased in size and each is about 600 μ in diameter in the primary yolk stage, which further increases in size and attains a size of about 1350 μ at the end of tertiary yolk stage. In the tertiary yolk stage yolk globules have greatly increased in number and size and yolk granules lie interspersed with these yolk globules.

The germinal vesicle is central in position and is more compact now being oval or spherical in shape. The nuclear membrane is hazy and crumpled.

5.Migratory nucleus stage (Plate. 5, Fig. L)

The distinguishing features of this stage are the peripheral migration of the germinal vesicle and liquefaction of the nucleolar material. The follicular layer is also very conspicuous, the individual cells being more distinguishable. The oocyte at this stage measures about 990 μ and the maximum size of the ovum is 1300 μ. The yolk globules almost completely fill the cytoplasm.

The germinal vesicle is now midway between the vitelline membrane and the centre of the oocytes indicating the migration to the periphery. Its size and form remain distinctly spherical. The nuclear membrane is hazy and the chromatin material powdery, granulous and deeply acidophilic.

6. The mature oocytes

The oocytes are now mostly spherical or oval in shape and covered by a fine outer sheath. Each oocyte now measures 1300 μ. Only some oocytes do not retain the spherical shape apparently due to the severe pressure from the adjoining oocytes. The vitelline membrane has now developed into a well recognizable membrane and is deeply basophilic. The yolk vesicles are few and scattered measuring about 100 μ and the interspace filled with yolk globules and granules. The germinal vesicle is still out of position, may be on its return journey.

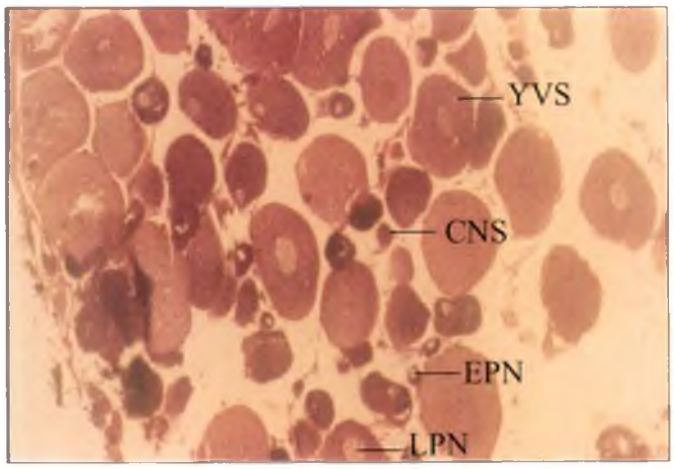
Another significant development is also visible on the periphery where there is a slight accumulation of cytoplasm, the vitelline membrane thickens in a conical fashion with the apex directed inwards. The cluster of the cells at this site looses their consistency and become liquefied forming a plug. A small depression is then formed at the surface of the plug, the oocytes thus being spherical with a dent at the area of the micropyle.

The ripe ova about to be extruded are more than 1500 μ in diameter, transparent, each with a single oil globule about one-third its size.

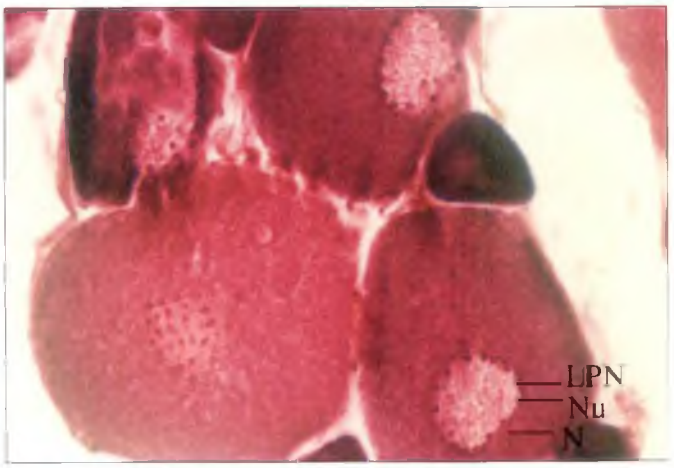
7. The fate of unextruded ova and empty follicles (Plate. 6, Fig. M)

In the partially spent and spent recovering ovary a few unspawned, ripe eggs undergo degeneration. The atresia starts with the liquefaction of the yolk, consequently, numerous minute, deeply staining ovoid bodies appear in the oocytes. The follicular layer gradually looses 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 vascularised and blood cells freely migrate into it.

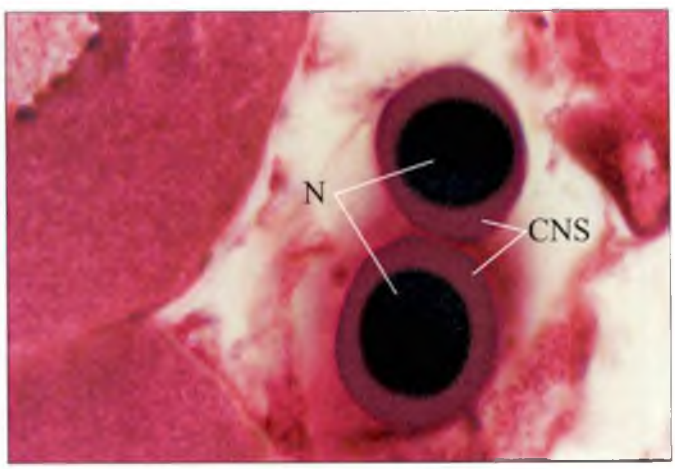
In the spent ovaries, in addition to the unspawned oocytes, which are degenerating, there are the empty follicles of the spawned ova. These empty pockets easily gets pushed out of existence by the fast developing immature oocytes, while the lone layer of the poorly staining cells around the empty bags, get reduced in size and shrivel out, their contents being gradually resorbed.



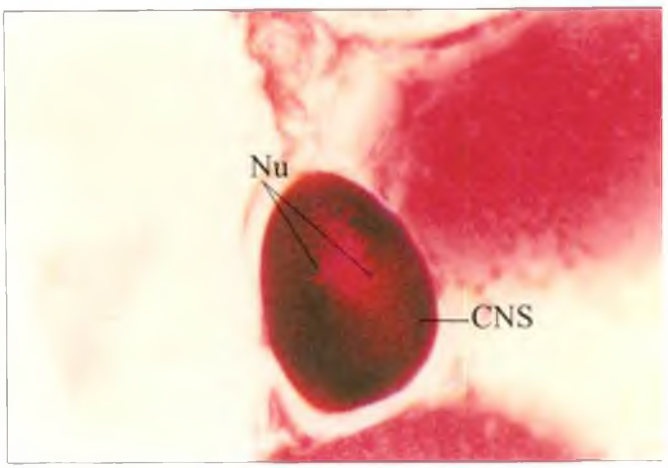
A. Maturing ovary showing oocytes of chromatin nucleolus, early and late perinucleolus stages x 10



B. Maturing ovary showing oocytes of chromatin nucleolus, early and late perinucleolus stages x 40

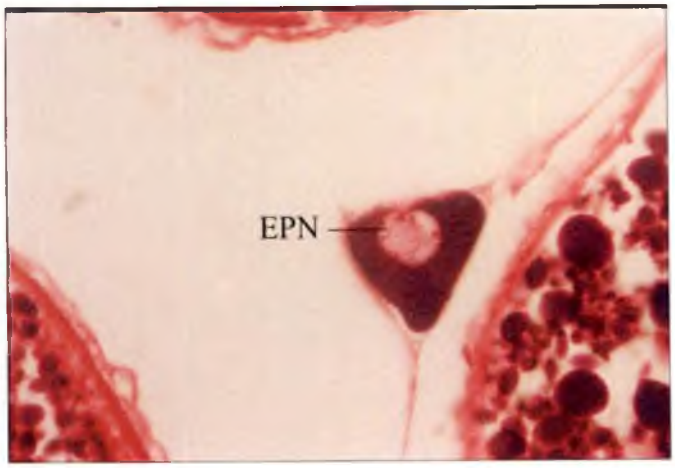


C. Maturing ovary showing oocytes of chromatin nucleolus stage x 100

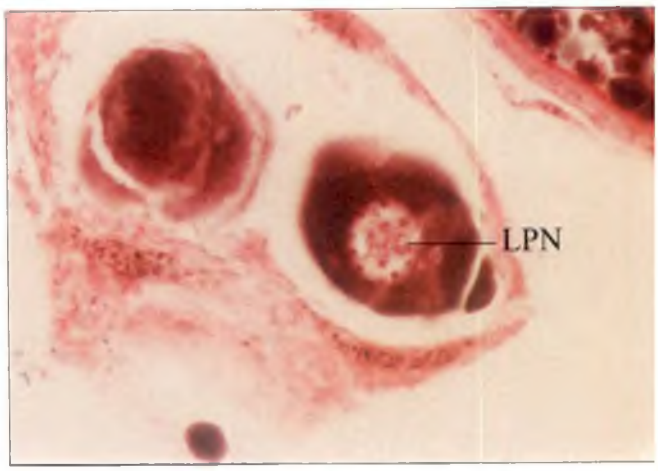


D. Maturing ovary showing oocytes of chromatin nucleolus stage x 100

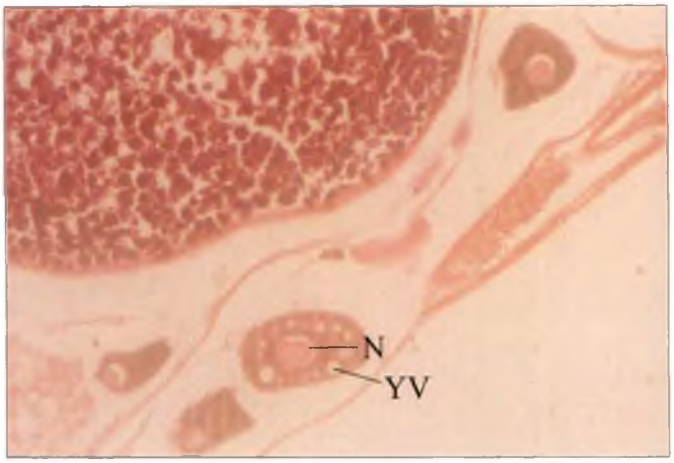
Plate 3. Transverse section of maturing ovary



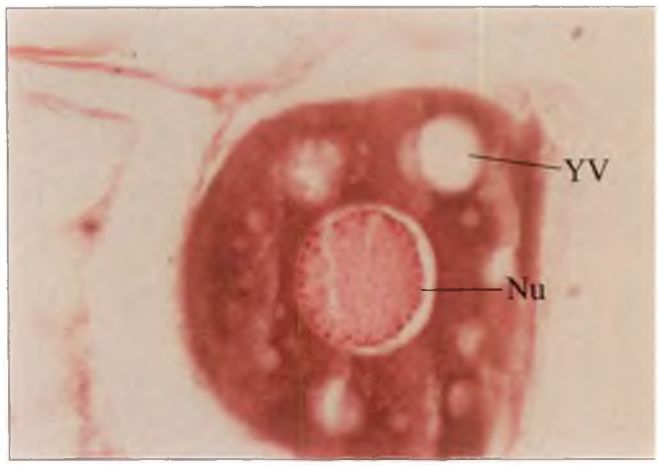
E. Ripe ovary showing oocytes of early perinucleolus stages x 40



F. Ripe ovary showing oocytes of late perinucleolus stages x 40

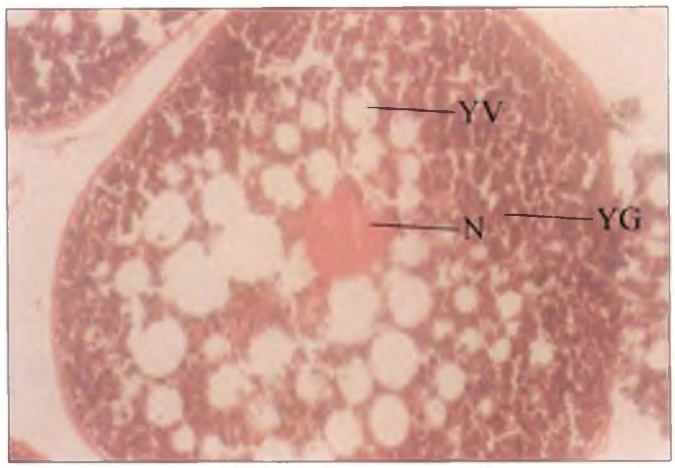


G. Ripe ovary showing oocytes of yolk vesicle stage x 10

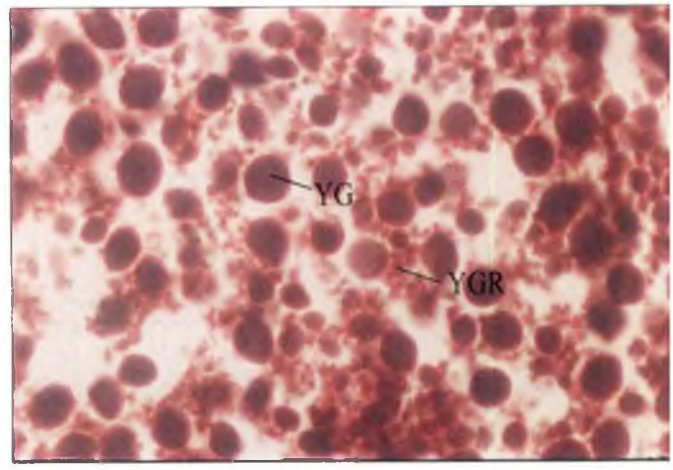


H. Ripe ovary showing oocytes of yolk vesicle stage x 40

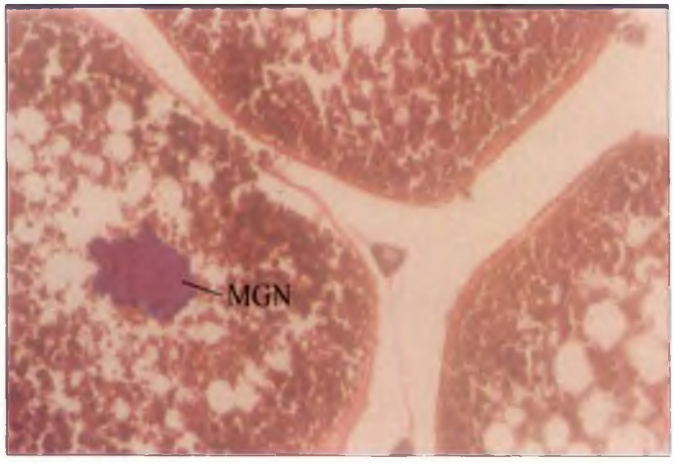
Plate 4. Transverse section of ripe ovary



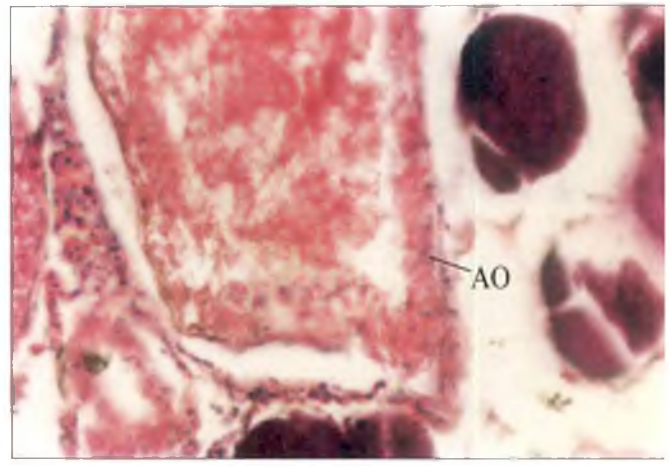
J. Ripe ovary showing oocytes in the tertiary yolk stage x 10



K. Ripe ovary showing oocytes in the tertiary yolk stage x 40



L. Ripe ovary showing oocytes in the migrating nucleus stage x 10



M. Partially spent ovary showing atretic oocytes and the new immature stocks x 40

Plate 5. Transverse section of ripe & partially spent ovary

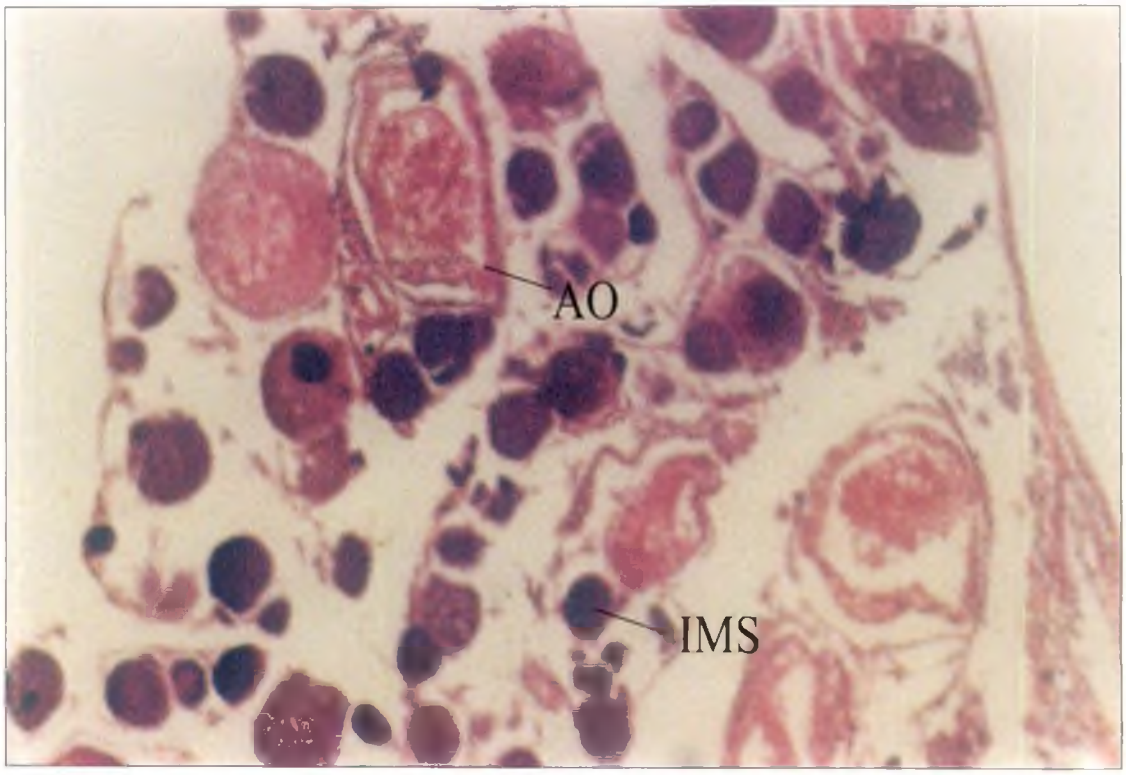


Plate 6. Origin of new crop of oocytes

4.3.2.2 Origin of new crop of oocytes **(Plate. 6)**

It has already been stated that tunica albuginea dips at several places forming the ovigerous lamellae. The free surfaces of these lamellae are lined by the ovigerous cells. After each spawning activity the germinal or ovigerous epithelium shows signs of increased activity. As a result of repeated mitosis a cluster of cells is formed at several points. The newly constituted primary oogonia divide further and differentiate into the primary oocytes, increasing in size.

Another site of oocyte formation is the empty follicles. The follicular layer which surrounds the empty pockets of the spawned eggs also give rise to fresh crop of oocytes. Thus it can be noted that there is no localized area, which can be termed a 'gamogen' present in the ovary from which alone a new crop of oocytes will be produced. The free surface of tunica albuginea and even some of the follicular cells produced new crop of oocytes in this fish.

4.4 MALE REPRODUCTIVE SYSTEM

4.4.1 External Morphology

The testis of the adult *P marginata* is a paired ovate or oblong structure similar in position to the paired ovaries. The immature testis is cylindrical in shape. As they mature the right lobe becomes longer than the left and each lobe appears triangular in cross section with lateral lobulated margin. Each testis gets narrowed towards the anterior and ends in a blunt point. Posteriorly, they open into separate sperm ducts, which are whitish in colour. The two sperm ducts open independently into urinogenital sinus, which is a wide tube running for a short distance before opening to the outside at the urinogenital papilla (Plate 2 (B)).

Testis appears as white to creamish white in ripe condition and dirty white in spent condition. When mature they are not so extensive as the ovaries and do not distend the abdominal wall to the same extent.

4.4.2 Internal anatomy (Plate. 7)

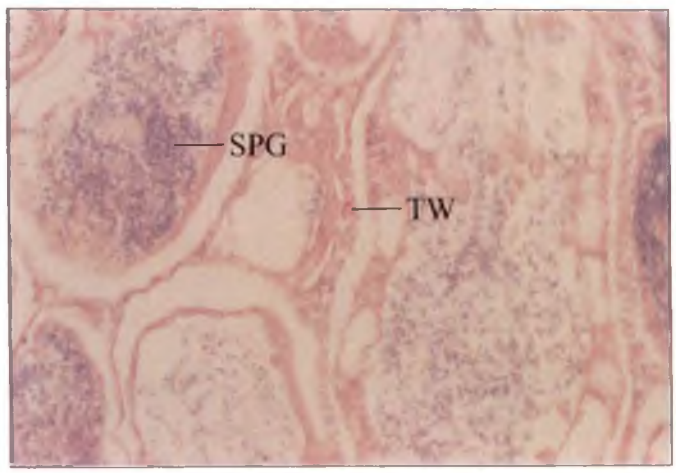
The testes are contained within a connective tissue layer, the tunica albuginea, the only layer in the testis wall, which has a firm consistency and lends strength and shape to the testes. When viewed under the microscope, the testes of the adult fish consist of numerous ill-defined lobules, closely held together by a thin covering of connective tissue formed by the extensions of the tunica albuginea. The lobules vary in their size and form. Each lobule is separated from the other by a thin connective tissue stroma in which germ cells are embedded.

During the growth period the germ cells embedded in the connective tissue matrix becomes active and divide and are transformed into sperm mother cells or spermatogonia. The spermatogonia are spherical cells containing a large, round, clear and centrally placed nucleus with a distinct nucleolus. In these cells, the cytoplasm stains with less intensity forming a hazy cytoplasmic collar around the nucleus.

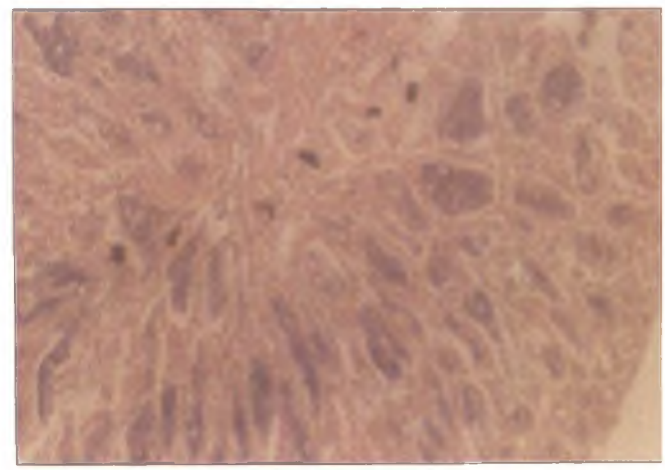
Each spermatogonium divides a number of times and form cysts of cells. Some of these cells grow in size and after spermatogonial divisions within the cyst, give rise to primary spermatocytes. The nuclei show more affinity for haematoxylin while the cytoplasm is only weekly staining.

The primary spermatocytes divide to form large numbers of secondary spermatocytes that are similar to the previous stage. The secondary spermatocyte stage is of short duration, which rapidly matures to form spermatids.

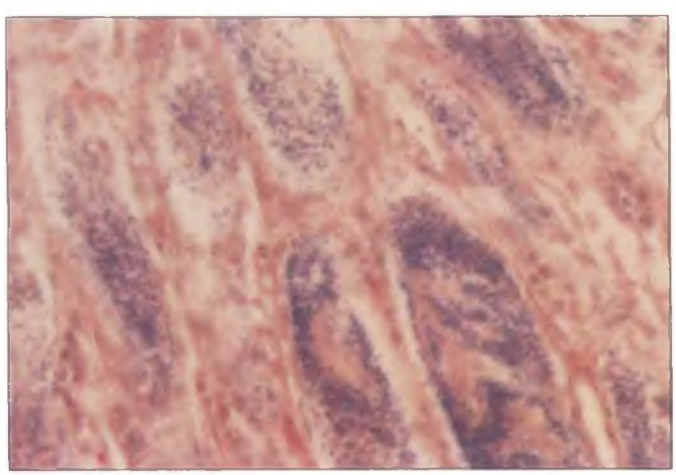
The spermatids have a reduced size and have no cellular outline. They are more deeply staining under haematoxylin. The spermatids give rise to the spermatozoans which are slightly smaller and deeply staining. They appear in sections as clusters in the lumen of the testis.



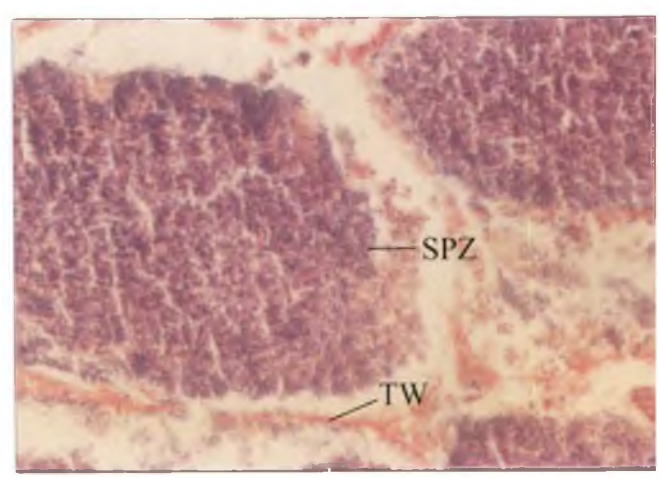
A. Immature testis x 40



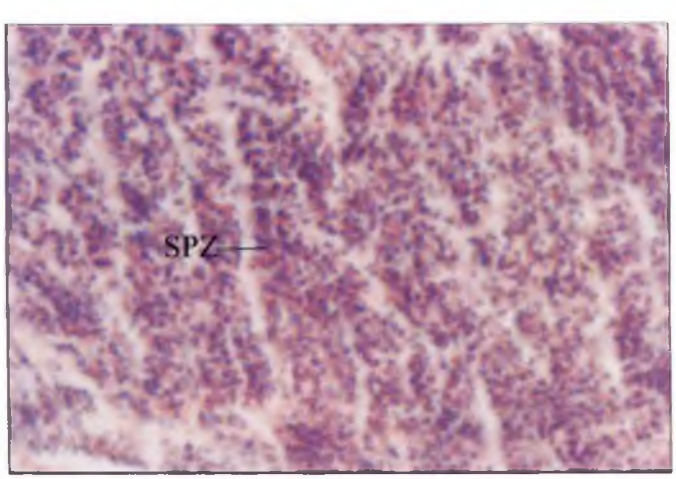
B. Maturing testis x 10



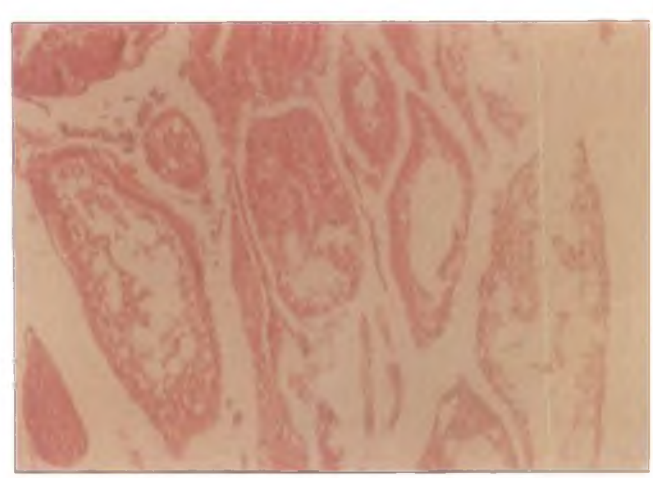
C. Maturing testis x 40



D. Mature testis x 40



E. Mature testis x 100



F. Spent testis x 40

Plate 7. Transverse section of testis in various maturity stages

4.5 LENGTH - WEIGHT RELATIONSHIP

Length weight relationship calculated separately for juveniles, males, females and both sexes combined in captivity are given below and are illustrated in the Figs. 5 (A) to 5 (D).

1. Juvenile: $\text{Log } W = -1.1882 + 2.84 \log SL$

   $R^2 = 0.97$

2. Males: $\text{Log } W = -1.06 + 2.69 \log SL$

   $R^2 = 0.9603$

3. Females: $\text{Log } W = -1.293 + 2.90 \log SL$

   $R^2 = 0.9451$

4. Both sexes combined $\text{Log } W = -1.3 + 2.91 \log SL$

   $R^2 = 0.9688$

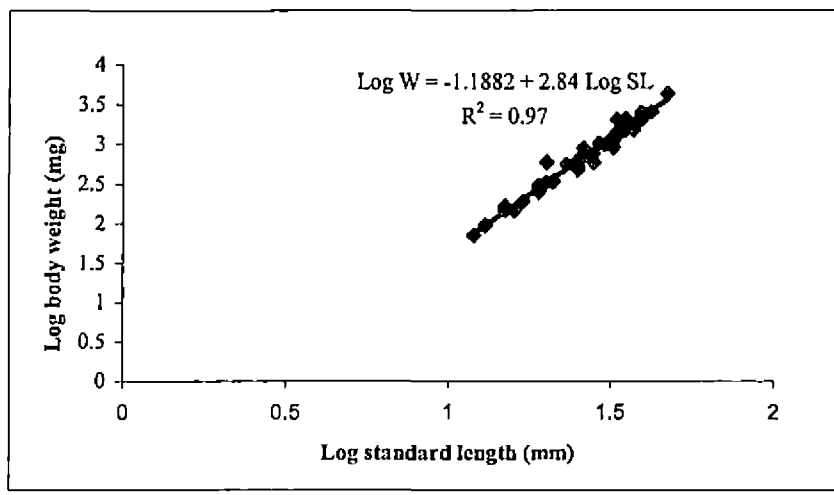


Fig.5 (A). Length-weight relationship in juveniles

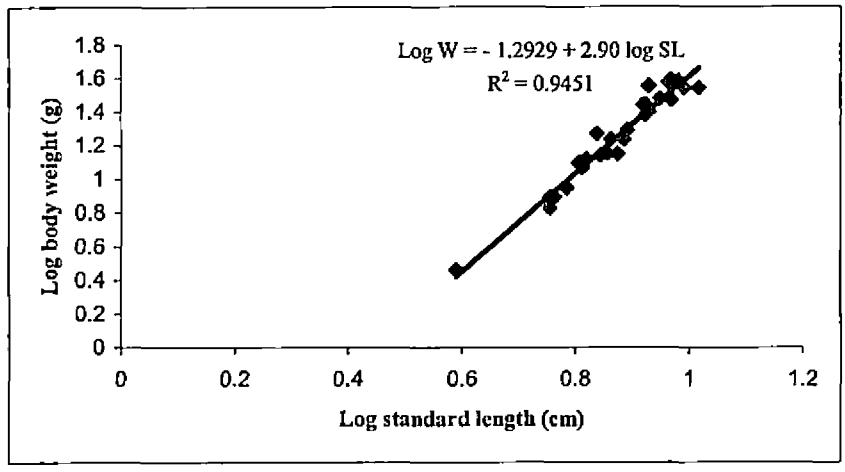


Fig. 5 (B). Length-Weight relationship in females

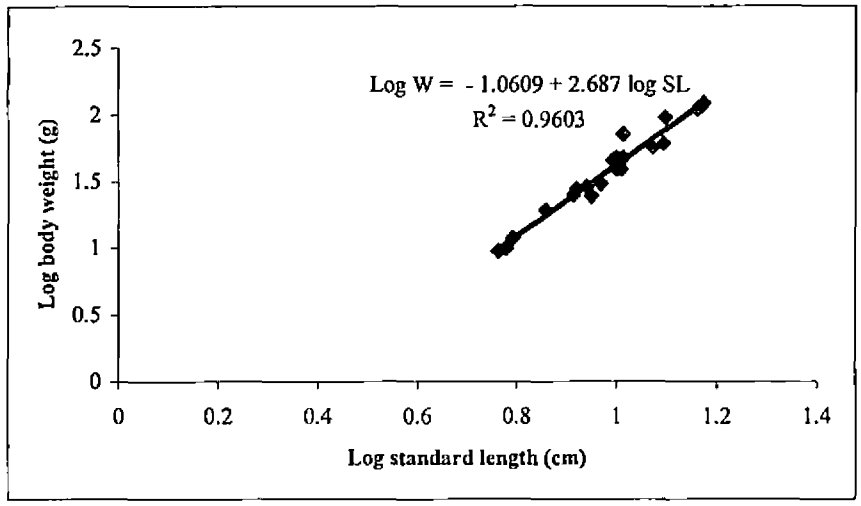


Fig. 5 (C). Length-weight relationship in males

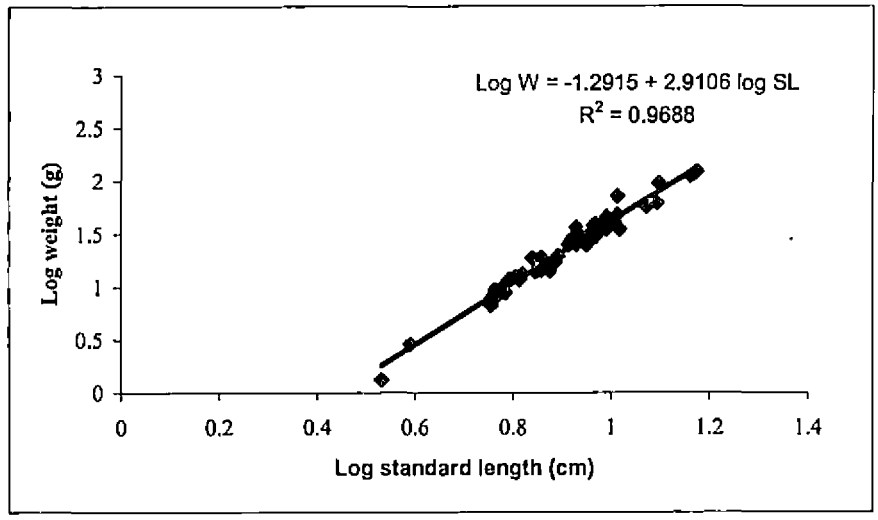


Fig. 5 (D). Length-weight relationship in both sexes combined.

4.6 CONDITION AND RELATIVE CONDITION FACTOR

Juveniles range in size from 1.2 to 4.7 cm (SL). The relative condition factor value ranges from 1.10 to 1.91.The average relative condition factor value is 1.46.

Males range in size from 5.8 to 14.9 cm (SL). The average relative condition factor value is 1.006. The relative condition factor value ranges from 0.81 to1.57. Repeat and recruit spawners were considered based on the size at first maturity. Males below 7.5cm were grouped under recruit and those above were considered as repeat spawners. Recruit spawners showed relatively high average relative condition factor values of about 1.5 whereas repeat spawners showed low average relative condition factor values of about 0.9.

Females range in size from 3.9 to 10.4 cm (SL). Average relative condition factor value for female is 1.01. For recruit spawners, the relative condition average values reaches above 1.06. In repeat spawners theses values range from 0.72 to 1.38. The average value

ranges from 0.9 to 1. Relative condition factor is comparatively higher for the recruit spawners. (Fig. 6.)

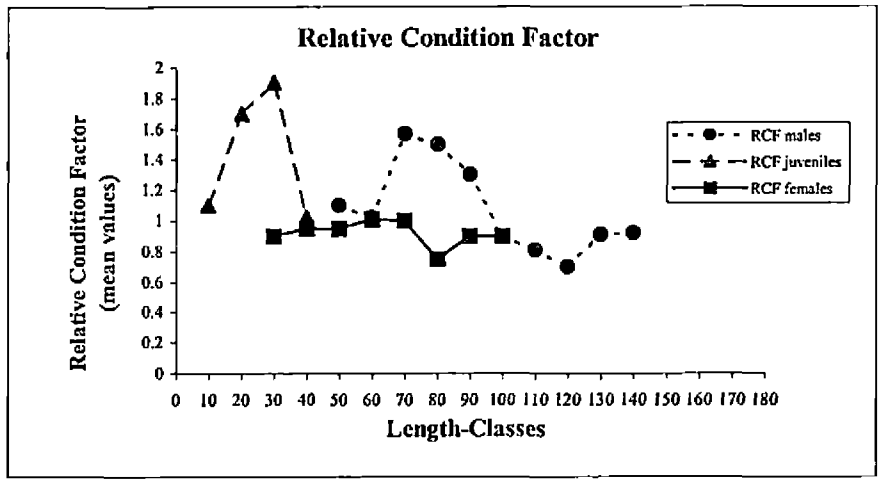


Fig. 6. Mean values of relative condition factor in different length groups of males, females and juveniles.

Discussion

5. DISCUSSION

5.1 BREEDING BIOLOGY

Studies on the size at first maturity, fecundity, spawning season and spawning frequency meant for determination of the breeding cycles with regard to population are essentially meant for elucidating both short and long term variation in the production of the broods which are finally recruited to the population as exploitable stocks. Under captive conditions size at first maturity, spawning frequency and spawning season shows a slightly different picture, as these physiological parameters are highly dependent on the external diet and ambient conditions provided. Hitherto, no study on the biology of the common catopra has been conducted. Hence in the present study an attempt was made to understand the nature of length- weight relationship, condition and breeding biology.

5.1.1 Size at first maturity

In the present study, the size at first maturity of males and females of *P marginata* is found to be around 54mm SL (50 – 60 mm) for females and 72mm SL (70 – 80) for males. The males mature only after reaching a much larger size. The largest female obtained during the present study was 10.4 cm SL and males above this size were very common. The largest male obtained being 15.5 cm (SL). This is a kind of protective adaptation. Captive studies have shown that male of the species showed aggressiveness and strong territoriality. Male of the species are solely engaged in building pebble or gravel nests. Ridley (1991) has classified male caring fishes into two types of mating systems. First, those in which male remains with the eggs (male site-attached with or with out territoriality), and second in which male carries the eggs with him. *Pristolepis marginata* can be included in the first

group in which the males remain with the eggs (site attached) with territoriality (Anna Mercy, 2003). The present study indicated that as the male remained with the eggs until the larvae become free swimming. Territoriality is to improve the probability of an individual offspring's survival to ensure reproductive success. In egg-laying fishes in which the males are territorial during the breeding season, the males are often larger than the females (as in salmon). This is usually the case when the male protects the offspring, so that larger size is a protective adaptation (Nikolsky, 1963). However in most fishes with sexual differences in size, it is the female that is larger or at least achieves a larger size. This has also been suggested by Keenleyside (1991). Size at first maturity in *Etroplus suratensis* (guarder, lithophil) was found to be 100mm (TL) in females and 119mm (TL) in males (Prasadam, 1971; Keshava *et al.*, 1988). Size at first maturity in *Pristolepis malabaricus* Gunther, a non-guarder have been determined to be 77 mm in males and 81mm in females (Sherly, 1993).

5.1.2 Distribution of ova in the ovary

Gross picture of distribution of ova stocks in the anterior, middle and posterior regions of ripe ovary remains almost same except for the slight variations in the percentage composition of the mode. Similar observations were made in *Chanda comersoni* (Nair & Nair, 1983), *Sphyraena jello* (Krishna Das, 1992), *Pristolepis malabaricus* (Sherly, 1993), *Priacanthus hamrur* (Sivakami *et al.*, 2001).

5.1.3 Spawning frequency

Based on the distribution of ova stocks, *P marginata* was found to release eggs more than once during an extended period of spawning. Ova diameter frequency studies show that this species comes under Category C of Karekar and Bal's classification (1960), characterized by

spawning more than once (multiple spawner) during a protracted spawning season. It can be placed under category II of Qasim and Qayyum (1961). The fishes which produce more than one brood annually provided that their cycles are extended over one or at the most two definite periods are included under this category. These fishes show protracted spawning season with individual spawning more than once. *Pristolepis malabaricus*, which spawns in a slightly estuarine habitat, also shows protracted spawning period, which coincides with the monsoon season. It is classified under Category B of Karekar and Bal (Sherly, 1993). On the contrary, *Etroplus suratensis* spawns throughout the year with two peaks, February to May and October to November (Prasadam, 1971; Keshava *et al.*, 1988).

In *P. marginata,* ovary possesses a batch of ripe stock and intermediate ripening stock in between the ripe and immature stocks. Ovary of *P. marginata* can be classified as 'Asynchronous', in which oocytes in all stages of development are present at the same time. The oocyte size frequency distribution is continuous except in ripe ovaries, where there may be a clear separation between the ripe and yolked oocytes. Similar pattern of oocytes size frequency distribution was observed by Wallace and Selman, 1981 in stickle backs.

These results were further strengthened by captivity studies in which fishes were reared for over an year and they were found to show protracted spawning activity, in which fishes, during a single spawning activity extrudes eggs in three or four batches. Interval between the release of ovulated clutches being three to four days. The fish is thus a multiple spawner which spawns in fractions or batches.

5.1.4 Fecundity

In the present study, absolute fecundity of *P marginata* ranged from 1102 to 4965 in fishes whose size ranged from 66 to 104mm (SL).

In repeat spawners, absolute fecundity ranged from 1957 to 4344 (80 to 104mm SL). These values are comparable to the fecundity of *Etroplus suratensis*. Fecundity of *Etroplus* ranged from 600 to 6000 (Bhaskaran, 1946; Prasadam, 1971; Keshava *et al.*, 1988). On the contrary, the fecundity in a non-guarder, *P malabaricus* ranged from 7814 to 19,000 in fishes whose size ranges from 9.7 cm 12 cm standard length. (Sherly, 1993).

Studies on fecundity shows that repeat spawners have larger fecundity than recruit spawners. The number of eggs released increases with age and size ranging from 1102 in first time spawner to 4344 for the oldest fish observed. Fecundity shows strong positive correlation with standard length and weight of the fish. It shows strong correlation with weight of the ovary while least correlation is shown between fecundity and ovary length. Similar relationships were observed in *P. malabaricus* (Sherly, 2005) and *E. suratensis* (Prasadam, 1971; Keshava et al., 1988).

Lower fecundity found in *P marginata* may be explained on the basis of 'K – selection' (MacArthur and Wilson, 1967). K selection is correlated with low fecundity and slow development. K-strategies tend to be multiple spawners who allocate a greater proportion of reserves to the non-reproductive effort such as territoriality, nest building, brooding and care of young, so tend towards greater efficiency.

5.2 REPRODUCTIVE SYSTEM

5.2.1 Female reproductive system

The present study reveals that the reproductive organs of *P. marginata* are built on the usual percoid plan with paired ovaries, short oviduct opening out by the urinogenital opening, the peculiarity being that the right lobe is conspicuously larger than the left lobe. Similar observations were made by Bennington (1936) on *Betta splendens*, James

(1946) on *Lepomis macrochirus*, Aravindan and Padmanabhan (1972) on *Tilapia mossabica*, Sherly (1993) on *Pristolepis malaabaricus*, Sivakami *et al.*, (2001) on *Priacanthus hamrur*, Goswami and Dasgupta (2004) on *Nandus nandus*.

5.2.1.1 Oogenesis

It can be seen from the present study that the ovaries of *P marginata* show asynchronous development. 'Asynchronous ovaries' show sufficient number of oocytes at various stages of development within the ovary. The oocyte size frequency distribution is continuous except in ripe ovaries, where there may be a clear separation between the ripe and yolked oocytes. Such fishes spawn more than once in a year as seen in sardines (Clarke, 1934; Ishida *et al.*, 1959), in *Tilapia mossambica* (Aravindan and Padmanabhan, 1972), and in *Chanda commersonii* (Nair & Nair, 1983).

From the present study it is discernible from the intraovarian development of ova that oocytes of at least 3 or 4 stages of development are present in the same ovary at a time. So the nature of the ovary indicates that the fish can spawn more than once in a year. De Vlaming (1983) considers that most species with asynchronous oocytes development have protracted spawning seasons with multiple spawnings. However, other parameters such as asynchronous breeding habit of the population and spawning frequency of the individual fish contribute to an extended, protracted and almost round the year breeding season of the species.

5.2.2 Male reproductive system

Asymmetry is shown in size and form of the two lobes of the testis of *P. marginata*. Male reproductive organs are pinkish white during the younger stages and creamish white on reaching the ripe stage. Partially spent testis appears dirty white and blood shot. Same

observations were made in *Lepomis macrochirus* (James, 1946), in *Pristolepis malabaricus* (Sherly, 1993), in *Nandus nandus* (Goswami and Dasgupta, 2004).

5.2.2.1 Spermatogenesis

The proliferation of germinal epithelium as seminiferous tubules in P. marginata takes place in the very early stages of testicular development. During this stage the seminiferous tubules are very thin and have weak affinity to dyes making it difficult to notice their identity. During the spermatogenesis there is a gradual decrease in the size of different stages from spermatogonia to spermatozoans, similar to the condition found in *Phoxinus leavis* (Bullough, 1939), *Oncorhynchus nerka* (Wiesel, 1943), *Chanda commersonii* (Nair, 1982), and *Pristolepis malabaricus* (Sherly, 1993). Spermatozoans appear in sections as clusters in the lumen of the testis. Presumably the clustering is maintained by the adhesion of the sperm tails as observed by Stenger (1959).

In *P. marginata*, it was observed that the germinal epithelium of the testis has an uneven thickened portion in the lamellar walls. This portion can be considered as the site of reserve stock of dormant cells suggesting that the origin of new crop of germ cells in *P. marginata* is from already existing dormant cells. Similar observations were made in *Phoxinus laevis* (Bullough, 1939), *Oncorhynchus nerka* (Weisel, 1943), *Mystus seenghala* (Sathyanesan, 1959), *Chanda commersonii* (Nair & Nair, 1983) and *Pristolepis malabaricus* (Sherly, 1993).

5 .3 LENGTH-WEIGHT RELATIONSHIP

The length-weight relationship of this potential ornamental fish is being estimated to ascertain the pattern of growth, general well-being

and gonadal development. Length weight relationship values show that juveniles are showing allometric growth with 'b' value equal to 2.83. This allometry is attributed to the increased growth during this stage in the life history. Males are showing allometric growth with a 'b' value equal to 2.68.Males are found to grow to a larger size than the females of the same age but the increase in volume or weight of the fish meat is not in pace with the increase in length resulting in allometry. This is because even when the fish is having a broad body, the girth is not proportional. Female of the species is showing isometric growth with 'b' value equal to 2.94. This may be because the females are having greater girth than males. The b values differ significantly in males (b = 2.74) and females (b = 2.62) as has been observed by Sivakami *et al.,* (2001) in *Priacanthus hamrur* and Goswami and Dasgupta (2004) in *Nandus nandus.*

5.4 CONDITION AND RELATIVE CONDITION FACTOR

Average relative condition values are higher in juveniles (1.43) due to the higher growth rate encountered during this stage in life cycle. Males are showing higher relative condition factor value compared to females. This can be attributed to the larger size attained by the males. This is also seen in *Etroplus suratensis* (Bhaskaran, 1946; Keshava *et al.,* 1988).

Males and females are classified on the basis of their length at first maturity into recruit and repeat spawners. In both males and females, recruit spawners exhibited better condition than repeat spawners. This may be because recruit spawners have large quantity of fat mass surrounding their gonads, which get exhausted during the course of reproduction (with age). Qasim (1957) suggested that the waxing and waning of the condition factor could probably be due to the building up or lose of reserve of the fish. Similar observations were also

noticed by Bennington (1936) on *Betta splendons*, Nair (1982) on *Chanda commersonii*, Sherly (1993) on *Pristolepis malabaricus*.

Summary

6. SUMMARY

The present study was made to understand the maturation and reproduction of *Pristolepis marginata* under captive conditions. The methodology, results and conclusion are as follows:

1. Captively reared fishes from the NBFGR – NATP programme (Germplasm inventory evaluation and Gene banking of fresh water fishes of India) were utilized for the present study. These fishes were reared under captive conditions providing ambient conditions and *ad libitum* feed.
2. A total of 209 fishes (12 to 149 mm) were used for the study of reproductive biology.
3. For studying the external morphology of gonads in males and females, both fresh and preserved specimens were used.
4. Spermatogenesis and oogenesis were studied using the standard histological procedures. Delafield's haematoxylin and eosin were used for the histological differentiation.
5. Spawning biology was studied using the standard procedures. A six-stage key was used for the classification of the maturity stages.
6. The size at first maturity for females and males were found to be 54 mm and 72 mm SL respectively.
7. Gross picture of distribution of ova stocks in the anterior, middle and posterior regions of the ovary remained almost the same except for the slight variation in the percentage composition of the mode.
8. Based on the ova diameter frequency studies, *P. marginata* was found to be a multiple spawner, which releases the eggs in two or three batches/ fractions.

9. Absolute fecundity of the fishes ranged from 1102 (first time spawner) to 4965 (repeat spawner) in fishes of size range 66 to 80 mm SL. Number and size of eggs were found to be directly proportional to the size and age of the fish. Fecundity showed a positive linear relationship with the length and weight of the fish as well as to length and weight of the ovary.
10. The reproductive organs of *P. marginata* were built on the usual percoid plan with paired ovaries and testis.
11. The ovaries of *P. marginata* showed asynchronous development, in which oocytes at all the stages of development were present in the same ovary at the same time.
12. During the spermatogenesis, there is a gradual decrease in size of different stages from spermatogonia to spermatozoans.
13. Length – weight relationship values showed that the juveniles are exhibiting allometric growth pattern with ' b' value equal to 2.83. Males also showed allometric growth pattern with a 'b' value equal to 2.68. Females showed isometric growth pattern with 'b' value equal to 2.94.
14. Average relative condition factor values were higher in juvenile (1.43) due to the higher growth rate encountered during this stage in life cycle. Males showed higher average relative condition value than females.

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STUDIES ON THE MATURATION AND REPRODUCTION OF *PRISTOLEPIS MARGINATA* JERDON UNDER CAPTIVE CONDITIONS

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ABSTRACT OF THE THESIS

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ABSTRACT

Pristolepis marginata Jerdon commonly called "Malabar catopra" or "Malabar sunfish" or "Chutichi" in the vernacular is an attractive ornamental fish belonging to the family Nandidae. This species, which is endemic to the Kerala part of Western Ghats inhabits clear and rapid streams. The species has been enlisted as vulnerable by – the Conservation Assessment and Mangement Plan Workshop (CAMP, 1998) for fresh water fishes of India held at NBFGR, Lucknow. Conservation of the endangered/vulnerable fishes cannot become successful without protection to the resources, which support the diversity and abundance. Hence *Pristolepis marginata* was one of the prioritized species for the development of captive breeding technology under NBFGR – NATP programme entitled "Germplasm inventory evaluation and gene banking of fresh water fishes of India". Anna Mercy *et al.,* (2003) developed the captive breeding technology for *Pristolepis marginata.* In the present study, an attempt was made to understand the maturation and reproduction of *P. marginata* in captivity. Captively reared fishes from the NATP project of College of Fisheries were utilized for the present study. A total of 209 fishes (12 to 149 mm) were used for the study of reproductive biology.

A six-stage key was used for the classification of the maturity stages. The size at first maturity for females and females were found to be 54 mm and 72 mm SL respectively. Gross picture of distribution of ova stocks in the anterior, middle and posterior regions of the ovary remained almost the same except for the slight variation in the percentage composition of the mode. Based on the ova diameter frequency studies, *P. marginata* was found to be a multiple spawner, which release eggs in two or three batches. Absolute fecundity of the

fishes ranged from 1102 (first time spawner) to 4965 (repeat spawner) in fishes of size range 66 to 80 mm SL. Number and size of eggs was found to be directly proportional to the size and age of the fish. Fecundity showed a positive linear relationship with the length and weight of the fish as well as to length and weight of the ovary.

The reproductive organs of *P. marginata* were built on the usual percoid plan with paired ovaries and testis. The ovaries of *P. marginata* showed asynchronous development, in which oocytes at all the stages of development were present in the same ovary at the same time. During the spermatogenesis, there is a gradual decrease in size of different stages from spermatogonia to spermatozoans.

Length – weight relationship values showed that the juveniles are exhibiting allometric growth pattern with ' b' value equal to 2.83. Males also showed allometric growth pattern with 'b' value equal to 2.68. Females have shown isometric growth pattern with 'b' value equal to 2.94. Average relative condition factor values were higher in juvenile (1.43) due to the higher growth rate encountered during this stage of the life cycle. Males showed higher average relative condition value than females.