OVARIAN MATURATION, BREEDING AND EARLY EMBRYONIC DEVELOPMENT OF AN INDIGENOUS ORNAMENTAL CYPRINID OF THE WESTERN GHATS - *CHELA FASCIATA* SILAS

By

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THESIS

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PANANGAD, COCHIN.

DEDICATED TO MY FAMILY

DECLARATION

I hereby declare that this thesis entitled – "**OVARIAN MATURATION, BREEDING AND EARLY EMBRYONIC DEVELOPMENT OF AN INDIGENOUS ORNAMENTAL CYPRINID OF THE WESTERN GHATS - *CHELA FASCIATA* SILAS**" is a bonafide record of research work done by me during the course of research and that the thesis has not formed the basis of award to me for any other degree, diploma, association, or other similar title of any other University or society.

Panangad,
12.3.2008

INDIRA DIVIPALA

CERTIFICATE

Certified that this thesis entitled – "**OVARIAN MATURATION, BREEDING AND EARLY EMBRYONIC DEVELOPMENT OF AN INDIGENOUS ORNAMENTAL CYPRINID OF THE WESTERN GHATS - *CHELA FASCIATA* SILAS**" is a record of research work done independently by **Miss INDIRA DIVIPALA** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or association to her.

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INTRODUCTION

1. INTRODUCTION

1.1 ICTHYOBIODIVERSITY IN INDIA

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

The vastness of Indian ichthyobiodiversity can be seen from the range of habitats they occupy. The fish diversity in the major river systems of India has been divided into four types based on the habitat preference. There are about 154 coldwater species, 433 warmwater species, 171 brackishwater species and about 1360 marine species. Of these 34 species

are commonly found both in cold and warm waters. About 67 species are commonly seen in warm water and brackish water, 73 species are common in warm water, brackish water and marine water, 16 species found only in brackish water and 82 species commonly seen in cold water, brackish water and marine waters. A total of 2118 species inhabit the waters of India (NBFGR, 2007).

An understanding of the biology and reproductive strategies of these fishes is essential for the preservation of the stock and its habitat, carrying out ranching programme in the case of endangered species, it is also essential for conducting other aspects of conservation and management of fish germplasm like declaration of parts of the rivers as aquatic sanctuaries, protection and preservation of endangered species and mitigation of anthropogenic activities (Ranjeet and Kurup, 2002).

1.2 BIODIVERSITY THREATS TO THE FRESHWATER FISHES

Fishes exhibit diversity in their morphology, habitat and biology. The habitat always has a direct impact on the diversity in morphology and physiology of the fish inhabiting therein. Habitat destruction for the betterment of human life leads to considerable reduction of many valuable species of fishes (Jameela Beevi and Ramachandran, 2002). Unsustainable and unethical fishing by using fish poisons, dynamiting and a wide array of prohibited fishing methods are rampant in the uplands and lowlands of most rivers. Habitat destruction of natural spawning and breeding grounds of the

fishes through sand extraction and construction of physical obstructions across rivers has contributed to the population decline and the endangerment of the freshwater fishes. Many of the species reported as endangered are now found only in areas protected under forest and wildlife jurisdiction, which clearly indicates the reasons for their endangerment (Ranjeet and Kurup, 2002).

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

1.2.1 Causes for declining freshwater fish biodiversity

1.2.1.1 Use of small meshed fishing gears:

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

1.2.1.2 Wanton destruction:

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

1.2.1.3 Destruction and modification of habitats:

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

1.2.1.4 Introduction of exotic species:

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

1.2.1.5. Over fishing:

Over fishing of potential ornamental species without assessing their population size could lead to their extinction in the near future. Unfortunately, with the targeting of half a dozen fishes for the domestic

and international trade, the stock size of these fishes has declined drastically and, as a result, most of them are now endangered. The decline in population of the fishes such as *Tor* species and *Schizothorax* species in upland waters (Nautiyal, 1994 and Mahanta *et al.*, 1998); *Notopterus chitala, Ompok pabda, Pangassius pangassius* etc in warm waters (Menon, 1989) and *Mugil cepalus, Liza tade, Nematalosa nasus* etc in brackish waters (Pandit and Mandal, 1994) are due to overexploitation of their stocks.

1.2.1.6. Aquatic pollution:

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

***1.2.1.7. Genetic problems in threatened species*:**

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

1.3 NEED FOR ICHTHYOBIODIVERSITY CONSERVATION

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

1.4 MANAGEMENT MEASURES RELEVANT FOR CONSERVATION OF THE FRESHWATER FISHES

Management measures aimed at conserving freshwater fish biodiversity should be inserted into the fishery policies. In addition, the

information given can be utilized by central and state government agencies and all those who are deeply involved in implementing various measures for the protection of the fish biodiversity of these ghats.

1.4.1 Strategies for conservation of ichthyobiodiversity

1.4.1.1 In-situ conservation

In-situ conservation of fish is useful where genetic diversity exists and where wild forms are present. This is done through their maintenance within natural or man-made ecosystems in which they occur. The major advantages of in-situ conservation are: (i) continued co-evolution wherein the wild species may continue to co-evolve with other forms providing the breeders with a dynamic source of resistance that is lost in ex-situ conservation, and (ii) national parks and biosphere reserves may provide less expensive protection for the wild relatives than ex-situ measures (Narain, 2000).

1.4.1.2. Ex-situ conservation

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

1.4.1.3. Cryopreservation

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

1.4.1.4. Habitat Restoration

Most of the damage done to the various fish habitats so far is irreversible, at least in short term, where the fish species or communities are severely threatened (Wang and Xie, 1997). In many cases unique stocks have completely disappeared. Even where habitat restoration is contemplated stock transfer could be an important interim measure. 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 (Surtida, 1998).

1.4.1.5. Captive Breeding

In the world conservation strategy, captive propagation is considered to be an integral part of the global strategy to conserve genetic diversity and is recommended wherever 'on site' conservation becomes untenable. Despite these recommendations and the strong advocation of captive

propagation as conservation option by the conservationists (Franklin, 1980), captive propagation is viewed doubtfully by numerous biologists. 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). Nowadays the science of captive propagation has a firm and respectable foundation. It is growing rapidly and benefiting from numerous successes.

1.4.1.6. National Conservational Strategies

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

In a huge country like India with diverse ecosystem where, enforcement of law is not an easy task, the most effective way of tackling the problem would be to arouse mass consciousness on the subject so that people themselves could protect the fish genetic resources (Das, 1989). Zoo Outreach Organization conducted a workshop on Conservation, Assessment and Management Plan (CAMP) for freshwater fishes of India in 1998, hosted by the National Bureau of Fish Genetic Resources, ICAR. The recommendations of this workshop became the base line data for a large number of research projects on the conservation of the fresh water fishes.

1.5. OBJECTIVES OF STUDY

Ornamental fishes are the most popular pets of the world and aquarium keeping is the second largest hobby next to photography. Tropical fishes have always attracted the attention of ornamental fish hobbyists. India being a tropical country has a vast potential of indigenous ornamental fishes. The Western Ghats of India is in fact a gold mine of ornamental fishes (Anna Mercy, 2004 a).

Chela fasciata Silas commonly called "Chela" or "Malabar hatchet chela" is an elegant ornamental fish of the family Cyprinidae. It is endemic to the Western Ghats of Kerala and inhabits the riffels of the River Bharathapuzha at Thootha. *Chela fasciata* has all the desirable qualities of being an ornamental fish. Being small it is not considered as a food fish.

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

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

(ii) To determine sexual dimorphism.

(iii) To estimate fecundity.

(iv) To determine the spawning frequency.

(v) To observe the cyclical changes of the oogonial maturation and their histological differentiation.

(vi) To study the early embryonic development.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

2.1 LIFE HISTORY TRAITS

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

2.2 REPRODUCTIVE STRATEGY IN FISHES

A complete knowledge of the reproductive system and the reproductive biology of fishes are essential to understand the reproductive strategy of any given species. Quite some work had been done on the fish reproductive system namely the gonads and their development. The notable of these early works are those of Yamamoto (1956 b), Yamamoto and Yamazaki (1961). Some of the later works are those on *Barbus tor* (Ham.) (Rath, 1965), on *Etroplus suratensis* (Ritakumari and Padmanabhan, 1976), on *Rhomboplites aurorubens* (Grimes and Huntsman, 1980), on *Ambassis*

commersoni (Nair, 1982). Recent works on the same aspects involve those of Dasgupta (2002), Kurian and Inasu (2002) and Goswami and Dasgupta (2004).

The literature on the reproductive organs and reproduction was reviewed by Raven (1961), Hoar (1969), Marshall (1979), Nagahama (1983), West (1990) and Jalabert (2005).

2.3 OOGENESIS IN FISHES

The most important step in the reproductive biology is gametogenesis. The formation, development and growth of the ova are oogenesis. Despite a great diversity in their reproductive strategies, the ovaries of numerous teleost species show a similar general structure. In most cases they are the elongated organs oriented longitudinally within the abdominal cavity. Ovaries of some species such as trout and salmon are not completely surrounded by the mesovarium and the ovigerous lamellae are open in the body cavity where mature oocytes are directly released at ovulation, and where they can remain for some time before being laid (Jalabert, 2005).

The ovaries are compartmentalized by numerous septa formed by folds of the germinal epithelium, usually called the ovigerous lamellae, projecting into the lumen. These lamellae are the site of nest of oogonia and oocytes at early stages of entry into the meiotic prophase. The posterior part of each ovary is prolonged by an oviduct connected to the genital papilla. At

ovulation, mature oocytes are released from their follicle into the ovarian cavity, before being laid outside through the oviduct and the genital papilla . Immature oocytes bud off from the ovigerous lamellae. This immature oocyte is characterized by a large nucleus, containing one large nucleolus. At the end of this stage; the nucleoli are mostly arranged towards the periphery of nuclear membrane. This is followed by vacuolization of cytoplasm, yolk deposition (vitellogenesis) migratory nucleus and mature ova. These mature oocytes with micropile are ready for ovulation (Jalabert, 2005)

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

Oogenesis developmental stages have been extensively described in numerous species with some differences depending on the species and the classification criteria. Some of the notable works are those of Yamamoto (1956 b) on *Liopsetta obscura*; Yamamoto and Yamazaki (1961) on *Carassius auratus*; Selman *et al.* (1993) on *Brachydanio rerio* ; Glasser *et al.*

(2003) on *Ctenopharyngodon idella*; Gui *et al.*(2003) on *Carassius auratus gibelio* ; Cek *et al.*(2001) on *Puntius conchonius* and Selman and Wallace (2005) on *Fundulus heteroclitus*. Some of the recent reviews are those of Wallace and Selman (1981); Guraya (1986); Patino and Sullivan (2002) and Jalabert (2005).

2.4 SIZE AT FIRST MATURITY

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

Besides the physiological and environmental factors, population density and quantity and quality of food available also have a significant role to play in determining the size at first maturity of the given species.

In case of expanding populations the females tend to mature at an earlier age. Their maturing earlier owes to the fact that expanding population usually is found in favorable environment and so attained larger size at a younger age. On the other hand where the environment is favorable for growth and adult survival, fishes tend to delay reproduction. The advantage in such cases is the increase in fecundity, which is proportionate to the somatic growth of the fish. The size at first reproduction has an important role in understanding the life history of a species during its evolution and gives a rough estimation of the ultimate (L_α) size of the species.

2.5 SPAWNING FRQUENCY IN FISHES

The success of any species lies in its ability to reproduce in times most conducive to the higher rate of survival of their off spring. Also the frequency of spawning depends on the environment and the reproductive strategies evolved by the given species over the time. The two basic strategies observed in nature are semelparity and iteroparity. Semelparity otherwise known as the "big bang" reproduction involves species which spawn only once in their lifetime and then die. The Pacific salmons are a very good example of this type of reproduction where all the adults take up reproductive migration and die by the end of spawning. On the other hand we have the iteroparity in which the species spawn repeatedly in a season or

in their life. This is so in case of most, of the fishes being even more in the tropical ones.

The frequency of reproduction forms the index of the predictability of the environment and can be elucidated by the ova diameter studies. It has been demonstrated that by studying the intraovarian egg dimensions of fishes in the ripe condition or penultimate stage of maturity, it is possible to elucidate the duration of spawning periods and individual spawning frequency (Clark, 1934; Hickling and Rutenberg, 1936; De Jong, 1939; June, 1953; Prabhu, 1956; Qasim and Qayyum, 1961; Grimes and Huntsman, 1980).

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

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

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

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

'Isochronal' or 'total' spawners is the other name given to the group synchronous spawners while it is 'partial', 'heterochronal', 'multiple', or 'serial' spawners for the asynchronous spawners. According to Holden and Raitt (1974) the oocytes will be shed within a short period – a week or so in the group synchronous spawners and only a compliment of the yolked oocytes is spawned in case of the asynchronous spawners. De Vlaming (1983) suggested that the 'multiple spawning' generally refers to more than one spawning in a season, and 'fractional spawning' is used for species that spawn part of an ovulated clutch.

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

(i) Category A: Spawning takes place once in a season during a short duration, the individual spawning once. Ovary contains

a ripe stock distinctly and clearly separated from immature stock.

(ii) Category B: Spawning takes place once in a season but with longer duration. Range in size of the ripe ova nearly one half of the total intraovarian eggs, the individual spawning once.

(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.

(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.

Histological studies provide very precise information on oocyte developmental stages, 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 (Fairbridge, 1951; June, 1953; Yamamoto, 1956 a; Crossland, 1977 and Forberg, 1982).

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

2.6 FECUNDITY IN FISHES

2.6.1 Absolute fecundity

Though it is an easy procedure for estimation of the reproductive potential of any given fish species, it does not give an accurate picture of the egg release as the fishes tend to be multiple spawners or even batch spawners. Hence a precise estimation of fecundity is almost impossible. Absolute fecundity is defined as the number of ripe eggs found in the female prior to spawning (Bagenal and Braun, 1968). On the other hand is fertility, which may be defined as the actual number of young produced rather then the number of eggs. Both the endogenous and exogenous factors have a profound effect on the fecundity. The variation in fecundity is dependent on season, climatic condition, environmental habitat, nutritional status and genetic potential (Bromage *et al.*, 1992). If suitable conditions are not found then the eggs might become atretic, degenerate and ultimately get resorbed into the body. Grimes and Huntsman (1980) used gravimetric sub sampling to estimate fecundity in vermillion snapper, *Rhomboplites aurorubens*. The same was followed in *Pricanthus hamrur* (Sivakami *et al.*, 2001), *Mystus gulio* (Dasgupta, 2002) and *Horabagrus brachysoma* (Kurian and Inasu, 2002).

Estimation of the absolute fecundity of multiple spawners is difficult. Usually all the yolked (ripe/ripening) eggs are counted based on the classic work of Hickling and Rutenberg (1936) for multiple spawners. The estimation of fecundity in open substrate spawners is also difficult owing to

the extremely high fecundities and protracted spawning periods. On the other hand fecundity estimation of the brood hiders and nest spawners and all the fishes showing parental care is easier due to low fecundity. Here eggs are larger with high survival rate. In case of mouth brooders the fecundity depends more on the brooding capacity of the parent than on the fertility. The fecundity is the least in live bearers where the young born are stronger than those that hatch out of an egg. The egg size, yolk content and incubation time are inversely related to fecundity.

2.6.2 Relative fecundity

Relative fecundity is the expression of absolute fecundity in terms of numbers per unit length and weight of the body or ovary of the given fish. The relationships are linear and are expressed as, $F = a X^b$ where X = length, weight or age and 'a' and 'b' are constants (Bagenal and Braum, 1968).

2.7 SEXUAL DIMORPHISM

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

The relative growth of body parts of a fish may vary with sex at different stages of its life history and this was exemplified by earlier workers (Godsil, 1948 and Pitcher, 1999). They stated that there were different ratios between different morphometric characters and total length of males and females in the fish species studied by them. Sexual dimorphism in the Western Ghats indigenous ornamental fishes like *Puntius amphibius, P. fasciatus, P. pookodensis, P. melanostigma, P. sarana, P. filamentosus, Chela dadiborjori* and *Danio malabaricus* was recorded (Anna Mercy *et al.*, 2007).

2.8 EMBRYONIC DEVELOPMENT IN FISHES.

The embryonic development commences with the fertilization of egg and ends with the exogenous feeding by the larvae. The need for knowledge of the embryonic development of fishes has fast gained momentum with the need for captive breeding of endangered fishes and their conservation. Special efforts have been made to breed some of the indigenous ornamental fishes of the Western Ghats in captivity (Anna Mercy, 2004 a).

A large number of freshwater ornamental fishes belonging to the family Cyprinidae are known to the world. This family includes the ornamental barbs, which are abundant in almost all freshwater ecosystems. *Brachydanio, Danio, Puntius, Barilius, Esomus, Rasbora, Garra, Chela, Labeo, Gonoproktopterus,* and *Amblypharyngodon* are some of the well-known ornamental genera from the Western Ghats. The freshwater habitats

of Western Ghats are rich in indigenous ornamental cyprinids and a few of these species show high degree of endemism (Anna Mercy, 2004 b). Besides these, there are many ornamental catfishes, which are equally popular. India is considered as the land of barbs, because several species of colorful barbs are available in the natural waters of India (Swain *et al.*, 2008).

The development of captive breeding of these barbs along with an understanding of their early embryonic development would greatly help in the reduction of damages caused to the fish populations in the wild. In the College of Fisheries, Kochi, the captive breeding technology for *Danio malabaricus*, *Puntius filamentosus, P. melanostigma, P. fasciatus, P. pookodensis, P. sarana, Chela fasciata, Garra mullya, Nemacheilus semiarmatus, Nemacheilus triangularis and Pristolepis marginata* have been successfully developed (Anna Mercy, 2004 c).

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 SYSTEMATICS

Specimens of *Chela fasciata* bred under captive condition at the hatchery, college of fisheries were used for the present study. They are the F_1 and F_2 generation of the fishes originally collected from River Bharathapuzha at Thootha and the distinguishing characters based on external morphology and colour were studied (Talwar and Jhingran, 1991).

3.2 BREEDING BIOLOGY

A total number of 144 specimens (94 females and 50 males), collected from Bharathapuha during the period of 2006 September to 2007 November, were used for the study of different aspects of breeding biology. The females ranged from 37.0 mm to 82.0 mm and the males ranged from 25.0 mm to 56.0 mm total length. The different aspects of breeding biology namely size at first maturity, spawning frequency based on ova-diameter studies and fecundity were studied using the methods followed by Nair and Nair (1984).

3.2.1 Quantification of maturity stages

The maturity can be defined as the cyclic morphological and histological changes undergone by the gonads to grow and ripen. For study of spawning biology quantification of maturity stages is a prerequisite.

Quantification was done based on external evaluation of the gonads. Macroscopic quantification characters used were colour and shape of gonad, space that it occupied in the body cavity, size of ova and texture of ovary and blood supply.

3.2 .2.Maturation of ovary and oogenesis

3.2.2.1 Oocyte distribution

The study was based on the progression of the oocyte stocks in the different maturity stages. Ova diameter measurements were made from the different maturity stages using an ocular micrometer. During the initial quantification of maturity stages itself a few ovaries belonging to all the stages of maturity were preserved in 5 % formalin for ova diameter measurements. The ova diameter measurements were done on a monocular microscope with 10x10 magnifications, after calibrating the ocular micrometer using a stage micrometer. A mixed sub-sample was taken from different parts of the ovary to eliminate the error due to differential distribution of ova stocks in the different parts of the ovary.

3.2.2.2 Histology

For histological studies fresh material was used. Small pieces of the ovary at different stages of maturity were fixed in alcoholic Bouins fluid. Paraffin sections at 6 – 8 microns thickness was stained with Harry's

haematoxylin and counter stained with eosin (Weesner, 1960). The sequence of histological changes during the origin, maturation and liberation of the germ cells were studied by sectioning the gonads in the different stages of maturity.

3.2.3. Size at first maturity

Size at first maturity was computed with a total of 144 fishes of which 94 were for the females (ranging from 37mm to 82mm) and 50 fishes for the males (ranging from 25mm to 59mm). The length at first maturity is the size at which 50 % of the population is mature.

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

3.2.4. Spawning frequency

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

percentage frequencies of different ova diameter classes (32 μm) interval were plotted to study the spawning frequency

3.2.5. Fecundity

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

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

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

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

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

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

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

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

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

3.2.6 Sexual dimorphism

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

3.2.7 Early embryonic development

Captive breeding technology was developed and standardized for *Chela fasciata* by Anna Mercy (2004 a). Fishes, which were bred in captivity, were reared in rectangular FRP tubs of two-meter length and 1 meter depth and width. 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 6.5 to 7.0. Fishes were maintained under ambient temperature conditions 26 to 29°C.

All the fishes were fed with a uniform diet; newly hatched larvae were fed with cladocera (moina), rotifera, and egg yolk. The early juveniles were given mosquito larvae till they reached a length of 2.0 cm. *Chela fasciata* 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 of the College of Fisheries. Feed was given *ad libitum* twice a day. The adults were also fed on mosquito larvae, egg yolk and moina as the maturation feed.

The time and duration of the early embryonic development of these fishes were observed by collecting the fertilized adhesive eggs clinging to the roots of floating aquatic plants like the Salvinia. The early embryonic developmental phases - the cleavage egg, inside egg embryo and free embryo were observed and photographed using a Nikon camera mounted on to the LABOMED binocular microscope.

RESULTS

4. RESULTS

4.1 SYSTEMATICS

4.1.1 Systematic position

The latest systematic position of *Chela fasciata* Silas, 1951 based on Nelsons (2006) classification is as follows:

Super order	:	Ostariophysi
Order	:	Cypriniformes
Suborder	:	Cyprinoidei
Family	:	Cyprinidae
Subfamily	:	Cultrinae
Genus	:	*Chela*
Species	:	*fasciata*
English name:		Malabar Hatchet Chela
Malayalam:		Chela

4.1.2 Description of the species

The photograph of the fish is given in Plate 1.

4.1.2.1 Distinguishing Characters

D ii 7; A iii 14-15; P i 8-9; V i 5-6

Body greatly compressed its depth 3.8 to 4.3 times in standard length. Head slightly turned upwards. Mouth small, obliquely directed upwards, its cleft not extending to below front edge of eye. Pectoral fin long extended much beyond origin of anal fin; outer ray of pelvic greatly elongated, extends beyond origin of anal fin. Lateral line complete, with 33 or 34 scales; lateral transverse scale rows $^{6\text{-}1}/_{11/2}$; predorsal scales 18.

4.1.2.2 Colour

In life, upper body is grayish. A dark violet broad lateral stripe commences just behind the eye and runs along the middle of the body up to the caudal base. Colour of the fins is dirty white.

4.1.2.3 Geographical Distribution (as per Talwar and Jhingran, 1991)

India: Western Ghats of Kerala. They inhabit the riffles of River Bharathapuzha at Thootha zone.

Plate 1: *Chela fasciata* Silas

4.2 BREEDING BIOLOGY

4.2.1. Quantification of the maturity stages in *Chela fasciata* (Female)

(i) Stage – I: Immature virgin

These are the young individuals that have not yet spawned. Differentiation of the gonads had just taken place. Ovaries in this stage were very small, triangular and translucent; pinkish occupying less than one-fourth of the body cavity. Ova were not visible to naked eye. A cursory examination under the microscope was required to differentiate ovary from the testis. Microscopically, the oocytes were oval/spherical in shape and completely transparent with a large nucleus. Under microscope the testis appeared as undifferentiated tissue. Maximum size of the ova was 136μ.

(ii) Stage – II: Maturing virgin

In maturing virgins ovaries were usually pinkish, translucent occupying about one – third of the body cavity. The length of both the lobes was equal. Ova were still microscopic oval/spherical in shape with large nucleus and with slight yolk deposition up to yolk vesicle stage. Ova size had gone up to 360μ. The testis occupied less than $^1/_4$ of the body cavity. It was thin and transparent.

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

Ovary usually off white/creamy in colour occupying half to less than three fourth of the body cavity (process of vitellogensis). Ovary was usually turgid with a transparent ovarian wall and ova quite visible to the naked eye. It increased in weight rapidly. The maximum size of ova observed in this stage was about 648µ. This stage may also be called as spent recovering stage for fishes that have at least spawned once. In this stage the testis was transparent occupying about ¼ of the body cavity and they are thin and transparent.

(iv) Stage – IV: Late Ripening

Ovary is pale yellow in colour, occupying three – fourth or more of the body cavity. The ovarian wall was thin, the ova being visible through the extremely thin ovarian wall. The ovary was turgid. The maximum size of ova seen in ovaries of this stage was about 808µ in diameter. The testis occupied less than ½ of the body cavity.

(v) Stage V: Ripe (Reproduction)

Ovary is yellow in colour. Ovarian wall was extremely thin and ova quite distinct. Occupied more than three–fourths or whole of the body cavity. Ovary was very turgid. Ovarian lobes were quite stout and equal in length. The maximum size of ova seen in this stage was around 872µ. The

testis occupied about more than a half of the body cavity and the lobes were translucent.

(vi) Stage VI: Partially Spent

Ovary at this stage was slightly flaccid, pale yellowish in hue not as stout as the ripe ovaries but still retaining a number of residual ripe ova/atretic ova after the spawning. The largest ova that could be found in this stage was 648μ. This stage enters the maturation cycle at early ripening stage. Here the testis looked flabby and occupied about ¼ of the body cavity.

4.2.2 Maturation of ovary and oogenesis in *Chela fasciata*

4.2.2.1 Oocyte distribution

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

i. Immature virgin: (Fig: 1)

All the transparent ova in this stage have been found to be below the size of 136 μ. The mode of the immature stock is at the ova diameter class group of 40 - 72μ.

ii. Maturing virgin: (Fig: 2)

The ova diameter ranged from 16 to 360μ by this stage. The percentage contribution of the immature stock (up to 200μ) is 85.27% with the mode at 40 – 72 μ. Up to an average ova diameter of 200 μ the oocytes are transparent without any yolk material and represent the immature stock. The remaining 14.73% was from the ripening stock. The early yolk vesicle eggs appeared at an average ova diameter of 216 μ and on reaching an average diameter of 552 μ the eggs have become mature. The ripening stock is thus represented by the ova diameter range of 216 – 552 μ.

iii. Early ripening: (Fig: 3)

The ova diameter size had gone up to 648μ by this stage. All the three stocks (immature, ripening and ripe) were seen in this stage. The percentage contribution of the immature stock had further gone down to 70.01% with a mode at 40 - 72μ. The ripening stock contribution had gone up to 27.04% which was divided into many batches with no distinct mode (216 – 552 μ). Above an average diameter of 568 μ, the ova constitute the ripe stock. There was about 2.94% of the ripe stock with a size range of 568 to 648μ.

iv. Late ripening: (Fig: 4)

The range of the ova had been extended to 808μ. Of this 69.1% was by the immature stock and had a mode at 72 - 104μ. The percentage

contribution by the ripening stock was 18.75% with its batches showing no mode. The remaining was from the ripe stock which had gone up to 12.14%.

v. Ripe: (Fig: 5)

The range of ova size was the highest with a maximum size of 840µ. The contribution of the immature stock had further dropped down to 45.46% with a mode of 104 - 136µ. The percentage of the ripening stock was 23.09% with its batches showing no definite mode. The ripe stock had gone up to 31.5% with a distinct mode of 616 - 648µ.

vi. Partially spent: (Fig: 6)

The ova diameter had come down to 648µ. The percentage contribution of the immature stock had gone up to 79.68% with a mode at 40 - 72µ. The ripening stock constituted about 17.68% with indistinct ripening batches with no clear mode. There was about 2.64% of ripe stock, which may be the unspawned residual ova.

Table 1: Oocyte distribution in different maturity stages of ovary of *Chela fasciata.*

Ova diameter class (µm)	Immature virgin		Maturing virgin		Early ripening		Late ripening		Ripe		Partially spent	
	No:	%	No:	%	No:	%	No:	%	No:	%	No:	%
8-40	139	30.95	362	37.01	112	9.15	78	7.6	18	1.92	202	21.2
40-72	211	46.99	256	26.17	336	27.45	195	19.24	52	5.5	265	27.89
72-104	79	17.59	118	12.06	204	16.66	202	19.94	118	12.59	158	16.63
104-136	20	4.43	44	4.49	96	7.84	105	14.8	140	14.94	77	8.1
136-168			32	3.27	59	4.82	57	5.62	66	7.04	36	3.78
168-200			22	2.24	50	4.08	18	1.77	32	3.41	19	2
200-232			36	3.68	39	3.18	5	0.49	22	2.34	15	1.57
232-264			34	3.47	46	3.75	9	0.88	22	2.34	19	2
264-296			38	3.88	46	3.75	10	0.96	27	2.88	19	2
296-328			26	2.65	47	3.83	18	1.77	26	2.79	22	2.31
328-360			10	1.02	20	1.63	12	1.18	22	2.34	16	1.68
360-392					9	0.73	7	0.69	18	1.92	11	1.15
392-424					18	1.47	30	2.96	18	1.92	15	1.57
424-456					11	0.89	18	1.77	17	1.81	10	1.05
456-488					37	3.02	49	4.83	20	2.13	17	1.78
488-520					30	2.45	11	1.08	12	1.28	14	1.47
520-552					28	2.28	21	2.07	12	1.28	10	1.05
552-584					25	2.04	33	3.25	24	2.56	14	1.47
584-616					6	0.49	20	1.97	41	4.37	6	0.63
616-648					5	0.4	40	3.94	81	8.64	5	0.52
648-680							7	0.69	57	6.08		
680-712							4	0.39	33	3.52		
712-744							10	9.87	24	2.56		
744-776							3	0.29	13	1.38		
776-808							6	0.49	15	1.6		
808-840									5	0.53		
840-872									2	0.21		
Total sample	499		978		1224		1013		937		950	

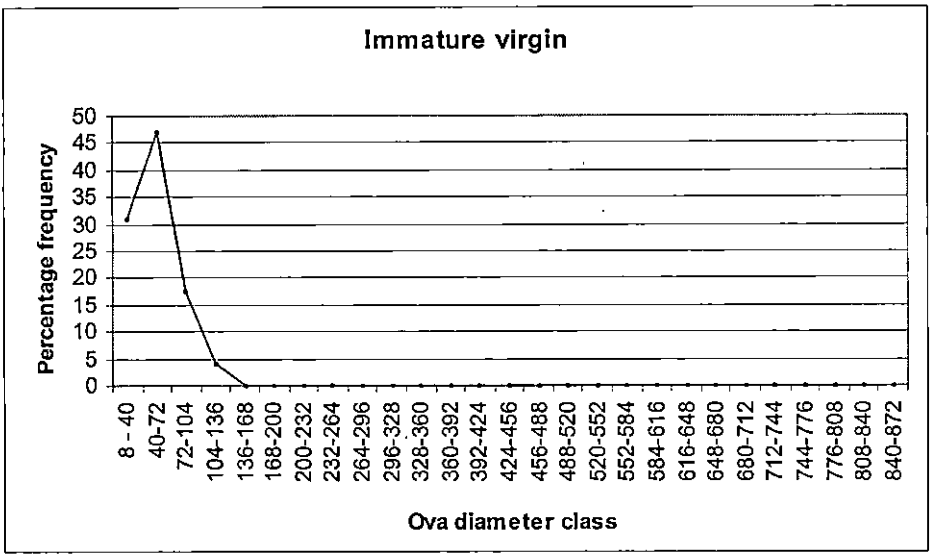

Figure 1: Graph showing percentage frequency of ova diameter classes in an immature ovary of *Chela fasicata.*

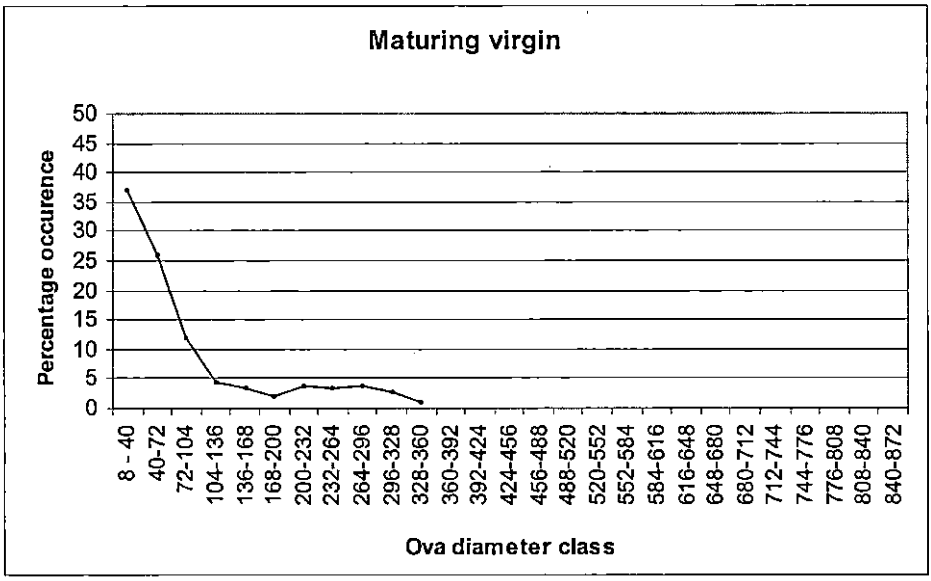

Figure 2: Graph showing percentage frequency of ova diameter classes in a maturing virgin ovary of *Chela fasicata.*

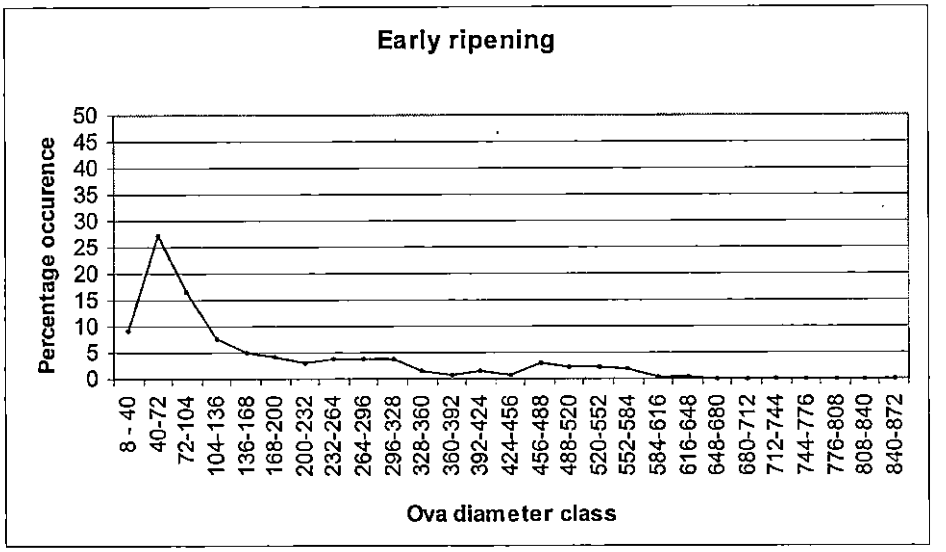

Figure 3: Graph showing percentage frequency of ova diameter classes in an early ripening ovary of *Chela fasicata*

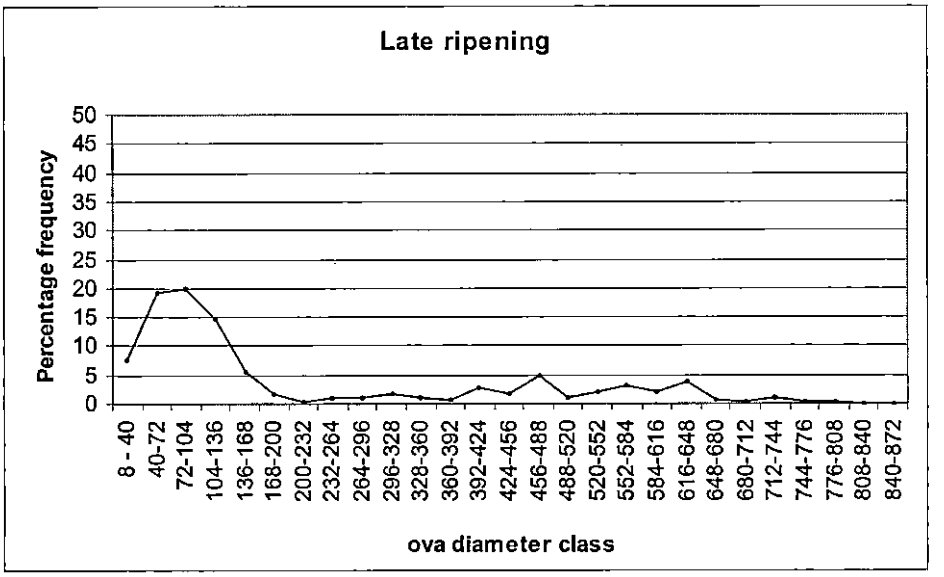

Figure 4: Graph showing percentage frequency of ova diameter classes in a late ripening ovary of *Chela fasicata*

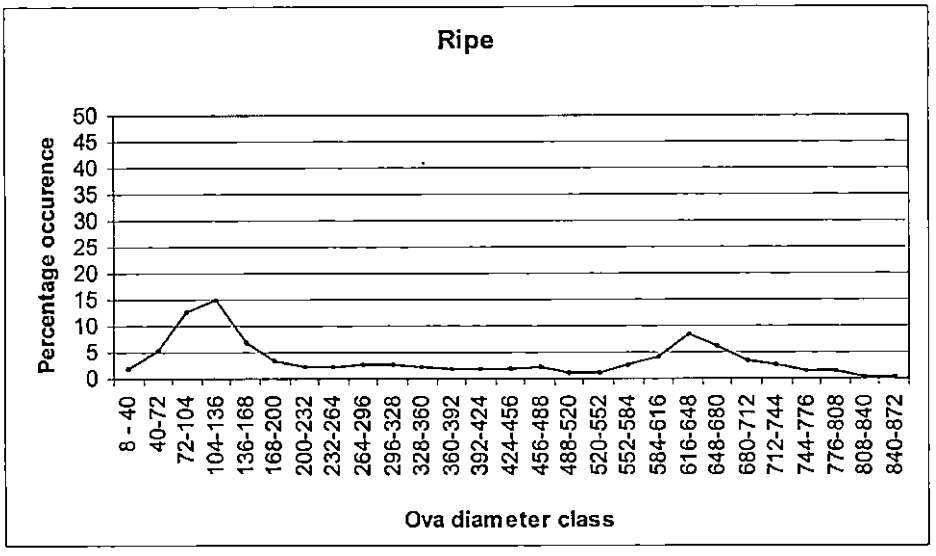

Figure 5: Graph showing percentage frequency of ova diameter classes in a ripe ovary of *Chela fasicata*

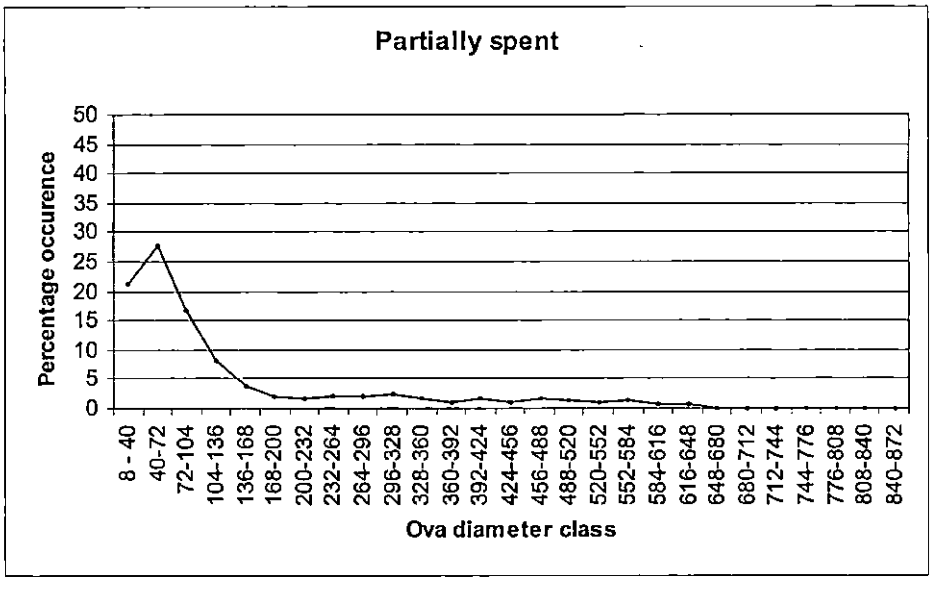

Figure 6: Graph showing percentage frequency of ova diameter classes in a partially spent ovary of *Chela fasicata*

4.2.2.2 Histology

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

1. Chromatin nucleolus stage:

Measuring about 32 μ in diameter, this stage was the youngest encountered in the different maturity stages of the ovary. With an indistinct cell membrane, yet with a distinguishable outer limit, the cells of these stages showed a thin collar of deeply basophilic, granular cytoplasm with a centrally placed large nucleus. The spherical nucleus was with a distinct nuclear membrane enclosing centrally placed large nucleolus and the chromatin material scattered in the nucleoplasm. These immature oogonia could be seen as clumps and occurred deep within the germinal epithelium. (Plate: 2 a)

2. The early perinucleolus stage:

The oocytes appear bigger, more spherical due to accumulation of more cytoplasm and measured an average of 120 μ at this stage. The thus thickened collar of cytoplasm was strongly basophilic. The centrally placed

nucleus had also grown into germinal vesicle acquiring a spherical shape and gaining a vesicular interior. The chromatin material was found to show less staining affinity and were seen to fuse together leading to the formation of the nucleoli, while the existing nucleolus has moved to periphery almost retaining its earlier size and structure. (Plate: 2 a)

3. The perinucleoulus stage:

The oogonium remained spherical in shape but had further increased in size to 184μ. The cytoplasm continued to be granulose but changes have occurred in staining affinity. The chromatin material was less basophilic than in the previous stage and took up a light pinkish hue with haematoxylin. (Plate: 2 b)

The chromatin nucleolar and perinucleolar stages are sometimes referred to as 'primary growth phase' (Wallace and Selman, 1981) or first growth phase (Forberg, 1982).

4. The yolk vesicle stage:

The follicular layer got closely pressed on to the outer surface of the oocyte, getting squeezed between the enlarged oocytes and thus was not as distinct as in the previous stage. The oocytes were much enlarged and were about 248 μ in diameter. The vitelline membrane had become distinct in this stage as a compact membrane of dense, homogenous cytoplasm surrounding the oocyte. The cytoplasm remained granular but a number of yolk vesicles

have appeared in it peripherally. The vesicle was of similar shape, empty, with an outer cytoplasmic shell. The cytoplasm was faintly basophilic and turns reddish with eosin. While the nucleaolar bits were deeply basophilic, the chromatin material remains poorly basophilic and turns pinkish with hematoxylin. (Plate: 2 b)

5. The primary yolk stage:

The appearance of clusters of minute granules, the yolk granules on the periphery of the cytoplasm and the complete proliferation of yolk vesicles were distinguishing characters of this stage. The oocyte had further increased in size and was about 336μ in diameter. Now, owing to mutual pressure, the oocytes began to loose their characteristic shape but were mostly oval or spherical. The follicular layer was thin.

The germinal vesicle was oval or elliptical in shape and central in position with an irregular, wavy nuclear membrane. All the nucleolar bits have migrated to the nuclear periphery and were closely aligned to the inner surface of the nuclear membrane and showed a clumping tendency. The nucleoli and the nuclear membrane were deeply basophilic while the chromatin material was faintly basophilic (Plate 3 a).

6. The secondary yolk stage:

The yolk vesicles clump together, fusing to form larger vesicles, may be due to the appearance of globules. The nuclear membrane remained highly

irregular and wavy as in the previous stage, but the membrane itself had become hazy and quit indistinct. The yolk formation in this stage began with the accumulation of yolk globules in the periphery of the oocytes below the vitelline membrane. Average ova diameter is 384 μ in this stage (Plate 3 b).

7. The tertiary yolk stage:

The follicular layer and vitelline membrane remained prominent as before and were almost equally thick. The oocytes were still irregular in shape on account of the pressure from the adjacent oocytes. They have further grown in size and now measured about 520 - 552 μ in diameter along the longest axis. The yolk vesicles too have further grown in size and were less in number. The yolk globules have greatly increased in number and size and almost completely fill the cytoplasm while the yolk granules lie interspersed with the yolk globules. (Plate: 4 a)

8. Migratory nucleus stages:

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

9. The mature oocyte:

The oocyte is now mostly spherical/oval in shape and covered by a fine outer sheath. The vitelline membrane had by now developed into a well recognizable membrane. The yolk vesicles are few and scattered and the interspaces were filled with yolk globules and granules. Yet another development at this stage in teleosts is the formation of the micropyle.

The final stages of oocyte maturation are often difficult to follow in histological material because of the shrinkage and distortion of these cells during normal processing. In addition, ovulated oocytes may be lost from the ovarian lumen during initial tissue preparation. (West, 1990).

The fate of the unextruded ova and empty follicles:

In the partially spent and spent recovering ovary a few unspawned eggs undergo degeneration. The atresia started with the liquefaction of the yolk, consequently, numerous minute, deeply staining ovoid bodies appears in the oocytes. The follicular layer gradually lost its compactness and strength, thinning down considerably and becoming pliable, convoluted and discontinuous at places (Plate 4 b). Through these the liquefied yolk partially extrudes. The follicular layer becomes much vascularized and the blood cells freely migrated into it. In spent ovaries, in addition to the unspawned oocytes, which were degenerating, there were the empty follicles of the spawned ova. These empty pockets easily got pushed out of existence by the fast developing immature oocytes.

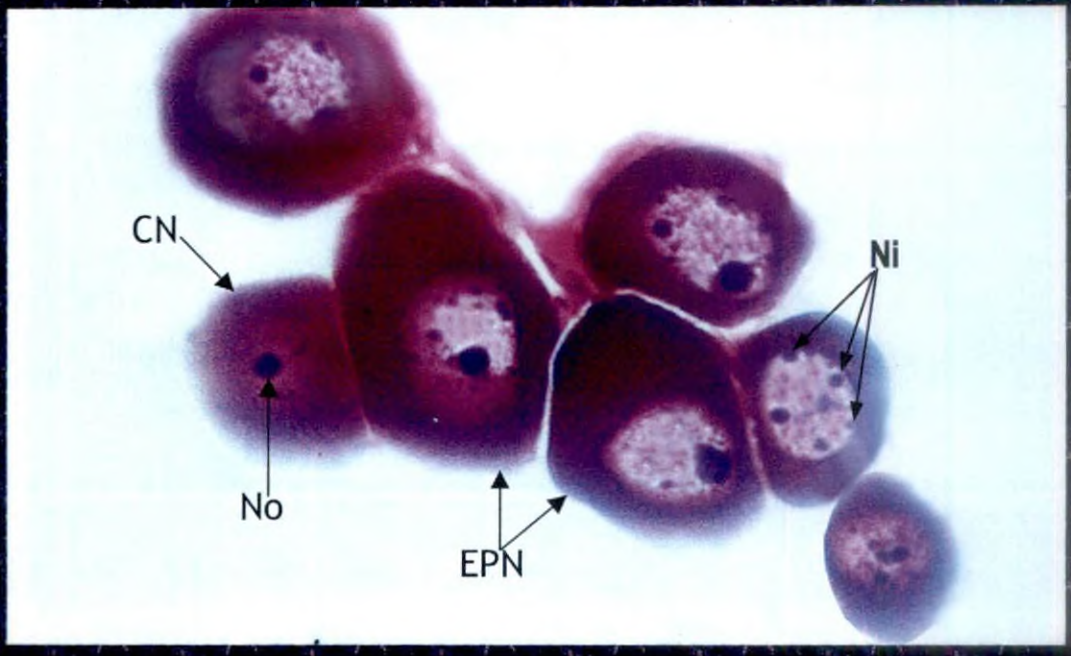

PLATE 2a: Section of ovary shownig - CN: Chromatin Nucleolar stage; No: Nucleolus; EPN: Early Perincleoulus stage and Ni: Nucleoli

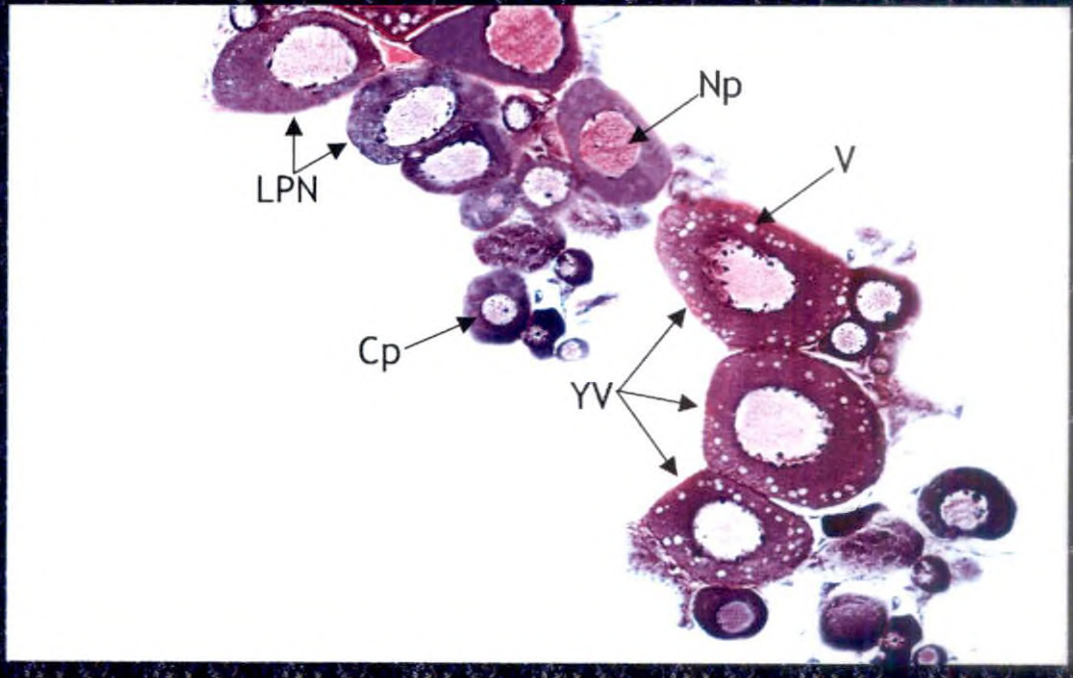

PLATE 2b: Section of ovary showing - LPN: Late Perinucleolus stage; Np: Nucleoplasm; Cp: Cytoplasm; V: Vesicle and YV: Yolk Vesicle stage.

PLATE 2

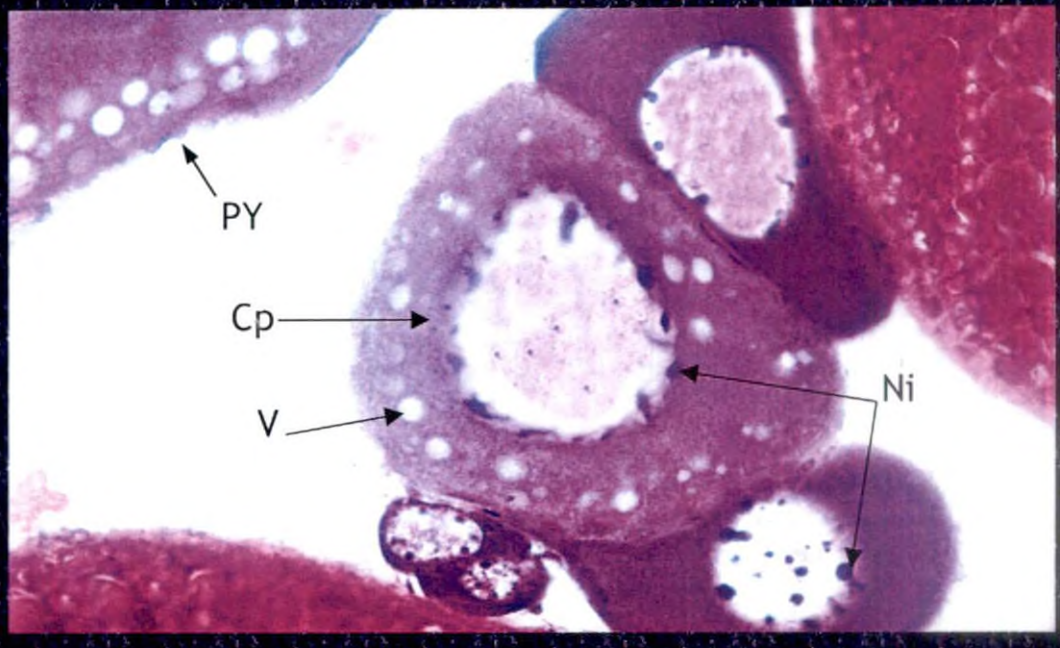

PLATE 3a: Section of ovary showing - PY : Primary Yol
stage; Cp: Cytoplasm; V: Vesicle and Ni: Nucleolii.

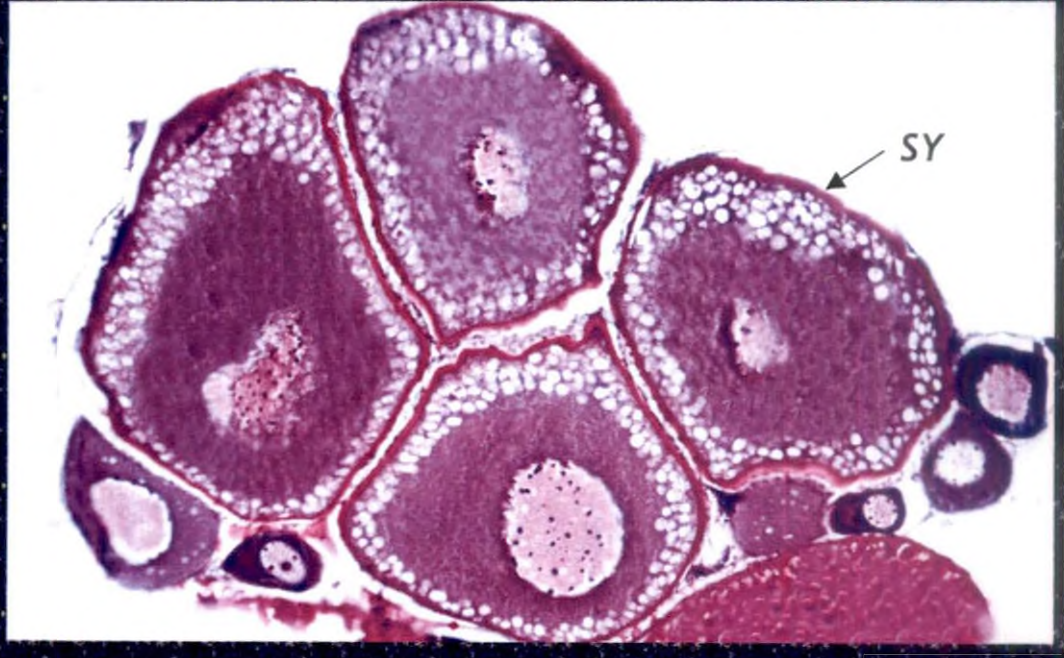

PLATE 3b: Section of ovary showing - Secondary Yolk sta

PLATE 3

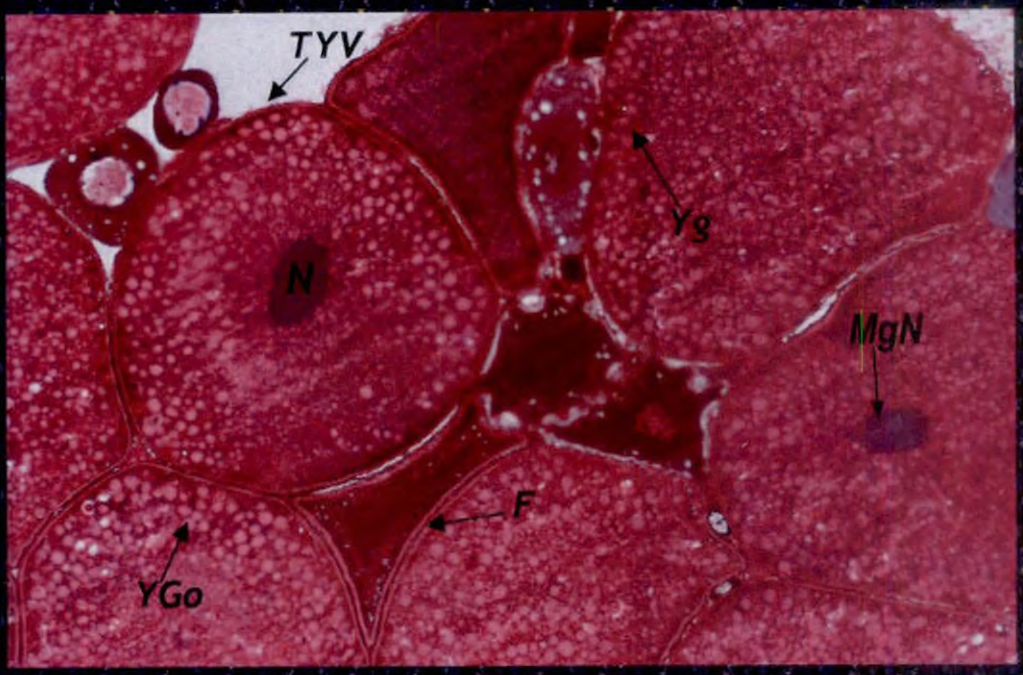

PLATE 4a: Section of ovary showing - TYV: Tertiary Yolk stage; Yg: Yolk granules; F: Follicular layer; MgN: Migratory Nucleus and YGo: Yolk Globules.

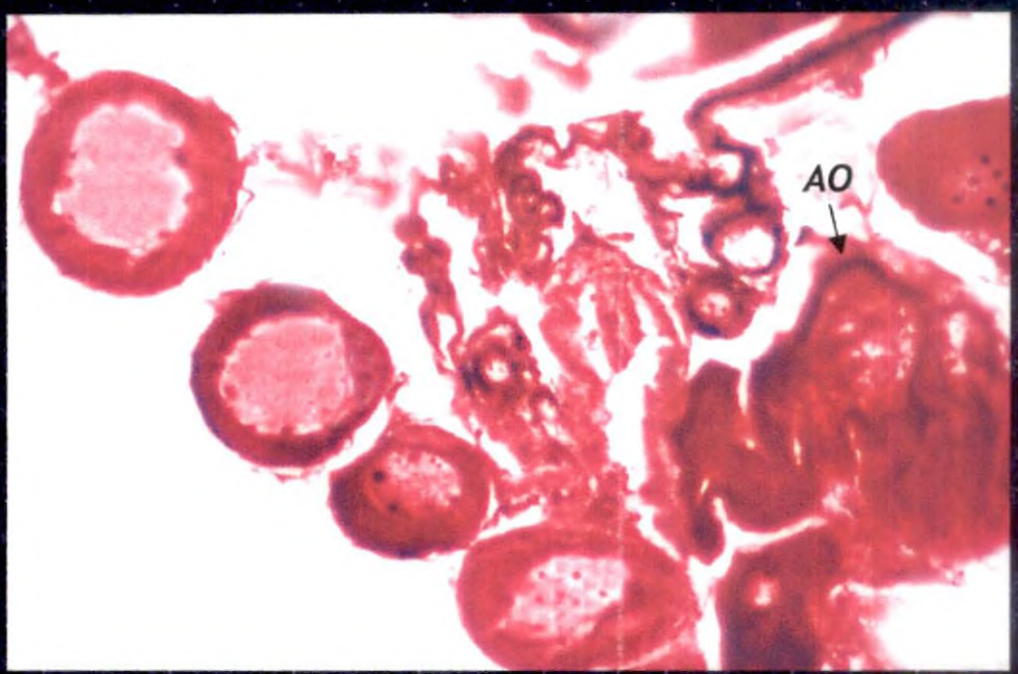

PLATE 4b: Section of ovary showing - Atretic oocyte.

PLATE 4

4.2.3 Size at first maturity

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

While the first mature male fishes appeared in the 25 – 30 mm (TL) group (16.6%), the first mature females appeared only in the group of 40 – 45 mm (22.2%). All male fishes were mature on reaching a total length of 45 mm and all female fishes on reaching a length of 60 mm total length. The size at first maturity for males was 36.25 mm TL (35 – 40 mm) and 45.75 mm TL for females (45 – 50 mm). (Fig: 7 and 8).

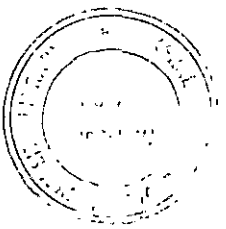

Table 2: Table showing the total length classes and percentage occurrence of mature female *Chela fasciata.*

S. No	Length class (TL) mm	Total No.	No. mature	% mature
1	35 - 40	20	0	0
2	40 - 45	18	4	22.2
3	45 - 50	22	14	63.63
4	50- 55	18	14	77.77
5	55 - 60	10	9	90
6	60 -65	2	2	100
7	65 - 70	4	4	100

Table 3: Table showing the total length classes and percentage occurrence of mature male *Chela fasciata.*

S. No	Length class (TL) mm	Total No.	No. mature	% mature
1	25 - 30	6	1	16.66
2	30 - 35	12	4	33.33
3	35 - 40	15	8	53.33
4	40 - 45	5	4	80
5	45 - 50	4	4	100
6	50 - 55	5	5	100
7	55 - 60	3	3	100

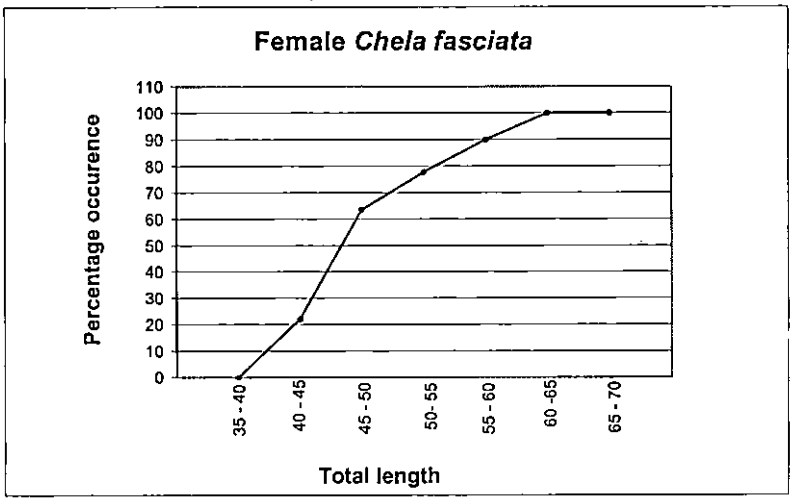

Fig 7: Graph plotted for the size at first maturity in females of *Chela fasciata.*

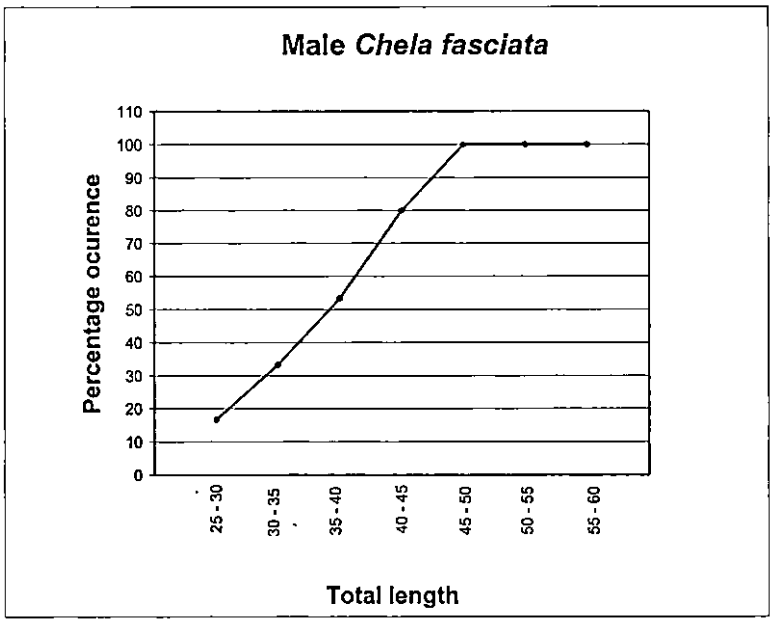

Fig 8: Graph plotted for the size at first maturity in males of *Chela fasciata.*

4.2.4 Spawning frequency in *Chela fasciata*

It is apparent from the ova diameter frequency distribution of a ripe ovary (figure 9) that there are three batches of eggs representing the immature, ripening and ripe ova stocks. The ova diameter size ranged from 16 – 200 μm for the immature ova stocks. The ova were transparent without any yolk material. The ripening stock had an ova diameter range of 216 – 552 μm. The first yolk vesicle eggs appeared at an ova diameter of 216 μ and on reaching a diameter of 552 μm the eggs had become ripe and ready to ovulate. Above an ova diameter of 568 μm, the eggs are constituting the ripe stock.

Ova diameter frequency showed that the immature and ripe stocks have clear-cut modes but the ripening stock showed many batches which were not well differentiated from each other. There was a large stock of immature ova constituting to about 45.5 % of the total ova count and ranged over a diameter of 16 – 200 μm with a mode at 104 – 13 6 μm diameter class. The ripe stock was about 31.5 % with ova size going up from 552 μm and with a mode at 616 – 648 μm diameter class. The maximum size of ova diameter recorded during the ova diameter studies carried out to determine the spawning frequency in *Chela fasciata* was 872 μm. There was always the presence of large percentage of immature stock in any developmental stage of the ovary and a distinguishable stock of ripe ova in the ripe ovary with clear-cut modes.

The ripening stock contributed to the remaining 23 % ranging from 216 – 552 μm. The ripening stock consisted of many batches with no clear-cut demarcation. This indicates that the spawning may be extended over a very long period of almost round the year with the individual spawning intermittently.

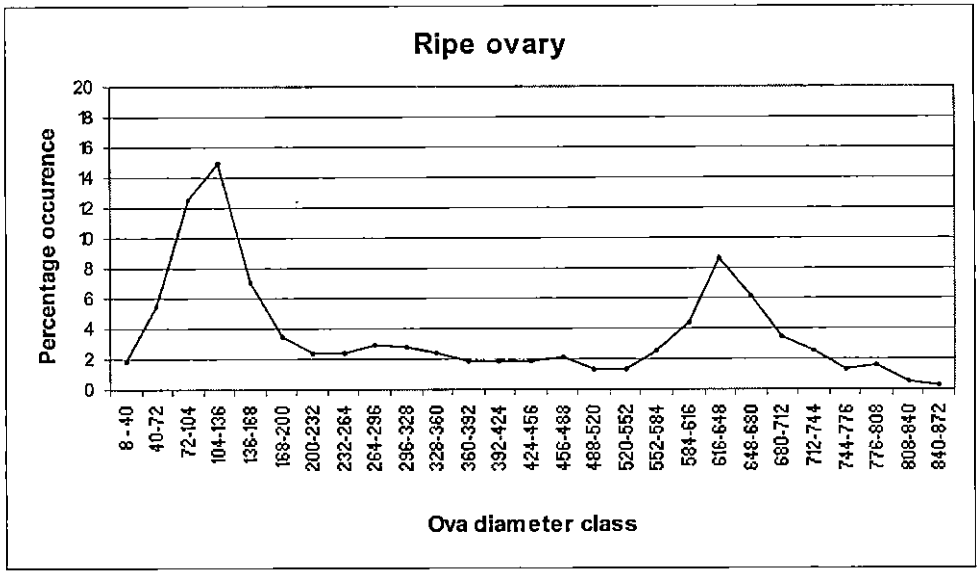

Figure 9: Graph showing percentage frequency of ova diameter classes in a ripe ovary of *Chela fasicata*

4.2.5 Fecundity in *Chela fasciata*

Results for the fecundity counts are given in the tables 4 and 5. Absolute fecundity ranged from 2669 – 4437 (49.5 - 82.0 mm TL and 1.033 - 2.933 gm body weight). The gonado somatic index (GSI) values ranged from 8.62 - 13.0. The relative fecundity values ranged from 501.91 – 587.74 per cm body length and from 1512.9 – 2617.6 per gm body weight of fish.

The ovary weight ranged form 112 - 253 mg and ovary length ranged from 1.10 - 2.70 cm. The relative fecundity values ranged from 15.20 – 22.91 per mg ovary weight and 1643.55 – 2468.41 per cm ovary length.

The relationship between Absolute fecundity and i) Total length, ii) Body weight, iii) Length of ovary and iv) Weight of the ovary are given in Table 6 and figures: 10-13. Fecundity increases with increase in the length and weight parameters. All the linear relationships are significant at 5% level.

Table 4: Absolute fecundity observed in *Chela fasciata.*

Sl. No.	Total body length (mm)	Body Weightt (gm)	Ovary length (mm)	Ovary weight (mg)	GSI %	Fecundity
1	49.5	1.033	1.40	118	11.42	2704
2	51.0	1.295	1.60	139	11.51	2997
3	52.0	1.112	1.10	112	10.72	2669
4	55.0	1.480	1.40	146	9.86	2919
5	57.5	1.630	1.50	153	9.39	2886
6	59.0	1.700	1.60	211	12.40	3209
7	67.5	2.071	1.65	188	9.08	3628
8	82.0	2.933	2.70	253	8.62	4437

Table 5: Relative fecundity observed in *Chela fasciata.*

S No.	Fecundity	Per cm body length	Per gm body weight	Per cm ovary length	Per mg ovary weight
1	2704	546.26	2617.61	1913.42	22.91
2	2997	587.74	2314.67	1873.43	21.56
3	2669	513.26	2400.17	2426.36	23.83
4	2919	530.85	1972.77	2085.51	19.99
5	2886	501.91	1770.55	1924.12	18.86
6	3209	543.89	1887.64	2468.41	15.20
7	3628	537.48	1751.81	2198.78	19.30
8	4437	541.17	1512.99	1643.55	17.54

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

S No.	Variant (x)	Equation Log Y= Log a + b Log x	Correlation coefficient (r)
1	Total length (cm)	Log F= 2.750 + 0.973 Log TL	0.963 *
2	Body weight (gm)	Log F = 3.403 + 0.477 Log BW	0.950 *
3	Ovary length(cm)	Log F = 3.374 + 0.622 Log OL	0.925 *
4	Ovary weight (mg)	Log F = 2.290 + 0.548 Log OW	0.909 *

* Significant at 5% level

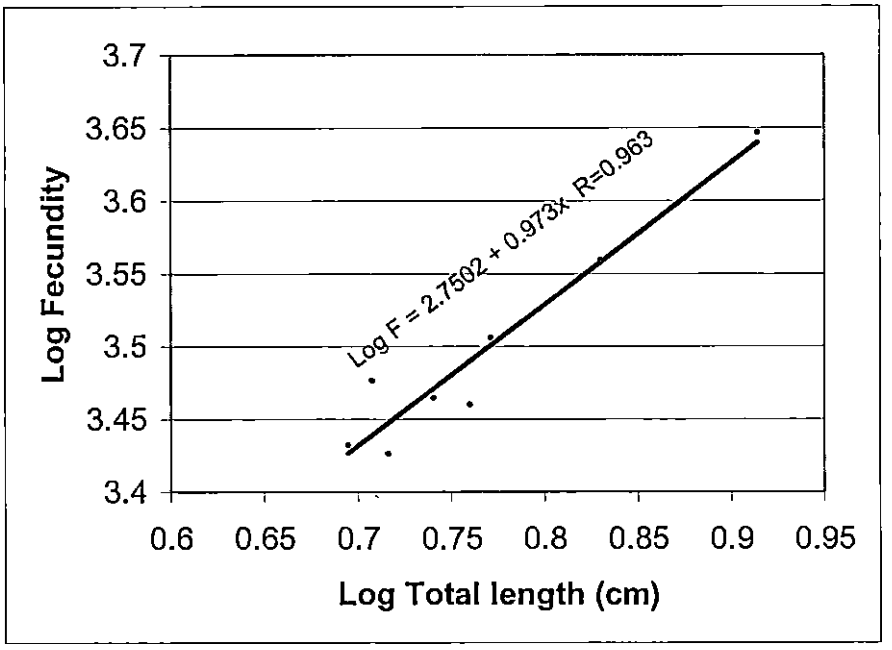

Figure 10: Relationship between fecundity and total length of fish.

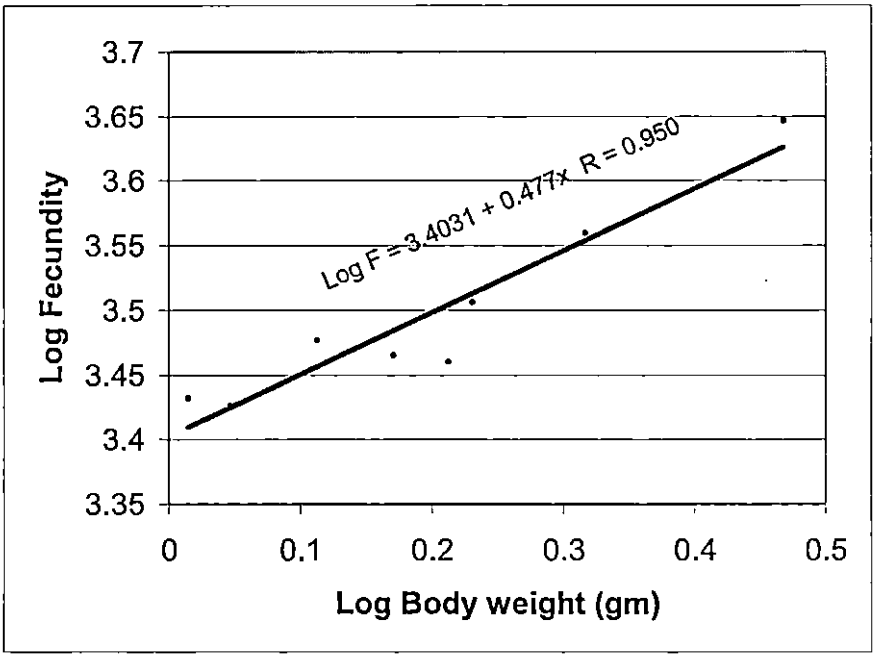

Figure 11: Relationship between fecundity and body weight of fish.

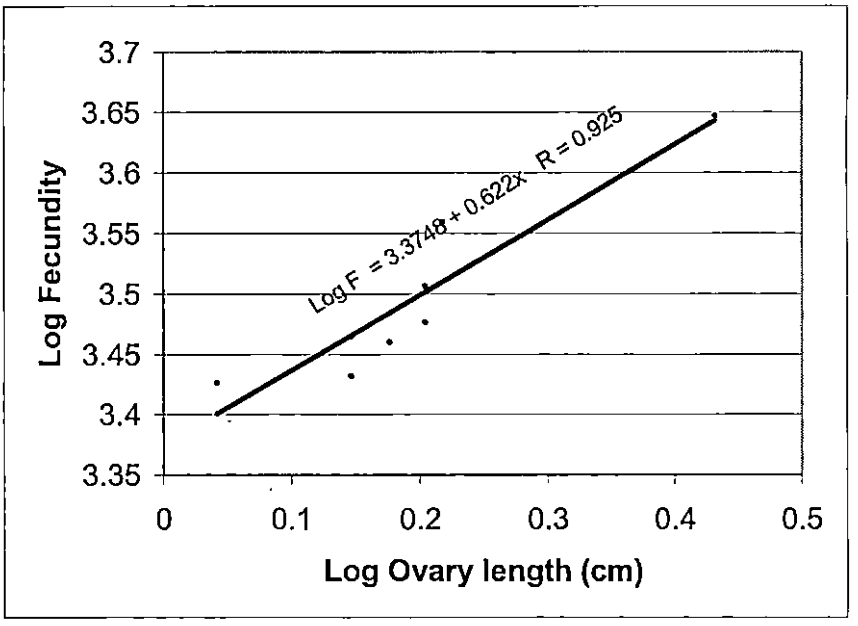

Figure 12: Relationship between fecundity and ovary length.

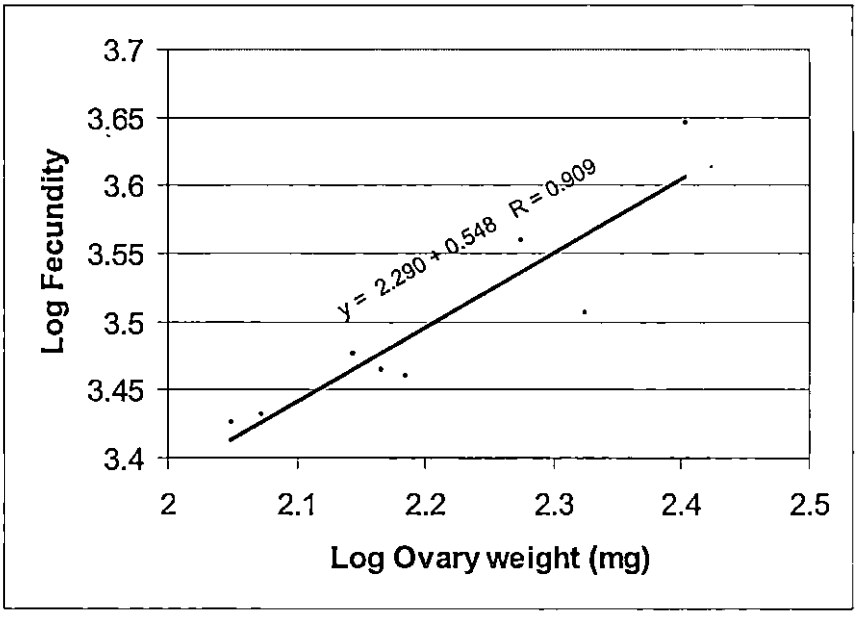

Figure 13: Relationship between fecundity and ovary weight.

4.2.6 Sexual dimorphism in *Chela fasciata*

It was observed that in the males, colour was intensified during the spawning. Usually it is the females which are larger as they mature late. The males are rather smaller and slender, while the females have a broader abdomen due to the presence of ripe ovary within. During the captive breeding experiments the females were identified by checking the soft, bulged belly during the ripe condition of the female.

4.2.7 Embryonic development in *Chela fasciata*

As per the eco-morphological classification of Balon (1975 a) *Chela fasciata* is a phytophil (morphotype) coming under the ecological group – Open substratum spawner and ethological class – Non-guarder. The time and duration of the early embryonic development of these fishes were noted by collecting the fertilized adhesive eggs clinging to the roots of floating aquatic plants like the *Salvinia*. The cleavage egg phase, inside egg embryo phase and free embryo phase and larval stage have been collected and photographed. The flow chart showing the different stages of development (as per the nomenclature of Balon, 1975 b) is given in plate 5.

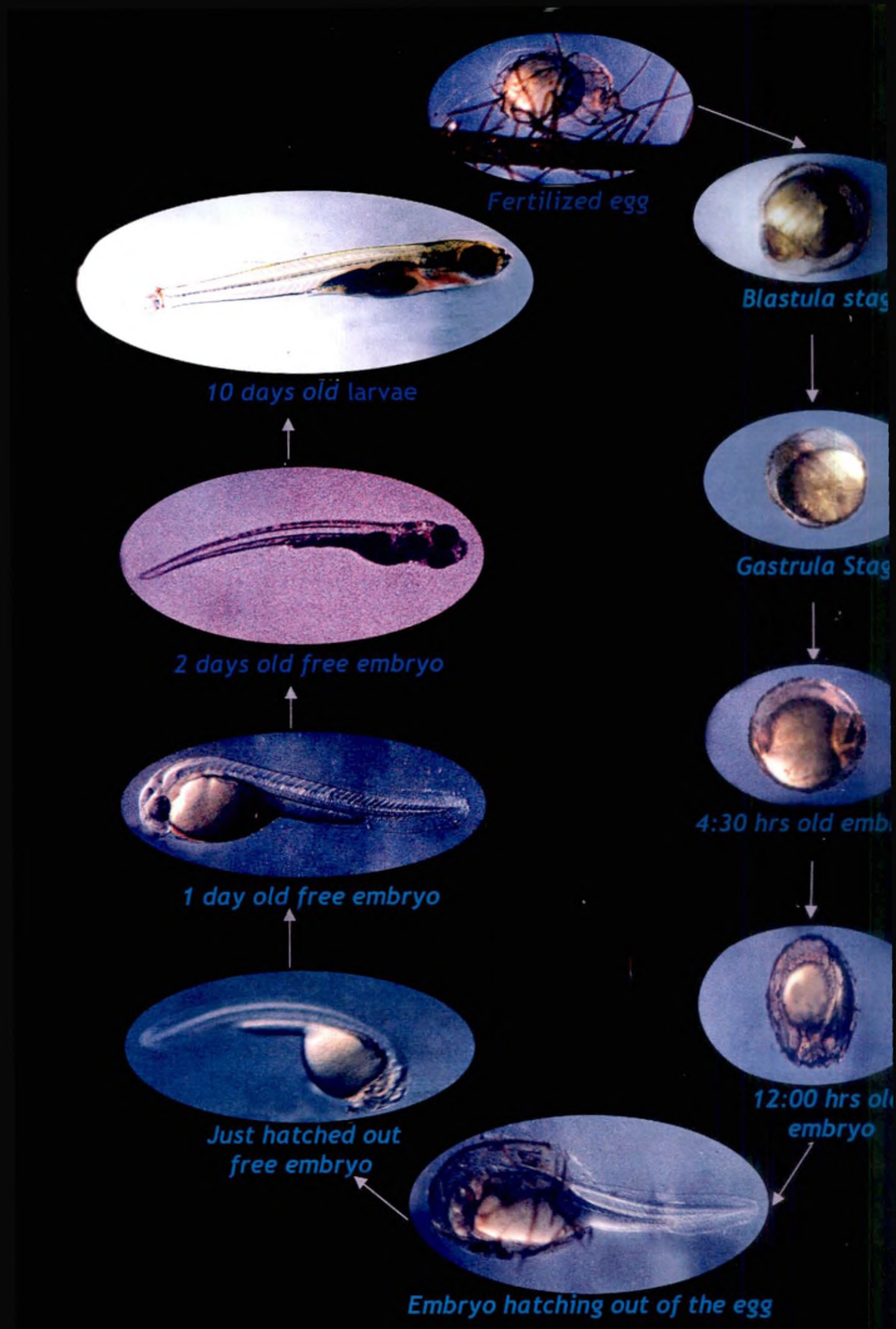

Plate 5 Embryonic development in *Chela fasciata*

1. Fertilized eggs:

The fertilized eggs were sticky due to the sticky secondary egg membrane of origin from the follicular envelope. These adhesive, fully swollen eggs were about 960μ in diameter and were translucent with a tinge of yellow.

2. Cleavage egg:

After fertilization the blastodisc was formed at the animal pole with in 30 min. The cleavage egg stage had lasted for about two hours. The formation of blastula had marked the end of the cleavage stage i.e., 2 hr10 min after fertilization. This was followed by the formation of the gastrula after an hour. The first appearance of the tubular embryo was noted about four and a half hours from fertilization of the egg. The myotomes had started to form about six and a half hours post fertilization while the notochord was visible in another two hours.

3. Inside-egg embryo:

The differentiation of embryo had started by about the tenth hour post fertilization and the gut had formed by the next half an hour. The optic cup had formed in the following three hours and this fifteen hour old inside egg embryo started to wriggle and also the continuous beating of heart could be observed. By eighteen hours post fertilization the unpigmented eye of the

embryo was observed. The embryo had finally hatched out tail first by rupturing the egg by its wriggling movement.

4. Free embryo (eleutheroembryo)

The hatched out free embryo was 1.5-2mm in length and was translucent with pale yellow yolk sack, unpigmented eyes and no mouth. By the end of twenty-four hours the fin fold was distinct and the gut was well defined. The eyes of one-day-old free embryo were pigmented and fin buds were visible by the second day. The yolk was completely absorbed by the end of third day the mouth was formed.

5. Larva

The larvae started feeding the third day after hatching. By the tenth day the fins were completely formed and the phase could be designated as a ptero-larva or fin formed larva.

DISCUSSION

5. DISCUSSION

5.1 BREEDING BIOLOGY

The reproductive strategy will be the summation of a suite of adaptive traits that enables individual fishes to leave the maximum number of offspring. These traits will encompass size and age at first reproduction, size and age specific fecundity schedules, reproductive effort or gonado somatic index, and the manner and timing of spawning (Mills, 1981). Hitherto, no study on the biology of the Malabar hatchet chela, *Chela fasciata* has been conducted. Hence in the present study an attempt was made to understand the various aspects of the breeding biology.

5.1.1 Quantification of maturity stages

For delimiting the different stages of the sexual cycle, a series of scales of ripeness have been worked out for each group of fishes. However, they are all based on an universal scale of six stages (Nikolsky, 1963) mainly for temperate species. In the present study a modified six-stage key is used, based on the peculiarities of the reproductive strategy, namely multiple spawning and protracted spawning season of the tropical species.

5.1.2 Maturation of ovary and oogenesis

5.1.2.1 Oocyte distribution

The quantification of maturity stages based on external morphology could be clearly demarcated by the distribution of oocyte stocks in the different maturity stages. It can be seen in the present study that the ovaries of *C. fasciata* show asynchronous development. 'Asynchronous ovaries' shows sufficient number of oocytes at various stages of development within the ovary (De Vlaming, 1983). The oocytes size frequency distribution is continuous, however in a ripe ovary a clear-cut mode for the ripe stock is obtained.

5.1.2.2 Oogenesis in Chela fasciata

Oocyte development in teleosts has been reviewed by Wallace and Selman (1981), De Vlaming (1983), Nagahama (1983), Guraya (1986), Wallace *et al.* (1987) and Jalabert (2005).

The yolk vesicles are not yolk in a strict sense since they do not serve as a nutrient source for the embryo (Selman *et al.*, 1993; Bromage and Cumaranatunga, 1988). Selman and Wallace (1989) recommended that yolk vesicles be replaced by the term 'cortical alveoli' in future studies. In most teleost species studied the yolk vesicles appear before the lipid droplets and yolk granules appear (Wallace and Selman, 1981). In *C. fasciata* the yolk vesicles appear first at the end of the perinucleolar stage. Of the yolk

inclusions, the yolk granules appear first at the primary yolk stage and the yolk globules at the secondary yolk stage. The appearance of yolk spheres, granules and globules is characteristic of vitellogenic oocytes (primary, secondary and tertiary yolk stages) as per Yamamoto (1956 b), Yamazaki (1965) and Davis (1977). The fusion of yolk granules begin soon after their initial formation or as late as final maturation (Wallace and Selman, 1981). In the case of *C. fasciata* the yolk granules and yolk globules retain their shape and structure even at the migratory nucleus stage. So the fusion of the yolk material may be during final maturation.

Atretic oocyte is indicated by an irregular shape, a change in the appearance of the yolk, the break down in the outer membrane (Forberg, 1982) as is also seen in the present study.

5.1.3 Size at first maturity

In the present study, the size at first maturity of males and females of *Chela fasciata* is found to be around 45.75 mm TL (45 – 50mm) for females and 36.25 mm TL (35 - 40mm) for males. The largest female obtained during the present study is 82.0 mm TL while the largest male is 59.5 mm TL. This is a kind of reproductive strategy to enhance the existence of the race by increasing the fecundity, which is directly related to the size of the female fish (Keenleyside, 1991). The larger the female grows the higher would be the fecundity. Here the females spend more energy first on

somatic growth and from there on, on gonadal growth and maturation. However, the early maturation of the males diverts all the energy reserves towards the gonadal development and its maturation at a relatively younger age. In *Chela fasciata* the males attained sexual maturity at a smaller length than females. Similar observations had been reported in many freshwater fishes like *Labeo boggut* (Selvaraj *et al.*, 1972), *Barbus sarana* (Murthy, 1975), *Nemachelius triangularis* (Ritakumari and Nair, 1979) and *Puntius denisonii, P. filamentosus, Nemacheilus triangularis,* and *N. semiarmatus* (Anna Mercy *et al.,* 2005 b).

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

5.1.4 Spawning frequency

Ova diameter studies reveal that this species comes under category D of Karekar and Bal's classification (1960), characterized by

spawning that is extended over a very long period of almost round the year with the individual spawning intermittently. Thus *Chela fasciata* may be designated as a multiple spawner with an asynchronous ovarian development. Asynchronous ovary in an ovary 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 oocyte (Wallace and Selman, 1981), a pattern very clearly exhibited in *C. fasciata*. De Vlaming (1983) considers that most species with asynchronous oocyte development have protracted spawning season with multiple spawnings. Multiple spawners are also termed partial, heterochronal, serial spawners (Holden and Raitt, 1974; Macer, 1974), implying that only part of the complement of yolked oocytes is spawned and that individuals spawn over a protracted period. . The ovary undergoes repeated maturation of the ripening ova stocks followed by ovulation and spawning. The classic examples for this strategy among cyprinids are the minnows largely restricted to the lake and river margins and to very shallow riffle areas (Mills, 1991). *Chela fasciata* is a typical tropical minnow distributed in the riffle zones of the Western Ghat river ecosystem. Cambray and Burton (1985) argues that early maturation and multiple spawning is an adaptation to enable recolonisation of unstable environments where adult mortalities may be high.

In *Chela labuca,* Sterba (1953) described the release of ovulated clutches of eggs in batches during a spawning activity in aquaria, indicating

the batch or fractional spawning activity in the tropical small cyprinids. Fractional spawning purely describes the batch release of ovulated eggs (De Vlaming, 1983). Under captivity, satinfin shiner, *Notropis analostanus* (Gale and Buynak, 1978) and fathead minnow, *Pimephales promelas* (Gale and Buynak, 1982) showed batch release of eggs.

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

5.1.5 Fecundity

In the present study, absolute fecundity of *Chela fasciata* ranged from 2669 to 4438 in fishes whose size ranged from 49.5 to 82 mm TL. In the European minnow, *Phoxinus phoxinus* with a maximum size of 78.0 mm, the maximum estimated egg production was 3172 eggs (Mills, 1987).

Studies shows that repeat spawners have larger fecundity than recruit spawners. The number of eggs released increases with age and size ranging from 2704 for 49.5 mm fish to 4438 for a fish of size 82mm. Similar studies have been done on *Danio malabaricus* (Anna Mercy *et al.*, 2005 b).

With increase in body length, the relative fecundity per mg ovary weight ranged from 23.83 to 15.2 in *C. fasciata*. This indicates an increase in size and weight of eggs from the small recruit spawners to the large repeat spawners. Hempel and Blaxter (1967) reported similar findings for the Atlantic herring (*Clupea harengus*) and Hislop (1975) for the haddock (*Merlanguis aeglifinnus*).

5.1.6 Sexual dimorphism

It was observed that in the males colour was intensified during the spawning. Usually it is the females which are larger then the males, as they mature late. The males are rather slender, while the females have a broader abdomen due to the presence of ripe ovary within. In the males of *Chela dadyburjori,* during the breeding season four to six minute wart-like protruberances on the opercle are seen (Anna Mercy *et al.*, 2007), but not seen in *Chela fasciata*. The sexual dimorphism is very slight and identification of the sexes has to be done mainly by checking the soft, bulged belly during the ripe condition of the female. Similar observation was made in *Danio malabaricus* (Sterba, 1953 and Anna Mercy *et al.*, 2005 b).

5.1.7 Early embryonic development

As per Balon (1975 a) *Chela fasciata* comes under the ethological class of Non-Guarders, ecological group of Open Substratum Spawners and

morphological type of Phytophils. Among cyprinids phytophils which include widespread species like carps (*Cyprinus carpio*), tench (*Tinca tinca*) and *Danio malabaricus* spawn on aquatic or flooded terrestrial vegetation. Adaptable organisms like roach (*Rilulus ritulus*) and bream (*Abramis brama*) are phyto-lithophils because they are non-obligatory phytophils which can also spawn on a rocky substratum (Mills, 1981). The fertilized, water hardened eggs were translucent yellow. They were sticky due to the follicular layer enveloping them. The cleavage egg stage was completed in about one and half hour post fertilization. The inside egg embryo stage lasted for about twenty-one hours post fertilization. Hatching of the egg was brought about by the wriggling movement of the embryo and it emerged tail first. The free embryo that hatched out was about 2mm in length, body was translucent, and eyes were unpigmented and had a pale yellow yolk sac. By the end of first day after hatching the eye developed pigmentation. By the second day fin buds were seen and by third day the yolk was fully absorbed, mouth was formed and the larva started exogenous feeding. Similar observations were made in *Danio malabaricus* (Anna Mercy *et al.*, 2005 b).

SUMMARY

6. SUMMARY

The present study was made to understand the ovarian maturation and breeding and embryonic development and other aspects of reproductive biology of *Chela fasciata* under captive conditions. The methodology, results and conclusion are as follows:

1. A total of 144 fishes (94 females and 50 males) ranging in size from 26 to 82 mm, collected from River Bharathapuzha at Thootha, were used for study of reproductive biology.
2. For studying the external morphology of gonads both fresh and preserved specimens were used. The reproductive organs of *Chela fasciata* were with paired ovaries and testis of equal lobes.
3. The gonads were quantified into six maturity stages based on external morphology (immature virgin, maturing virgin, early ripening, late ripening, ripe and partially spent).
4. The oocyte distribution of the six stages of maturity were studied to understand the maturation of ova in the ovary.
5. The ovaries of *Chela fasciata* showed asynchronous development in which oocytes at all stages of development were present in the same ovary at the same time.

6. Oogenesis was studied using the standard histological procedures. Harries hematoxylin and eosin were used for the histological differentiation.
7. The oocyte development was classified into nine different oogenic stages (chromatin nucleolus stage, early perinucleolus stage, late perinucleolus stage, yolk vesicle stage, primary yolk stage, secondary yolk stage, tertiary yolk stage, migratory nucleus stage and mature oocyte).
8. The first mature females appeared in the length group of 40 – 45 mm and males in the group of 25 – 30 mm. The size at first maturity for female was found to be at 45.75 mm TL (45 – 50 mm) and for males at 36.25 mm TL (35 – 40 mm) respectively.
9. Based on ova diameter frequency study of the ripe ovary, *Chela fasciata* was found to be a multiple spawner, with a protracted spawning season, the individuals spawning intermittently.
10. Absolute fecundity of the fishes ranged from 2669 to 4437 in fishes of size range 49.5 mm to 82 mm TL. The 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, at 5% level of significance, with the length and weight of both the fish and ovary.
11. The sexual dimorphism was very slight in *Chela fasciata* and involved intensification of color in adult males. Differentiation is possible only in

the breeding season when the abdomen of the female is broader due to the presence of ripe ovary.

12. Captive reared fishes from the NBFGR – NATP programme (Germplasm inventory evaluation and Gene banking of fresh water fishes of India) were utilized for the study on embryonic development. These fishes were reared under captive conditions providing optimum ambient conditions and *ad libitum* feed.

13. The embryonic development studies showed that the cleavage stage of the egg lasted for about 2 hrs 10 min. post fertilization. The inside egg embryo stage had lasted for 21 hrs, post fertilization. The eggs hatched at the end of 21 hrs post fertilization and the embryo emerged tail out first. Pigmented eye was seen in one-day-old free embryo. Free embryo stage lasted for three days. By the end of third day, post fertilization, the mouth developed, the yolk sac was empty and the exogenous feeding started, the free embryo entering the larval period.

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OVARIAN MATURATION, BREEDING AND EARLY EMBRYONIC DEVELOPMENT OF AN INDIGENOUS ORNAMENTAL CYPRINID OF THE WESTERN GHATS - *CHELA FASCIATA* SILAS

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ABSTRACT OF THESIS

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ABSTRACT

ABSTRACT

Chela fasciata is an indigenous ornamental cyprinid endemic to the Western Ghats of Kerala. It inhabits the riffle zones of River Bharathapuzha at Thootha. A total of 144 fishes (94 females and 50 males) ranging in size from 26.0 to 82.0 mm were collected from the wild and used for the study of reproductive biology.

The gonads were quantified into six maturity stages based on external morphology as immature virgin, maturing virgin, early ripening, late ripening, ripe and partially spent. Studies on oocyte distribution of the six stages of maturity were done to understand the maturation of ova in the ovary. The ovaries of *Chela fasciata* showed asynchronous oocyte development. The oocyte development was classified into nine different oogenic stages namely, chromatin nucleolus stage, early perinucleolus stage, late perinucleolus stage, yolk vesicle stage, primary yolk stage, secondary yolk stage, tertiary yolk stage, migratory nucleus stage and mature oocyte).

The first mature females appeared in the length group of 40 – 45 mm and males in the group of 25 – 30 mm. The size at first maturity for female was found to be at 45.75 mm TL (45 – 50 mm) and for males at 36.25 mm TL (35 – 40 mm). All the females were mature by 60.00 mm TL and the males by 45.00 mm TL.

Based on the ova diameter frequency study of the ripe ovary, *Chela fasciata* was found to be a multiple spawner, with a protracted spawning season, the individuals spawning intermittently. Absolute fecundity of the fishes ranged from 2669 to 4437 in fishes of size range 49.5 mm to 82 mm TL. The 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 (5% level of significance) with the length and weight of both the fish and ovary.

The embryonic development studies showed that the cleavage stage of the egg lasted for about 2 hrs 10 min., post fertilization. The inside egg embryo stage had lasted for 21 hrs, post fertilization. The eggs hatched at the end of 21 hrs, and the embryos emerged tail first. Pigmented eye was seen in one-day-old free embryo. Free embryo stage lasted for three days post fertilization. By the end of third day, the mouth developed, the yolk sac was empty and the exogenous feeding started, the free embryo entering the larval period.

A complete understanding of basic reproductive biology of this fish will definitely help in the commercial production under captivity for the domestic and export market of this indigenous ornamental fish.