# STUDIES ON SOME ASPECTS OF REPRODUCTION OF FEMALE ANABAS TESTUDINEUS (BLOCH)

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**COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY**

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**IN**

**MARINE BIOLOGY**

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**BY**

**Pius. K. Jacob**

DEPARTMENT OF MARINE BIOLOGY, MICROBIOLOGY AND BIOCHEMISTRY
COCHIN UNIVERSITY OF SCIENCE AND TECHNOLOGY
COCHIN – 682 016, INDIA

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# CERTIFICATE

This is to certify that the thesis entitled **Studies on some aspects of reproduction of female *Anabas testudineus* (Bloch)** is an authentic record of the research work carried out by Mr. Pius. K. Jacob, under my supervision and guidance in the Department of Marine Biology, Microbiology and Biochemistry, Cochin University of Science and Technology, Kochi, in partial fulfilment of the requirements of the degree of **Doctor of Philosophy** in Marine Biology of the Cochin University of Science and Technology and no part thereof has been presented for the award of any other degree, diploma or associateship in any university.

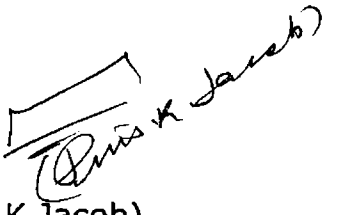
**Dr.C.K.Radhakrishnan**

Reader,
Department of Marine Biology,
Microbiology and Biochemistry,
School of Marine Sciences,
Cochin University of Science
and Technology,
Kochi – 682 016.

17-12-2005

# DECLARATION

I hereby declare that the thesis entitled **Studies on some aspects of reproduction of female Anabas testudineus (Bloch)** is an authentic record of the research work carried out by me under the supervision and guidance of **Dr.C.K.Radhakrishnan**, Reader, Department of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology, Kochi, for the Ph.D. degree in Marine Biology of the Cochin University of Science and Technology and no part thereof has been presented for the award of any other degree, diploma or associateship in any university.



Kochi,
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(Pius. K.Jacob)

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*... to my family*

# PREFACE

Fishes constitute more than half the number of thirty eight thousand vertebrates recognized world over. The number of valid fish species is placed at 24,618 under 482 families and 4258 genera. In our Indian region alone there are two thousand and five hundred species, of which nine hundred and thirty are inhabitants of fresh waters and the rest in seas (Jayaram, 1999). In Kerala, 207 fresh water fish species have been identified from various fresh water bodies (Ponniah & Gopalakrishnan, 2000).

As an additional source of human diet and as a component of various animal feeds, there is now a greater emphasis on fish production. Commercial aquaculture and sustained harvest thereof needs knowledge of reproduction and its control. For the development of domesticated brood stocks for consistent production of fry and fingerlings, it is necessary to gain control over all phases of fish lifecycle, especially reproduction.

Great diversities are seen in the reproductive patterns among the Indian teleosts; both fresh water and marine. Although majority of the species studied so far are known to breed annually during the monsoon, there are a few that breed biannually and a small number of species exhibit continuous breeding. Furthermore, among the annual spawners, different species utilize different seasons such as summer, monsoon and winter for their reproduction. Such diversities among the fishes suggest that the factors affecting gametogenesis and breeding may differ with the species.

About nine hundred fresh water fish species are known from different habitats of the Indian subcontinent. Out of these, only a few scores have been worked out for their reproductive cycles. In this context, it is evident that a lacuna exists that needs to be covered for better knowledge and scientific resource management.

Air breathing fishes in India form an economically important group of fishes; *Anabas testudineus* (Bloch) is one of them. This fish is highly esteemed for its highly nourishing quality and prolonged freshness out of water. It is suitable for cultivation in ponds, reservoirs and rice fields, and a sound knowledge on its reproduction and development is highly warranted.

A survey on literature showed that, barring one study on influence of estrogen on intermediary metabolism, and another on scale morphology, the species in Kerala has not been taken up for intensive investigations, especially for its reproductive biology, so far. It is against this background; the present study on the reproductive biology of the female *A. testudineus* (Bloch) was initiated. In the present work, basic aspects of reproduction, maturation and larval development, of *A. testudineus* (Bloch), an important air-breathing fish of Anabantid family, have been investigated.

The thesis is presented in seven chapters. There is no separate chapter on materials and method; instead, the same are dealt as part of each chapter. The first chapter surveys the literature on distribution, taxonomy, biology and reproduction, and presents a review of relevant works done in theses areas and important investigations on Anabantids in general, and on *Anabas species* in particular.

The second Chapter deals with the various aspects of the reproductive biology, such as the structure and organization of female reproductive system, classification of maturity stages, variation in gonadosomatic and hepatosomatic indices, the condition factor 'K' and relative condition 'Kn'. Oocyte size frequency distributions across various maturity stages, size at first maturity and sex ratio are also examined in this chapter. Fecundity and its correlation with the length, body weight, ovary weight, mean ovary length and mean ovary diameter are also discussed using the regression equations.

The third chapter is on the histology of the ovary, oogenesis, ovarian atresia and postovulatory follicles. A number of developmental stages of the oocytes are described and the histological observations related to macroscopic ovarian maturity stages. The fourth chapter describes the results of an experiment on induction of ovarian recrudescence during post spawning season, by photothermal treatment. In this chapter, ovarian responses to altered temperature and photoperiod are described in terms of variation in gonadosomatic index and histological changes in the ovary.

Results of experiments on induced breeding, forms the basis of the fifth chapter. The optimum dosage of the hormone, ovulation success, rate of fertilization and percentage of hatching are investigated. The sixth chapter deals with the development of larvae and their rearing. Suitability of *Artemia franciscana* as a feed for the larvae of *Anabas testudineus*, is examined using larval growth and survival as indices.

A summary of the results of investigations is presented in the seventh chapter of the thesis, which is followed by a detailed list of references.

# Chapter 1

# *Introduction*

# CHAPTER I

## 1. 1. INTRODUCTION

The labyrinth fishes, belonging to the suborder Anabantoidei, derive their name for having a labyrinth-like accessory breathing organ on either side of the head. Two widely known Asian members of the group are climbing perch (Anabas) and gouramy (Osphronemus). The climbing perch, *Anabas testudineus* (Bloch), also popularly known as '*Koi*' is a well known air breathing edible fish, inhabiting fresh waters and brackish waters of Southeast Asia. The common name, climbing perch, originated from the Asian legend that Anabas climbs palm trees to suck juice. Probably the origin of this myth is that birds pick Anabas when they travel overland and place it on palm trees (Norman, 1975).

Wakiama *et al.*, (1997) studied the genetical relationships of the 17-anabantoid fishes, and based on similarity of their alleles, divided them into three groups comprising: 1) *Anabas testudineus, Ctenopoma acutirostre*, *Osphronemus goramy*, *Ctenops nobilis* and *Luciocephalus pulcher*; 2) *Trichogaster leeri*, *T. trichopterus*, *T. microlepis*, *T. pectoralis*, *Colisa lalia*, *Sphaerichthys osphromenoides* and *Helostoma temmincki*; 3) *Betta splendens*, *Macropodus opercularis*, *Parosphromenus deissneri*, *Trichopis vittatus* and *Pseudosphromenus dayi*. Based on these studies, the Anabantidae, Macropodinae (excluding Ctenops) and Trichogastrinae are considered to be suitable taxonomic units.

**1. 2. General characteristics (Plate: 1. 1)**

The fish has a posteriorly compressed oblong body with a rather broad head and an anterior part. The mouth is not protractile and has small conical teeth on jaws and vomer. The gill covers are serrated and, unlike other teleosts, the opercular and subopercular bones are not fused into a single operculum. Instead, a thin, flexible membrane binds them, so that the fish has two sections of the gill cover hinged separately: the opercular on the suspensorium and the subopercular on the rear part of lower jaw. The gill covers open very widely and the subopercular rotates ventrally as well as laterally.

Dorsal and anal fins are long and composed of spines and soft fin rays. Dorsal and anal spines are strong with a larger soft ray portion. The caudal fin is rounded, scales are ctenoid. Fish are grayish black to dark brown in colour. Young fish have transverse dark stripes on hind part of the body and tail and a large dark spot at the base of caudal fin and a small one at hind border of the operculum.

*Anabas testudineus* shows great variability in morphological characters and body colouration. In body proportion, the Bengal forms are rather elongated than the Madras forms (Day, 1889). Based on morphometric characters, Das (1964) identified three ecological sub-species, *viz*; *Anabas testudineus riveri* (river Anabas), *A. testudineus lacustri* (lake and tank Anabas) and *A. testudineus ricei* (swamp Anabas). Rao (1968) revealed for the first time the presence of two species of Anabas in India, *A. testudineus* Bloch, 1792 that is distinguishable from *A. oligolepis* Bleeker, 1855 by less body depth,

longer pectorals, shorter snout and a dark spot at the base of the caudal fin.

Ramaseshaiah and Dutt (1984) compared electrophoretically both these species, which are found in a single habitat- lake Kolleru of Andhra Pradesh, and found them to be closely related. *A. oligolepis* has 46 chromosomes, while *A. testudineus* has 48 chromosomes (Dutt and Ramaseshaiah, 1980). The diploid number of chromosomes is 48 in either sex of *A .testudineus* (Kaur and Srivastava, 1965; Nayyar, 1966; Natarajan and Subramanian, 1974).

**1. 3. Distribution**

*A. testudineus* has a wide range of geographical distribution in nature. It is found in both fresh and brackish waters of India, Pakistan, Bangladesh, Nepal, China, Myanmar, Thailand, Cambodia, Philippines, Indonesia, Singapore, and Sri Lanka (Day,1889; Misra, 1959; Hora and Pillay, 1962). In India, they are found in Bihar, W. Bengal, Orissa, Uttar Pradesh, Andhra Pradesh, Tamil Nadu and Kerala (Besra, 1997). According to Myers (1937), Anabas has been carried by man across Wallace's line to the eastern part of the Indo-Australian Archipelago. Of the two spp. of Anabas, Shaji *et.al.*, (2000) and Gopi (2000), have included only *A. testudineus*, in their lists of fresh water fishes of western ghats and Kerala respectively.

**1. 4. General biology**

*A. testudineus* is an obligatory air-breathing fish. The fish gets asphyxiated, if denied access to water surface for longer periods. In

addition to four pairs of gills, it bears special structures called accessory air-breathing organs consisting of a pair of labyrinthine organs and the respiratory membranes covering the suprabranchial chamber. Each of the accessory respiratory organs situated on either side of suprabranchial region on the head is composed of a spacious air chamber communicating freely with bucco-pharyngeal cavity on one side and opercular cavity on the other. The detailed morphology and anatomy of the accessory respiratory organs of Anabas have been dealt by many authors (Misra and Munshi, 1958; Saxena, 1964; Munshi, 1968; Reddy and Natarajan, 1970, 1971; Hughes and Munshi, 1973).

### 1. 5. Habitat

*A. testudineus* is found in all types of waters of tropical and subtropical areas. They are more prevalent in derelict and swampy waters which are regarded as their habitual abode. Even though it is primarily a fresh water form, it shows a high salinity tolerance. *A. testudineus* fry measuring 14.0 mm can survive up to 11.5 % salinity (Khan *et al.,* 1976). Though the optimum tolerance of water temperature of the fish is 20-30°C, it can resist very low temperatures (Hora and Pillay, 1962). *A. testudineus* is quite hardy and is able to aestivate during the dry season, by burying in mud, like the African Lungfish (Thiraphan, 1984).

### 1. 6. Food and feeding habits

The natural food spectrum of *A. testudineus* is very wide and it may vary from a diet of filamentous algae to that of pure carnivorous nature. Larvae and young fry feed on phytoplankton and

zooplankton, large fry and adults feed on crustaceans, worms, molluscs, algae, soft higher plants and organic debris (Potongkam, 1972). Anabas has been described as a predator, carnivore (Pandey *et al.*, 1992) or an insectivore (Ahyaudin, 1992). However, gastric contents analysis of 204 specimens of Anabas showed that the stomach contained 19% crustaceans, 3.5% insects, 6% molluscs, 9.5% fishes, 47% plant debris and 16% semi-digested matter (Nargis and Hossain, 1987).

Major food items in the gut were found to be more or less consistent irrespective of spatial and seasonal distribution in Bangladesh (Nargis and Hossain, 1987), indicating that Anabas is an omnivore. The gastric pH was in the range of 5.96-6.58 indicating that Anabas is a stomached fish (Pandey *et al.*, 1992). During the larval period, 3-17 days from the inception of feeding, they can be fed only on live food (Doolgindachabaporn,1994).

**1. 7. Reproduction**

*A. testudineus* is bisexual. Sexual dimorphism is generally observed with the approach of the breeding season. Mookerjee and Mazumdar (1946 b) marked the sexual differences on the basis of the body and fin colourations. According to them, female '*Koi*', from west Bengal waters puts on a light brown color on the body and fins, particularly the pelvics which turn deep brown during breeding season. This colouration, which indicates the ripening of the ova, appears during pre-spawning season and gradually disappears after spawning. Mature males acquire glazy black colour during breeding season. Dehadrai *et al.*, (1973 b), where as, mark out the colour of the mature males of Bihar stocks as reddish hue on the body, particularly on the pectoral and

ventral fins, while females do not show reddish hue. Banerji and prasad (1974) reported that the colour differences as given by Mookerjee and Mazumdar (1946b) has not been noticed in any of the specimens examined from Bihar waters. However, colour pattern differs considerably in different localities.

According to Hora and Pillay (1962), '*Koi*' matures in about six months of age at about 8.0 cm of length. Chanchal *et al.* (1978) estimated on the basis of the relative condition factor the attainment of the first sexual maturity of male and female at 8.0 cm/11.3 g and 8.2 cm/12.2 g respectively.

Mookerjee and Mazumdar (1946 b) have described the mating behaviour of '*Koi*' in detail and observed that unlike other Anabantidae, the '*Koi*' neither builds nest of bubbles for laying eggs nor keeps guard or take care of the young. The fecundity of laboratory reared '*Koi*' is reported to be as low as 4,200 to 5,000, with an average of 4,591 and that of pond reared specimens varying between 8,500 and 10,804 with an average of 9,935 (Mookerjee and Mazumdar, 1946 b). Khan and Mukhopadhyay (1972) whereas found the ova number ranging from 10,002 to 36,477 in the fish procured from Port Canning in West Bengal. Fecundity of '*Koi*' from Bihar region have been recorded by Banerji and Prasad (1974) and Chanchal *et al.*, (1978).

*A. testudineus* is a seasonal breeder with long period of breeding. Spawning occurs once in a year. The spawning season in different localities of India and Bangladesh commence from April and extends to October, and in Indonesia from December to May (Hora and

Pillay, 1962). In Sri Lanka, the egg laden fish is found in January (Willey, 1910).

Khan (1972a), Khan and Mukhopadhyay (1975a), Banerji and Prasad (1974a) were successful in the induced breeding of '*Koi*' using carp pituitary extracts. Moitra *et al.*, (1979) recommended an optimum ambient temperature of 28.6 ± $1^0$C for breeding under laboratory conditions.

*A. testudineus* is a slow growing fish. It attained a maximum size of 114.5 mm/25g under laboratory conditions and 149 mm/54g in natural environment in a year (Mookerjee and Mazumdar, 1946b). Alikunhi (1957) and Chacko (1955) reported maximum lengths of 225 mm and 250 mm respectively. According to Hora and Pillay (1962), in ponds, '*Koi*' attains a length of about 102 mm and 200 mm at the end of the first and second year respectively. Laboratory feeding trials of Ray and Patra (1989), Sangrattanakhul (1989) and Doolgindachabaporn (1994) in *A. testudineus* fry indicated extremely efficient food conversion ratios.

A survey on the extant literature on *A. testudineus* reveals that the studies on this fish has been mostly done in India, barring some works in Thailand and Bangladesh. Majority of the studies in India, on '*Koi*' pertains to its biology, toxicology, endocrinology, biochemistry, apart from genetics, and ethology. Shashi and Akela (1996), Chanchal *et al.*, (1978) and Ramaseshaiah (1985), are the important contributors on its reproductive biology. Biology of the species has been explored by Pandey *et al.*, (1992), while Ray and Patra (1989) studied the growth response of '*Koi*' to different dietary protein sources. Thakur and Das

(1986) have presented a synopsis of biological data on '*Koi*'. Endocrinological investigations include those of Guin *et al.*, (1993), Halder *et al.*, (1991), Chakraborti and Battacharya (1982, 1984), Chakraborti *et al.*, (1983,1986), Battacharya and Sen (1981), Jana and Battacharya (1993), Deb and Battacharya (1988) and Bandyopadhyay and Battacharya (1994). Toxicological works include those of Tulasi *et al.*, (1989), Panigrahi *et al.*, (1978), Choudhury *et al.*, (1993), Nuradha and Raju (1996), Bakthavalsalam (1986) and Vijayram *et al.*, (1991). Biochemical investigations have been made by Dasgupta and Sircar (1986), Sen *et al.* (1985), Murugaboopathy *et al.*, (1985) and Vijayaraghavan and Rao (1984). Electrophoretic studies on the two species of Anabas by Ramaseshaiah and Dutt (1984) and works on their karyology by Dutt and Ramaseshaiah (1980) deserves special mention. Pandey and Sukla (1982), Pal and Pal (1985) Pal (1988), Binoy and Thomas (2004), are among the contributors to ethological investigations on the species. Some other studies on the species include those of Sinha *et al.*, (1982), Pathak *et al.*, (1980), Sen *et al.*, (1979), Agarval *et al.*, (1980), Jose and Sathyanesan (1980), Chanchal and Pandey (1979), Mishra and Singh (1979), Khan *et al.*, (1976), Pandey *et al.*, (1978), Pal and Pradhan (1990), Rao *et al.*(1981), Hughes *et al.*(1986), Kaippallil and Oommen (1994), Prakasm and Johnson (1988), Baksi and Pal (1993), Halder *et al.*, (1991), Sen and Battacharya (1982), Sen *et al.*, (1979), Rahmani and Khan(1981), Pal *et al.*, (1978), Ojha *et al.*, (1975), Pandey and Mishra (1992), Srivastava *et al.*, (1990) and Jha (1993).

The foregoing review of the literature on *A. testudineus* indicates that the species has been studied well only in North India. Further, the knowledge on the reproductive biology of the species is fragmentary, whereas information on the development and maturation of

the gonad at cellular level are scant. The species has not been considered for detailed investigations in Kerala. The present study therefore, is an attempt to learn more about the reproductive biology and the related aspects of reproduction of *Anabas testudineus* (Bloch).

**1. 8. Systematics**

| | |
|---|---|
| Order | : Perciformes |
| Suborder | : Anabantoidei |
| Family | :Anabantidae |
| Genus | :Anabas Cuvier, 1817 |
| Species | : *Anabas testudineus* Bloch, 1792 |

**1. 9. Synonyms:** *Perca scandens* Daldroff, 1797; *Cojus cobojius* Hamilton, 1822; *Anabas scandens* Bleeker, 1850.

**1. 10. Vernacular names:** *Koi*, *Koyee* (Bengali), *Kai* (Assamese), *Kou* (Oriya), *Kobhai*, *Kavai* (Hindi), *Sennal* (Tamil), and *Karoopu*, *Karippidi* (Malayalam).

**1. 11. Distinguishing Characters:**

D XVI-XVIII, 8-10; A VII-XI, 9-11; P 13-14; V 1, 5.

Anterior part of the body and head are broad while the posterior part is compressed. Mouth is terminal and relatively large. Jaws bear villiform teeth. Dorsal fin has 16-18 strong spines and 8-10 soft rays. Pectoral fins are blunt and rounded. Pelvic fins are with one spine and five soft rays and Caudal fin is rounded. Live fish is dark to pale

yellowish/greenish in cvolour; posterior margin of opercle bears a dark spot; base of caudal fin also possess a dark spot. Iris is golden reddish in colour; while, scales are large and ctenoid.

### 1. 12. Culture prospects

Extremely efficient food conversion ratio (FCR) values, air-breathing ability and tolerance to adverse environmental conditions make *A. testudineus*, an excellent candidate for aquaculture, perhaps more so than any other tropical fish. However, slow growth rate may be a constraint for commercial viability.

**Status as per IUCN:** *LRnt* (Low risk, nearly threatened).

Plate. 1. 1

**Fig.** Photograph of *Anabas testudineus* (Bloch)

# Chapter 2

# *Reproductive Biology*

# CHAPTER II

## REPRODUCTIVE BIOLOGY

### 2. 1. INTRODUCTION

Reproduction is a very complex process that involves synchronized gametogenesis, development of the accessory reproductive organs and secondary sexual characters, migration to breeding grounds, courtship behaviour, breeding etc. It also ensures that the offspring have abundant food supply and optimum climatic conditions for their growth and survival. This means that the animals should be able to predict the climatic changes several months in advance so that the onset of gametogenesis and the breeding times are precisely determined.

The reproductive cycle of different species of fish have developed in response to their natural range and habitat. The timing of spawning in annual spawning fish has developed as a response to ultimate factors that will maximize the survival of the eggs and fry. Some of these ultimate factors include water quality, food availability, reduced number of predators *etc* (Sumpter, 1990).

There are three basic strategies for the production of eggs and their subsequent spawning. The first strategy, which is used by pacific salmon, is "synchronous spawning". One crop of eggs and sperms are produced and spawned at one time after which the fish dies. A second strategy is called "group synchronous", in which groups of eggs are produced and spawned at one time; but several cycles of development and spawning occur. The cycle can take a year in annual spawning fish

or it may take a few weeks with spawning occurring several times during a given season. The last strategy is "asynchronous spawning", where there is continuous development and spawning of oocytes. In some species, individuals can spawn a few eggs every day throughout a spawning season.

The survival or existence of any species is ultimately determined by the ability to reproduce successfully in a fluctuating environment. A thorough knowledge of maturation cycle and depletion of gonads will help to understand and predict the annual changes that population undergoes. The process of maturation, size at first maturity, frequency and extent of spawning and fecundity are some very important biological aspects to be studied for economically important fishes. Variations in any or in more than one of these aspects due to variations in geographical locations or in the environment are not uncommon. An understanding of these factors is essential for the preservation, development and exploitation of the stock.

From a review of the literature, it is apparent that, studies on the reproductive biology of fishes are vast and mostly concerning the marine teleosts. A review of studies on the breeding biology of marine teleosts from Indian waters by Qasim (1973) deserves special mention. Bagarinao and Chua (1986) studied the relationship between the egg size and larval size among teleosts. Witthames and Walker (1987) developed an automated method for counting and sizing fish eggs, while Shoesmith (1990) compared the different methods for estimating mean fecundity. A gross measure of reproductive condition (ovary weight adjusted for body size and oocyte volume) was developed by Martini and Lau (1999), as an

alternative to commonly used gonadal indices for classifying the maturity stages in two species of etelline snappers (Lutjanidae).

Some other important works on the reproductive biology of marine teleosts are those of Clark (1934), June (1953), Yuen (1955), Sobhana and Nair (1974), Htun-Han (1978a,b), Wallace and Selman (1979), Delahunty and De Vlaming (1980a), Chan and Chua (1980), De (1980), Blay Jr and Eyeson (1982), Robb (1982), Cyrus and Blaber (1984), Chubb and Potter (1984), Marcus and Kusemiju (1984), Pankhurst *et al.*, (1988), Choudhury *et al.*, (1990), Koya *et al.*, (1995), Kamisaka *et al.*, (1999).

Highlighting a few studies on reproductive biology of freshwater fishes, Devaraj (1973) and Babiker and Ibrahim (1979) have worked on gonadal maturation and fecundity of *Ophiocephalus marulius* (Ham.) and *Tilapia nilotica* (L) respectively. Najim and Bhatti (1979) investigated annual changes in the ovarian activity of *Barbus luteus* (Heckel). Hails and Abdullah (1982) have studied the breeding cycle of an exotic Anabantid *Trichogaster pectoralis* (Regan). Bhimasena Rao and Karamchandani (1986) studied the spawning biology of *Ompok bimaculatus* (Bloch). Mollah (1986) investigated the cyclic changes in the ovary of a fresh water catfish *Clarias macrocephalus* (Gunther). De graph *et al.*, (1999) found no relation between the condition factor and the size at first maturity or relative fecundity, in Nile tilapia, O*reochromis niloticus* (L.)

There are very few studies available on the reproductive aspects of *A. testudineus*. Chanchal *et al.* (1978) have worked out the size at maturity, sex ratio, and relationship of the gonad weight, gonad length

and fecundity with the weight and length of the body in *A. testudineus* from Bihar. Ramaseshaiah (1985) has estimated the fecundity of *A. testudineus* from lake Kolleru of Andhra Pradesh. Shashi and Akela (1996) used gonadosomatic index and ova diameter measurements to determine maturity and spawning period in *A. testudineus*.

Available literature indicates that *A. testudineus* from Kerala has not been considered for any study on its reproductive aspects and therefore the present study was taken up to shed some light on various aspects of its reproduction such as gonadal maturation, spawning periodicity, spawning season, size at maturity, sex ratio, and fecundity.

## 2. 2. MATERIALS AND METHOD

The present work was carried out at the Department of Marine Biology, Microbiology and Biochemistry of Cochin University of Science and Technology, Kochi.

### 2. 2. 1. Collection of specimens

Regular fortnightly collections of fresh specimens were made between July 1998 and June 2000 from Trichur '*kole*' lands (Lat. $10^0$ 34' N and $10^0$ 36' N and Long. $76^0$ 06' E and $76^0$ 09' E) and adjoining fresh water bodies. Collections from July 1998 to June 1999 were mainly used to fix the maturity stages and for studies on sex ratio. Collections of the succeeding year comprising of 319 females were used for the rest of the analyses. The fish ranging in size from 6.8 cm and 19.0 cm in total length and 4.2 g and 152.6 g in total body weight were caught using cast nets or hook and line or during the operation of large sized traditional pumps. Surface water temperature was noted and salinity measured using a salinometer. Live specimens were then brought to the laboratory for further analyses.

### 2.2. 2. Morphometry

The specimens were wrapped in blotting paper to remove excess moisture from their bodies. They were then measured to the nearest millimeter (mm) for their total length (from the tip of the snout to the tip of the caudal fin) and then weighed to the nearest 0.01gram (g). The females above 6. 8 centimeters (cm) alone were selected for further analyses, as ovaries could be visibly distinguished, with some ease, from

testes only at this size. Each specimen was then dissected and the gonad examined. Each specimen was then assigned a number. Colour and appearance of the ovary was recorded, weighed to the nearest milligram and its length and width measured to the nearest mm using vernier calipers.

The number of maturity stages assigned to the developing gonads by different workers vary between 4 and 14. In the present case, the ovaries were grouped into five maturity stages, following the scheme of Qasim (1973), and Geevarghese and John (1982). The characters used for the classification of the ovary were appearance, colour, size, relative space occupied in the body cavity, size of the ova and their yolk content and microscopic observations such as ova diameter measurements.

### 2. 2. 3. Gonadosomatic index (GSI)

Gonadosomatic index (GSI) or the maturity coefficient, for each fish was calculated using the formula,

$GSI = \frac{GW}{BW} \times 100$, Where GW = gonad weight of fish in grams (g) and

BW = Body weight of fish in grams.

The range and average values of GSI were determined maturity stage wise and month wise.

### 2. 2. 4 Condition factor 'K'

The ponderal index or condition factor 'K' for each fish was calculated by the following formula, as suggested by Hile (1936):

$K = \frac{W}{L3} \times 100$ where,

K = condition factor,

W = Body weight of fish in grams

L = Body length of fish in cm

The range and average values of 'K' were determined maturity stage wise and month wise.

### 2. 2. 5. Length-weight relationship

Length-weight relationship was worked out by the method of least squares and any deviation from "cube law" (spencer, 1871) was found out by performing Student's t-test (Snedecor and Cochran, 1967).

t = (b – B) / sb where, b = regression coeffiecient, sb = standard error of b, and B = 3

Relative condition, 'Kn' was found following the method of Le Cren (1951), using the formula,

$'Kn' = \frac{W}{\hat{W}}$ where, W= observed total weight and $\hat{W}$ is the calculated weight.

The value of $\hat{W}$ for each fish was derived from the parabolic equation,

$W = aL^b$ , the logarithmic transformation of which gives the well known linear equation:

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

where, W = weight, L = length, a = a constant being the initial growth index and b = growth coefficient. Constant 'a' represents the point at which the regression line intercepts the y-axis and 'b' the slope of the regression line. The 'Kn' values in different maturity stages, months and length groups were found out. The variations in relative condition with

length, was also taken into consideration as an indicator of sexual maturity.

### 2. 2. 6 . Hepatosomatic index

The hepatosomatic index (HSI) for each fish was calculated by the following formula as suggested by Htun-Han (1978 c) and Crupkin *et al.*, (1988).

$$HSI = \frac{\text{Liver weight}}{\text{Whole body weight}} \times 100$$

The range and average values of the liver index was found with respect to each maturity stage and for the different months.

### 2. 2. 7. Spawning periodicity

Spawning periodicity was determined by ova diameter measurements done in ovaries belonging to various developmental stages following Clark (1934), Prabhu (1956), Sobhana and Nair (1974) and Acharya (1990). Ova diameter–frequency polygons were drawn to trace the development of oocyte from immature stage to ripe condition. Test measurements of ova from the anterior, middle and posterior regions of the ovary showed no significant variation in their relative size and hence not less than 200 ova from the middle portion of each ovary was found to be sufficient to represent all stages. A representative piece from the fresh ovary was gently teased on a large drop of 0.6 % saline on a clean glass slide and diameter of ova were measured along the horizontal axis using a calibrated ocular micrometer. Pooled averages of ova diameter measurements from ovaries of the same stage of maturity

were plotted at 60 μm class intervals. The diameter of the largest ova in each ovary was also found out.

### 2. 2. 8. Fecundity

The potential fecundity or the number of eggs available to be spawned in a single breeding season was estimated from ovaries of fishes in late mature stage III and early ripe stage IV. About 500 mg ovary sample taken from the middle portion of the ovary was put in modified Gilson' s fluid (Simpson, 1951) for later counting. The method described by Grimes and Huntsman (1980) was followed which involved counting of the number of mature ova in a portion of known weight of ovary and then calculating the total number of mature ova by the formula,

$$\text{Fecundity} = \frac{\text{Weight of the ovary}}{\text{Weight of sub sample}} \times \text{number of mature ova in sub sample}$$

The fecundity (F) thus estimated was studied in relation to several variables such as the total length of fish (TL) in mm, fish weight (W) in gram, ovary length (OL) in mm and ovary weight (OW) in gram, by the method of least squares.

### 2. 2. 9. Size at first maturity

Size at first maturity, which is defined as the total length (mm) at which 50% of the samples were in mature group, was determined using the samples collected from December to June, being the period when fishes from stage III (mature) and above are observed. The fishes were grouped into 10 mm size classes and the percentage mature in each size class was calculated.

## 2. 2. 10. Sex ratio

Sex ratio was calculated for various months. The percentage occurrence of males and females was calculated by employing the chi-square test ($\chi^2$-test) that tests the homogeneity of the distribution of either sex in the population.

$$\chi^2 = \Sigma \left[ \frac{(O-E)^2}{E} \right]$$ Where,

O = Observed number of males or females in each month

E = Expected number of males or females in each month.

## 2. 3. RESULTS

### 2.3.1. Sexuality

*A. testudineus* is bisexual. Sexual dimorphism is not marked, except that often males tend to be smaller and slender than the females. However, with the approach of the breeding season, the ripe females could be observed to have bulged abdomen due to the enormous increase in size of the ovaries, and upon slight pressure on the abdomen, eggs are extruded. Similarly, ripe males milt upon applying gentle pressure on their abdomen. The largest female recorded in the present study measured 19.0 cm in total length and weighed 152.6 g. The heaviest ovary weighing 14.0 g was found in a fish of 17.3 cm total length and 97.7 g body weight. Breeding marks, like pigmentation on body parts, reported in some teleosts were not observed in any of the specimens collected during the present study.

### 2. 3. 2. Structure of the female reproductive system (Pl: 2. A-C)

Ovaries of *A. testudineus* consisted of two slightly unequal sized cylindrical lobes resting on the ventral wall of body cavity, ventral to the kidneys. In immature fishes, the ovaries were ribbon like and closely resembled the immature testes in appearance. The ovaries are attached to the dorsal coelomic wall by a thin membranous mesovarium. The two lobes opened posteriorly into a common oviduct leading to the vent. Judged from their mean values, the left ovary appeared to be slightly longer in stage II (Fig: 2. 1). Therefore, Student's t-test was performed (Table. 2. 2) and observed difference was found to be not significant ($p < 0.05$).

### 2. 3. 3. Maturity stages of the ovary (Table- 2. 1)

Juveniles of *A. testudineus* did not exhibit internal differentiation of sex. At about a size of 6.5-7.0 cm (total length) the females could be distinguished from males by the presence of pinkish, translucent jelly like soft gonadal tissue, occupying one-fourth of the posterior part of the body cavity. In males of similar size, the testes appeared as thin whitish strands in the body cavity. An arbitrary five-stage maturity scheme was assigned based on gross morphology of ovary such as size, colour and texture and microscopic observations on the oocytes.

Stage I (Immature):

Pinkish, translucent and jelly like ovary measured 6.0 to 21.0 mm in length and 1.0 to 2.0 mm in width and occupied about one fourth of the body cavity of the fish. Ovary weighed a minimum of 7.0 mg; GSI ranged from 0.03 to 0.35 with a mean of 0.13 and the minimum size of the fish was 6.8 cm in total length. Ova were not visible to the naked eye. Oocytes under magnification appeared polygonal, translucent, with a central clear nucleus and without yolk. Diameter of the prominent groups of ova ranged from 61 to 120 µm.

Stage II (Maturing virgins and recovering spent):

Orange red nearly opaque cylindrical ovary measured 12 to 30 mm in length and 1.5 to 5.0 mm in diameter and occupied about one third of the body cavity of the fish. Ovary weighed a minimum of 30 mg; GSI ranged from 0.05 to 0.74 with a mean of 0.25 and the minimum size of the fish was 7.3 cm in total length. Ova were not clearly visible to the

naked eye. Most of the oocytes under magnification appeared rounded and translucent. Diameter of the prominent groups of ova ranged from 61 to 180 µm. A few residual atretic oocytes were observed in some of the recovering spent fishes.

Stage III (Mature):

Yellowish enlarged ovary measured 14 to 41 mm in length and 2 to 12 mm in width and occupied nearly two third of the body cavity of the fish. Ovaries appeared granular due to the eggs that were visible to the naked eye. Ovary weighed a minimum of 50 mg; GSI ranged from 0.33 to 7.3 with a mean of 2.95 and the minimum size of the fish was 8.1 cm in total length. Most oocytes were yolk laden and appeared opaque under the microscope. Diameter of the prominent clutches of ova ranged from 121 to 360 µm.

Stage IV (Ripe):

Pale yellow to grayish or brownish, bulky, thin walled ovary, were found packed with ripe eggs filling entire body cavity of the fish. Eggs were clearly visible through the thin ovary wall. Ovary measured 22 to 50 mm in length and 8 to 25 mm in width and weighed a minimum of 1440 mg. GSI ranged from 4.02 to 14.6 with a mean of 9.84 and the minimum size of the fish was 9.7 cm in total length. The female fishes had bulged bellies and extruded eggs upon mild pressure on their abdomen. Under the microscope, eggs appeared translucent. Diameter of the prominent clutches of eggs ranged from 481 to 780 µm.

Stage V (Spent):

Collapsed and highly shrunken, blood shot ovary had reddish colour towards its posterior end and contained a few whitish opaque residual eggs. Ovary measured 15 to 35 mm in length and 2.5 to 8.5 mm in width and weighed a minimum of 142 mg. GSI ranged from 0.28 to 1.77 with a mean of 0.93 and the minimum size of the fish was 10.9 cm in total length. Residual eggs ranging from 661 to 780 µm were found in the midst of many smaller oocytes measuring up to 180 µm.

### 2.3.4. Gonadosomatic index (GSI) (Table-2. 3-4; Fig. 2. 2-4)

The mean gonad weight increased steadily from 0.02 g in stage I to reach a high of 5.83 g in stage IV and declined to 0.62 g in stage V. Similarly, the mean GSI increased steadily from 0.13 in stage I to reach a high of 9.84 in stage IV and declined to 0.93 in stage V. The mean GSI in the month of July was 0.13, which gradually increased with a distinct upward trend in February to reach a high of 1.92, and further increased to reach the peak value of 8.67 in May. It then declined to 2.03 in June, which indicated spawning activity.

### 2. 3. 5. Condition factor 'K' and Relative condition 'Kn' (Table-2. 3-4; Fig. 2. 5-9)

Values of condition factor 'K' in the different maturity stages remained around 1.80 ± 0.08. Value of 'K' fluctuated marginally between months. Since deviation from "cube law" was found to be statistically significant ($p > 0.001$), relative condition 'Kn' was worked out.

Relative condition 'Kn' was the highest in stage II with a value of 1.03, followed by 1.0 each in stage I and V, and 0.95 each in stage III and IV respectively. Monthly 'Kn' values fluctuated in a cyclical pattern. High 'Kn' values of 1.09, 1.02 and 1.06 were found in the months of July, August and September respectively. It then declined to a value of 0.94 in October, returned to closely similar previous values from November to February, and again declined to 0.90 in March, which further increased for the next couple of months to decline further to a value of 0.94 in June.

The lowest 'Kn' value was found in the 6.1-7.0 cm length group (total length) with a value of 0.81 and highest value of 1.16 in 7.1-8.0 cm size group. This increase in relative condition in 7.1-8.0 cm size group indicates the building of reproductive materials prior to spawning. The first spawning or first sexual maturity is attained when the relative condition dropped to the lowest figure of 0.96, which occurred from 9.1 cm (total length) onwards.

### 2.3.6. Hepatosomatic Index (HSI) (Table-2. 3-4; Fig. 2. 10-11)

The mean hepatosomatic index (HSI) was the highest in stage IV with a value of 1.16 that declined to 0.86 in stage V. The mean value of HSI in stage I was at a low of 0.97 that increased to 1.00 in stage II and declined to 0.84 in stage III. Seasonal values of HSI fluctuated with almost regular vicissitudes between 0.59 and 1.49, the highest value being found in June.

### 2. 3. 7. Spawning periodicity (Table- 2. 5; Fig. 2. 12)

The observations were on 8-10 ovaries for each stage of maturity. The percentage frequency distribution of different sizes of oocytes in the ovaries of the five maturity stages is shown in fig: 2. 12. The stacked areas represent average frequencies from samples of specimens representing the same stage of maturity. Majority of ova in the stage I ovary were of the size group 61-120 µm, with the largest oocyte diameter (LOD) at 152 µm and a modal size of 90 µm. This modal size increased to 140 µm in stage II, with a range of 61-180 µm and the LOD at 254 µm.

In the subsequent stages, only two distinct groups of ova were present in the ovaries representing the immature and maturing/ mature ova (fig: 2. 12). In stage III, the maturing oocytes had a modal size of 240 µm within a range of prominent group of oocytes of 121-360 µm size and with the LOD at 630 µm. Ripe ovary (stage IV) had a distinct group of eggs with a mode at 600 µm within a range of prominent group of oocytes of 481-780 µm and the LOD at 870 µm. An immature reserve stock of oocytes was always observed in ovaries belonging to all stages of maturity. In stages III and IV, the modes representing mature and ripe eggs remained well separated and there was no sign of a second group of maturing ova. Stage V (spent) had an immature stock of oocytes within a range of 1-180 µm, plus a few residual eggs.

### 2. 3. 8. Fecundity (Fig. 2. 14 - 18)

Fecundity estimates were based on the enumeration of mature eggs from 25 specimens with mature (stage III) or ripe (stage IV)

ovaries. Fecundity varied from 575 to 59022 in individuals of total length from 89 to 187 mm, whole body weight between 12.74 g and 125.4g, ovary weight between 0.27 g and 14 g, ovary length between 15 and 50 mm and ovary diameter between 4 and 21.5mm. The mean fecundity was 20384. Fecundity showed a decreasing trend above a total length of 17.6 cm. The relationship between fecundity (F) and total length (L) is depicted in Fig. 2. 14, which in the logarithmic form could be expressed as

$$\text{Log } F = -5.2340 + 4.3283 \text{ Log } L$$

$$r = 0.687$$

The relationship between fecundity (F) and total body weight (W) is shown in Fig. 2. 15 and related as follows:

$$\text{Log } F = 1.7482 + 1.3749 \text{ Log } W$$

$$r = 0.698$$

Fecundity data were analyzed in relation to ovary length (OL), which is presented in Fig. 2. 16. The relationship could be expressed as

$$\text{Log } F = -1.449 + 3.7249 \text{ Log OL}$$

$$r = 0.953$$

The correlation between fecundity and ovary diameter (OD) is expressed in Fig. 2. 17 and is related as follows:

$$\text{Log } F = 1.5441 + 2.5056 \text{ Log OD}$$

$$r = 0.948$$

The fecundity and ovary weight (OW) relationship, presented in Fig. 2. 18 could be expressed in the form of an equation:

$$\text{Log } F = 3.5742 + 1.0758 \text{ Log OW}$$

$$r = 0.980$$

The correlation was found to be positive in all cases. Highest correlation existed between fecundity and ovary weight.

### 2 . 3 . 9. Size at first maturity (Fig. 2. 9, 19)

The smallest mature (Stage III) fish was 81.0 mm in total length; smallest ripe fish (Stage IV) was 97.0 mm long and smallest spent fish (Stage V) measured 109.0 mm. For finding the size at first maturity, fishes in stage I and II were treated as immature and stages III and above as mature. Since 50 % of the females were found to be mature at 97.8 mm ( Fig. 2. 19), the latter was regarded as the size at first maturity. For a comparison, the values of relative condition 'Kn' for different size groups may be consulted (Fig. 2. 9). An increase in 'Kn' value to 1.16 was found in 71-80 mm size group, indicative of the building of reproductive materials prior to spawning. The first sexual maturity is attained when 'Kn' value dropped to the lowest figure of 0.96, which occurred from 91 mm onwards. All females above 130 mm were found to be mature.

### 2. 3. 10. Sex ratio

The monthly sex ratios and their chi- square values are shown in Table- 2. 6. When all the samples were pooled, a ratio of 1.0 male : 0.94 female was obtained. On a monthly basis, except for February and April, the ratio did not differ significantly from the expected 1:1 ratio.

**Table. 2. 1.** Different ovarian maturity stages in Female *A. testudineus*

| Stage of maturity | Description |
|---|---|
| I (Immature) | Pinkish, translucent, jelly like ovary occupying 1/4 of the body cavity. Oocytes not visible to the naked eye, but under magnification appear polygonal and translucent; with a central nucleus and without yolk. |
| II (Maturing virgins and Recovering spent) | Orange red, nearly opaque cylindrical ovary occupying about 1/3 of the body cavity. Oocytes not visible to the naked eye, but under magnification appear rounded and translucent. |
| III (Mature) | Yellowish enlarged ovary, occupying about 2/3 of the body cavity. Ovary appears granular due to the eggs that are visible to the naked eye. Most oocytes yolk laden, which appear opaque under the microscope. |
| IV (Ripe) | Pale yellow to grayish or brownish bulky thin walled ovary packed with ripe eggs filling entire body cavity of the fish. |
| V (Spent) | Collapsed and highly shrunken blood-shot ovary with reddish colour towards its posterior end; many whitish opaque residual eggs visible. |

**Table. 2. 2.** Student's t- test for deviation from equivalence of ovarian lengths between left and right ovaries of *Anabas testudineus*

| Maturity stage | t-value | df | Remarks |
|---|---|---|---|
| I | 0.388 | 46 | P < 0.05* |
| II | 1.667 | 119 | P < 0.05* |
| III | 0.288 | 40 | P < 0.05* |
| IV | 0.068 | 46 | P < 0.05* |
| V | 0.21 | 14 | P < 0.05* |

*** not significant**

**Table. 2. 3.** Gonadosomatic index (GSI) , condition factor `K` and Hepatosomatic index (HSI) at different maturity stages in Female *Anabas testudineus.*

| Maturitystages | GSI (%) | Condition factor `K` | Relative condition 'Kn' | HSI (%) |
|---|---|---|---|---|
| Stage I | 0.13 ± 0.06 | 1.73 ± 0.29 | 1.00 ±0.18 | 0.97 ± 0.21 |
| Stage II | 0.25 ± 0.16 | 1.88 ± 0.17 | 1.03±0.08 | 1.00 ± 0.31 |
| Stage III | 2.95 ± 2.01 | 1.74 ± 0.18 | 0.95±0.08 | 0.84 ± 0.18 |
| Stage IV | 9.84 ± 3.52 | 1.77 ± 0.18 | 0.95±0.09 | 1.16 ± 0.56 |
| Stage V | 0.93 ± 0.54 | 1.87 ± 0.23 | 1.00±0.11 | 0.86 ± 0.16 |

All values are mean ± standard deviations

**Table. 2. 4.** Gonadosomatic index (GSI), Condition factor `K`, Relative condition 'Kn' and Hepatosomatic index (HSI) during different months in female *Anabas testudineus.*

| Month | GSI (%) | Condition factor `K` | Relative condition 'Kn' | HSI (%) |
|---|---|---|---|---|
| Jul | 0.13 ± 0.06 | 1.87 ± 0.44 | 1.09±0.28 | 1.05 ± 0.19 |
| Aug | 0.13 ± 0.05 | 1.84 ± 0.20 | 1.02±0.10 | 0.81 ± 0.15 |
| Sep | 0.27 ± 0.16 | 1.81 ± 0.19 | 1.06±0.11 | 1.32 ± 0.21 |
| Oct | 0.17 ± 0.07 | 1.78 ± 0.26 | 0.94±0.13 | 0.83 ± 0.16 |
| Nov | 0.19 ± 0.07 | 1.75 ± 0.10 | 1.02±0.06 | 0.88 ± 0.16 |
| Dec | 0.56 ± 1.20 | 1.73 ± 0.17 | 1.05±0.07 | 1.19 ± 0.25 |
| Jan | 0.26 ± 0.17 | 1.70 ± 0.14 | 1.03±0.06 | 0.83 ± 0.15 |
| Feb | 1.92 ± 2.57 | 1.67 ± 0.16 | 1.03±0.10 | 0.69 ± 0.15 |
| Mar | 2.62 ± 1.86 | 1.64 ± 0.13 | 0.90±0.05 | 0.81 ± 0.18 |
| Apr | 6.86 ± 4.67 | 1.61 ± 0.16 | 0.99±0.08 | 0.59 ± 0.11 |
| May | 8.67 ± 4.80 | 1.58 ± 0.14 | 0.96±0.07 | 0.84 ± 0.28 |
| Jun | 2.03 ± 3.53 | 1.55 ± 0.20 | 0.94±0.11 | 1.49 ± 0.42 |

**All values are mean ± standard deviations.**

**Table. 2. 5.** Percentage frequency of Oocytes at different stages of maturity in *A. testudineus*.

| Oocyte diameter range (µm) | Percentage of oocytes | | | | |
|---|---|---|---|---|---|
| | **Stage I** | **Stage II** | **Stage III** | **Stage IV** | **Stage V** |
| 1-60 | 15.40 | 7.94 | 2.10 | 1.22 | 28.10 |
| 61-120 | 66.80 | 35.11 | 8.30 | 0.22 | 32.13 |
| 121-180 | 16.70 | 38.78 | 32.67 | 0.11 | 16.12 |
| 181-240 | 1.00 | 14.44 | 28.33 | 0.84 | 11.80 |
| 241-300 | 0.10 | 2.61 | 13.67 | 1.83 | - |
| 301-360 | - | 0.83 | 6.33 | 1.83 | - |
| 361-420 | - | 0.11 | 3.33 | 1.90 | - |
| 421-480 | - | 0.17 | 2.00 | 1.55 | - |
| 481-540 | - | - | 0.67 | 9.10 | - |
| 541-600 | - | - | 1.91 | 34.93 | - |
| 601-660 | - | - | 0.67 | 17.97 | 0.56 |
| 661-720 | - | - | - | 10.13 | 9.10 |
| 721-780 | - | - | - | 11.72 | 2.16 |
| 781-840 | - | - | - | 4.33 | - |
| 841-900 | - | - | - | 1.89 | - |
| Oocyte diameter mode (µm) | 90 | 140 | 240 | 600 | 70 |
| Largest Oocyte diameter(LOD) (µm) | 152 | 254 | 630 | 870 | 210 |

**Table. 2. 6.** Monthly sex ratio of *A. testudineus* (1998-1999).

| Month | No. of fish sexed | No. of males | No. of females | sex ratio (male:female) | $\chi^2$ |
|---|---|---|---|---|---|
| Jul | 29 | 15 | 14 | 1:0.93 | 0.03* |
| Aug | 27 | 15 | 12 | 1:0.80 | 0.33* |
| Sep | 20 | 6 | 14 | 1:2.33 | 3.20* |
| Oct | 73 | 41 | 32 | 1:0.78 | 1.11* |
| Nov | 58 | 24 | 34 | 1:1.42 | 1.72* |
| Dec | 63 | 27 | 36 | 1:1.33 | 1.29* |
| Jan | 71 | 32 | 39 | 1:1.22 | 0.69* |
| Feb | 33 | 25 | 8 | 1:0.32 | 8.76** |
| Mar | 24 | 13 | 11 | 1:0.85 | 0.17* |
| Apr | 43 | 30 | 13 | 1:0.43 | 6.72** |
| May | 34 | 16 | 18 | 1:1.13 | 0.12* |
| Jun | 50 | 26 | 24 | 1:0.92 | 0.08* |

*** Not significant at 5% level of probability.**

**** Significant at 5% level of probability.**

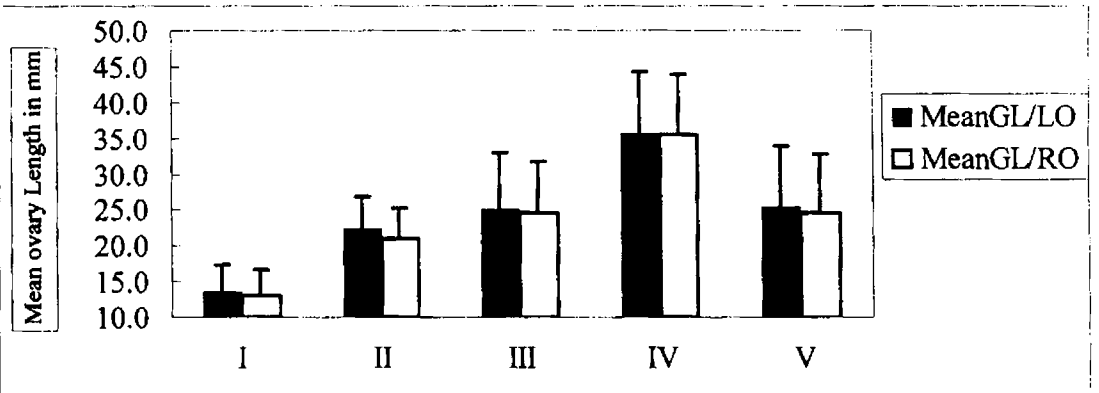



**fig: 2. 1.** Variation in the lengths of the two lobes of the ovaries in different maturity stages of *A. testudineus* , GL=Gonad length,LO=Left ovary,RO=Right ovary.

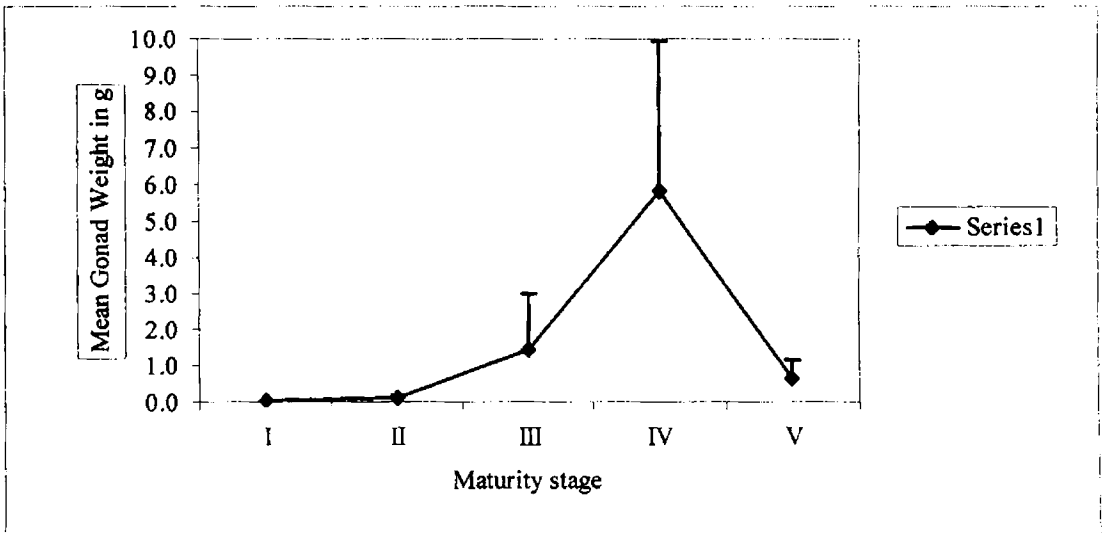



**Fig: 2. 2.** Variation in gonad weight in Different maturity stages of female *A. testudineus*

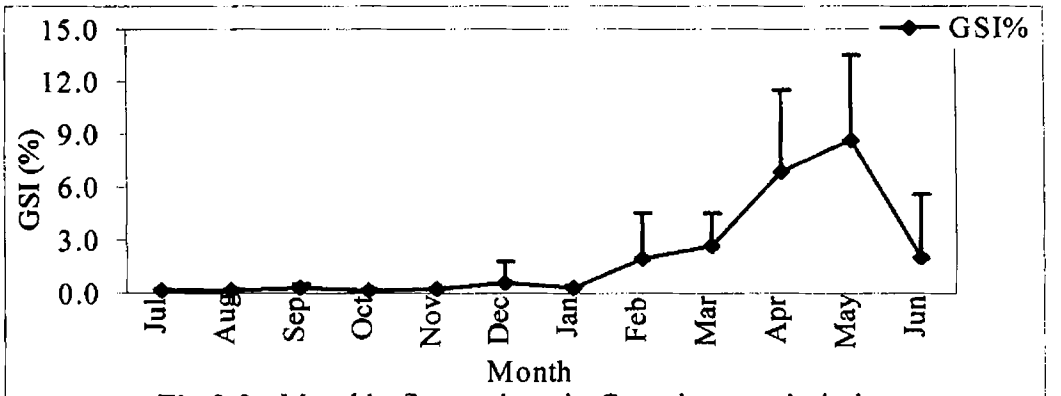



**Fig.2.3** . Monthly fluctuations in Gonadosomatic index (GSI) in Female *A. testudineus* .

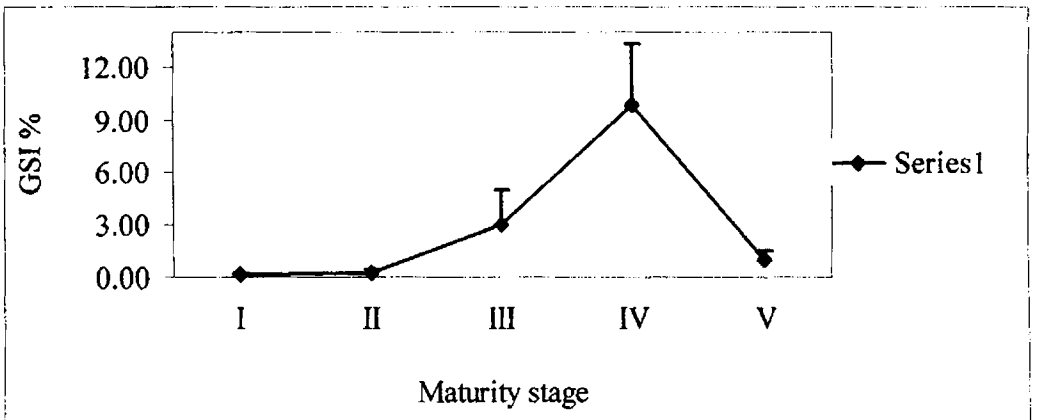



**Fig: 2. 4.** Variation in GSI in various maturity stages of Female *A. testudineus* (Bloch).

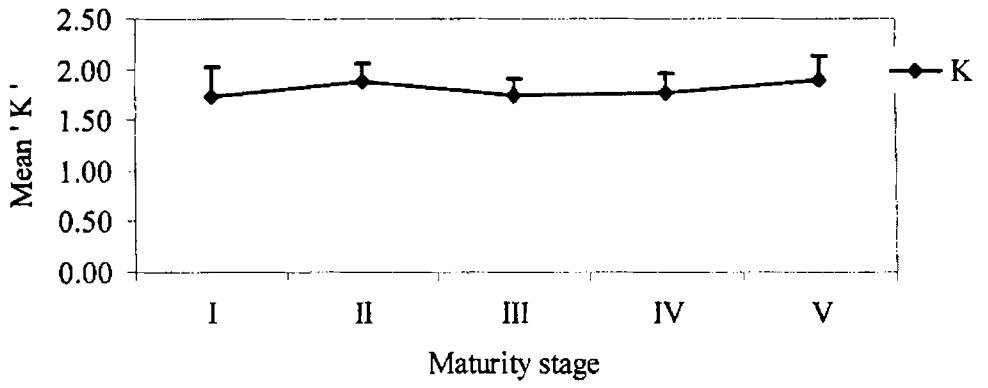



**Fig: 2. 5**. Variation in condition factor ' K ' in different maturity stages of Female *A. testudineus* .

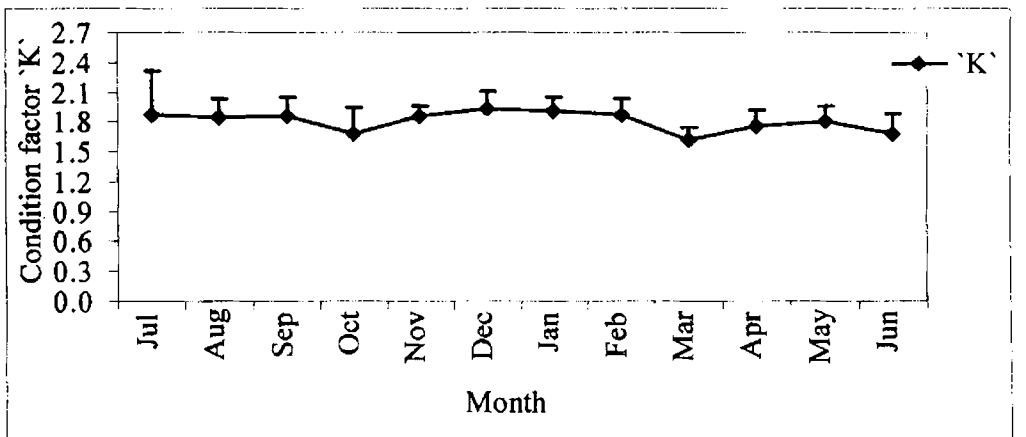



**Fig. 2. 6**. Monthly variation in Condition factor 'K' in Female *A. testudineus* .

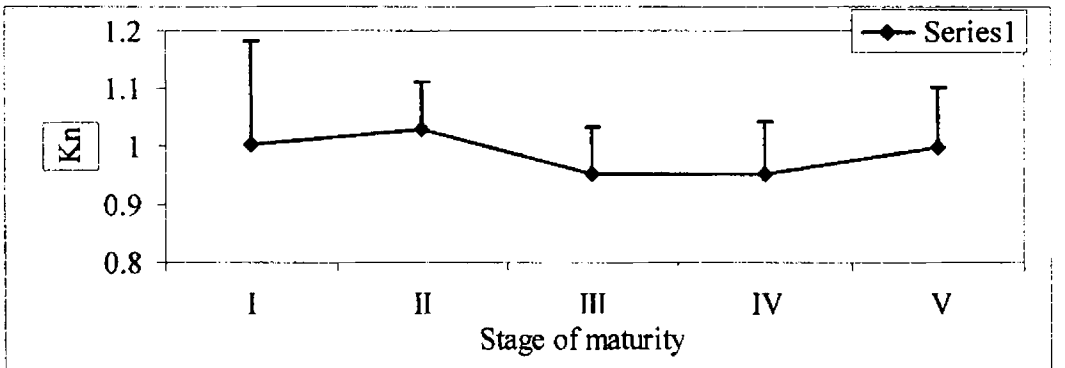



**Fig: 2. 7.** Maturitywise relative condition(Kn) of Female *A. testudineus*

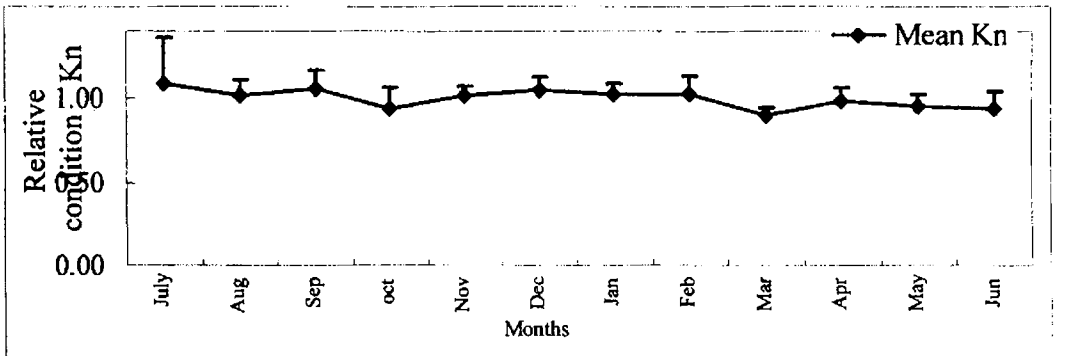



**Fig: 2. 8.** Monthly variation in relative condition of Female *A.testudineus*

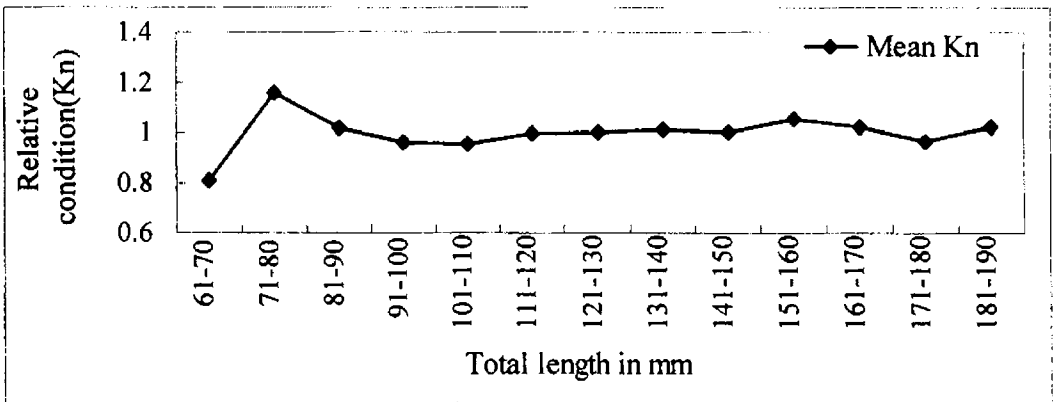



**Fig: 2. 9.** Changes in relative condition, Kn in Female *A. testudineus* with respect to length.

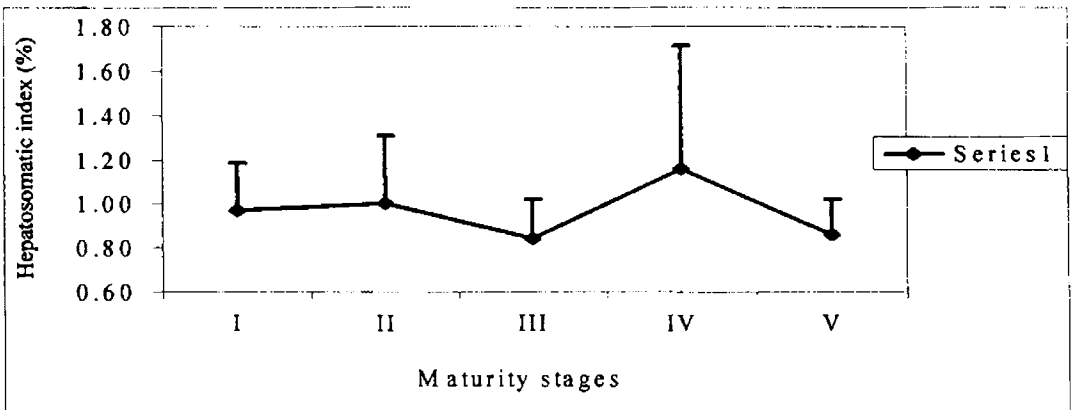



**Fig.2. 10**. Variation in hepatosomatic index (HSI) in different maturity stages of Female *A. testudineus*.

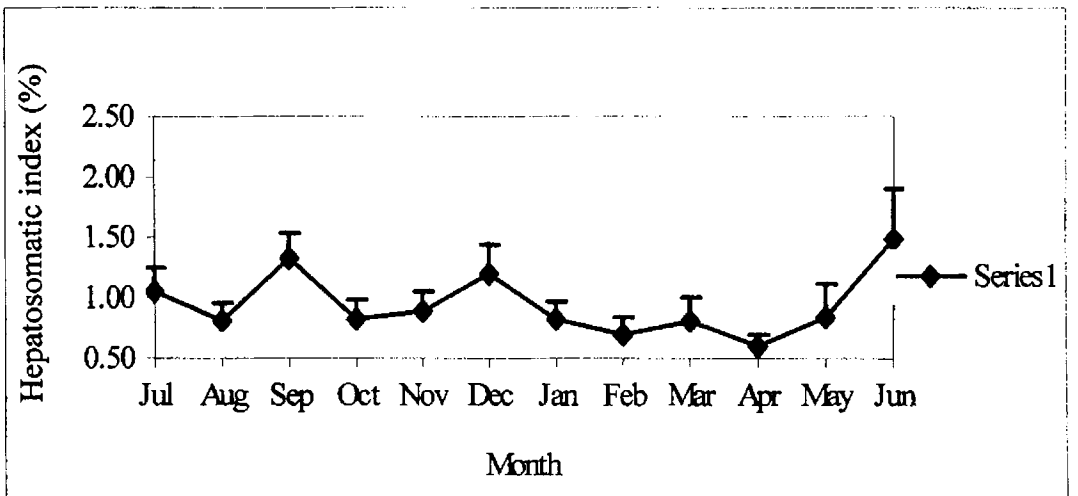



**Fig.2.11**. Monthly variation in Hepatosomatic index in Female *A. testudineus* .

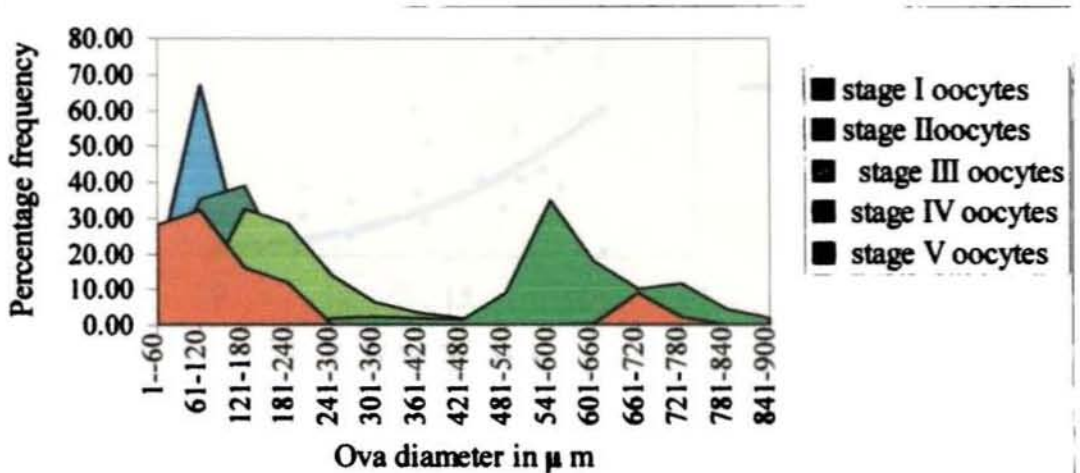



**Fig: 2.12.** Percentage frequency of different size groups of ova in different stages of maturity of *A. testudineus* .

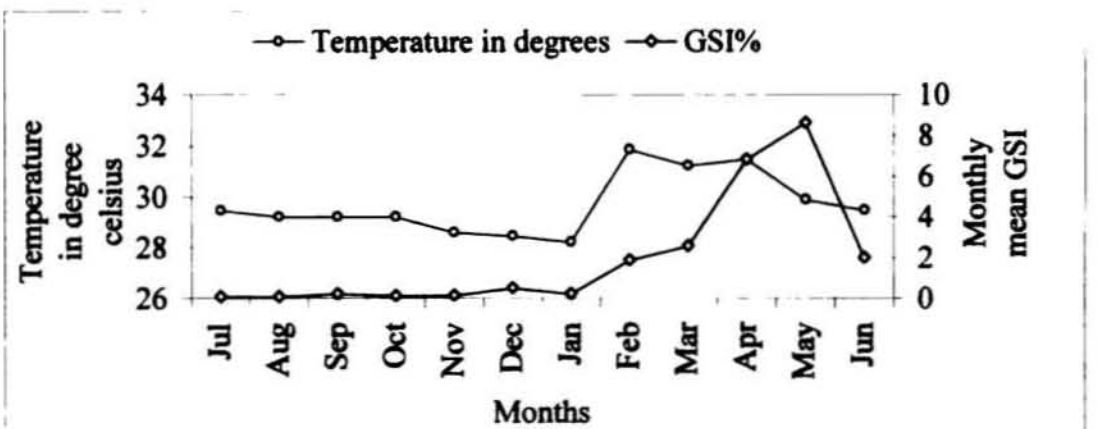



**Fig: 2.13**. Influence of water temperature on gonads in Female *A. testudineus* .

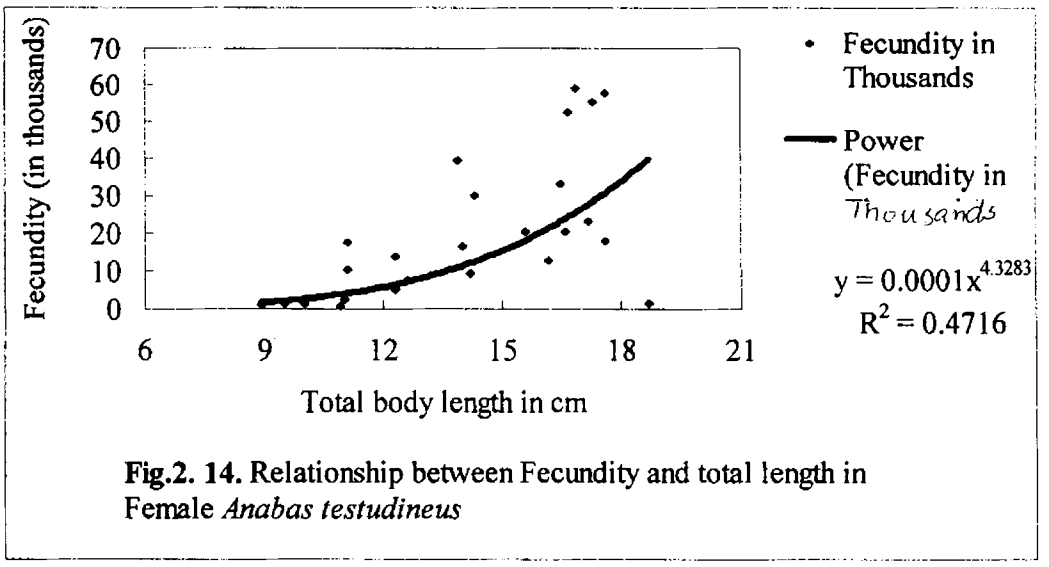



**Fig.2. 14.** Relationship between Fecundity and total length in Female *Anabas testudineus*

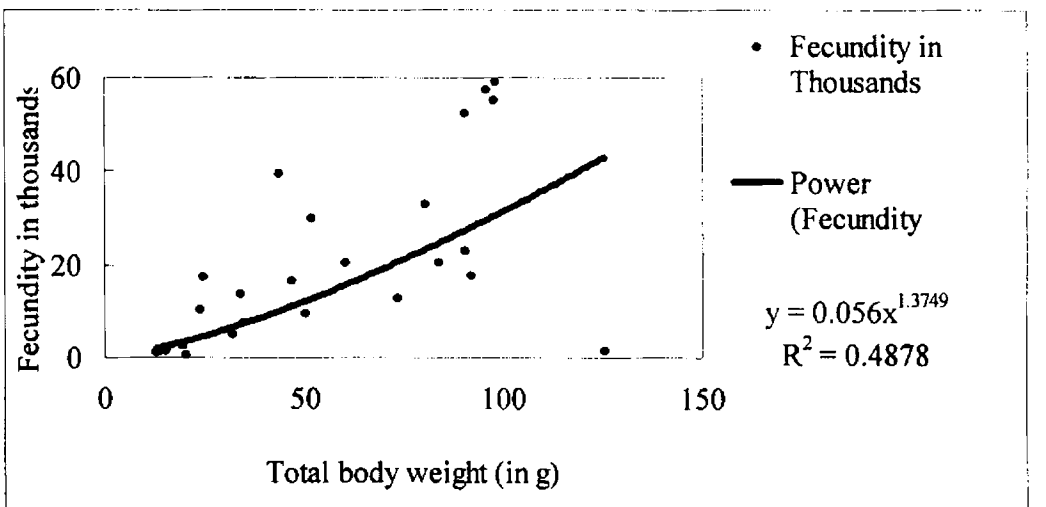



**Fig. 2. 15** .Fecundity-body weight relationship in Female *A.testudineus*

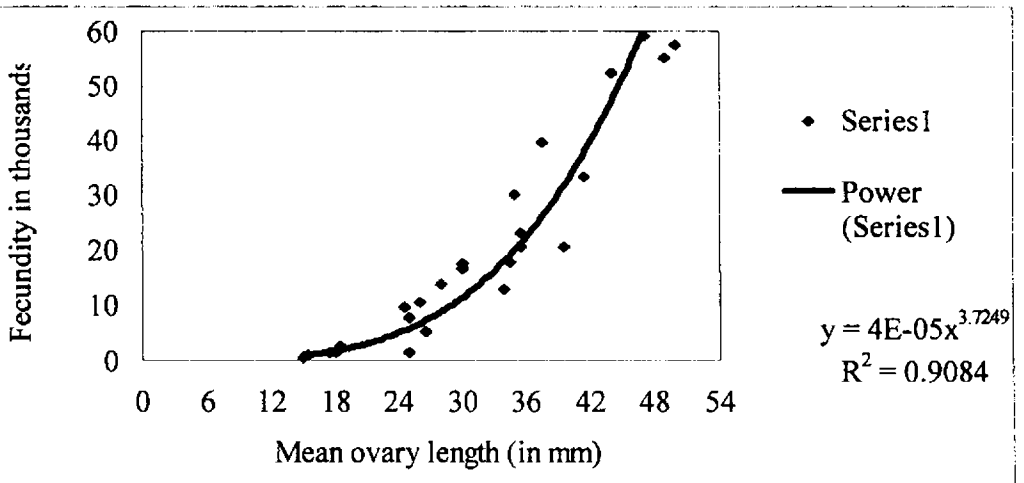



**Fig.2. 16.** The relationship of fecundity with length of ovary in *Anabas testudineus* .

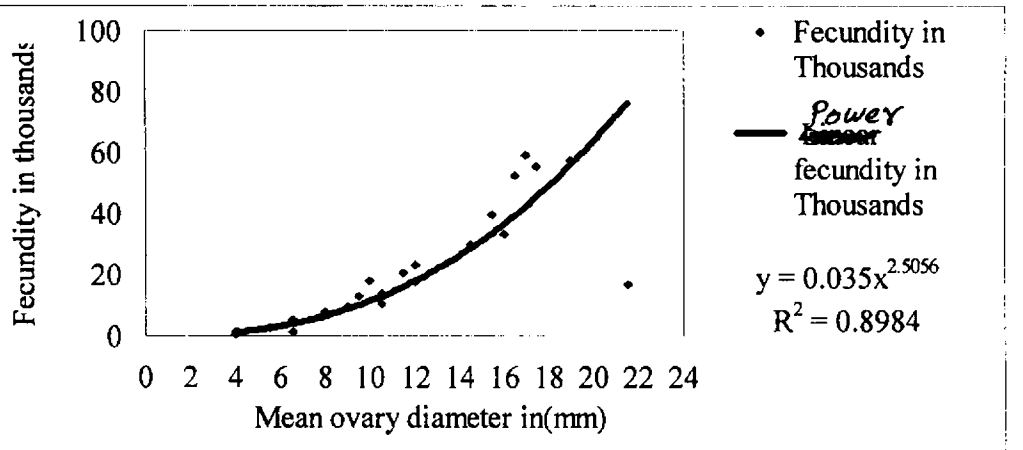



**Fig. 2.17.** Relationship between Ovary mean diameter and fecundity in *A. testudineus*

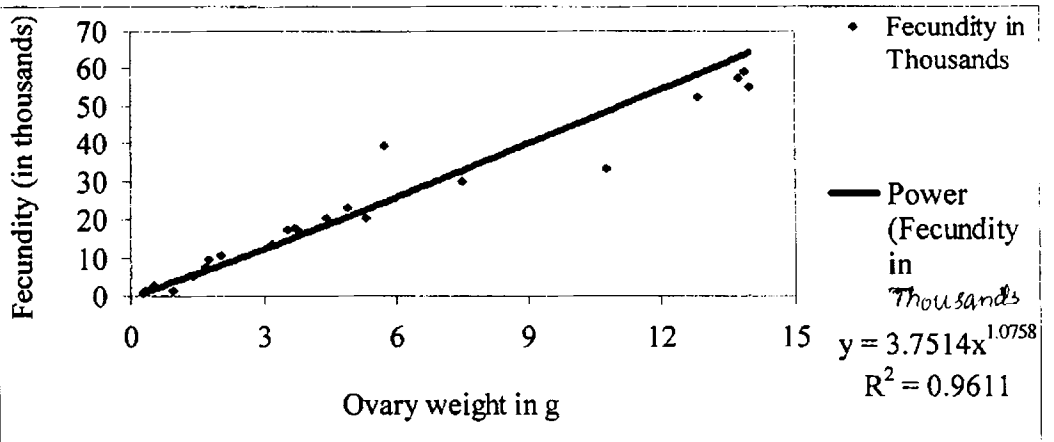



**Fig. 2. 18.**Relationship between fecundity and ovary weight in *A. testudineus* .

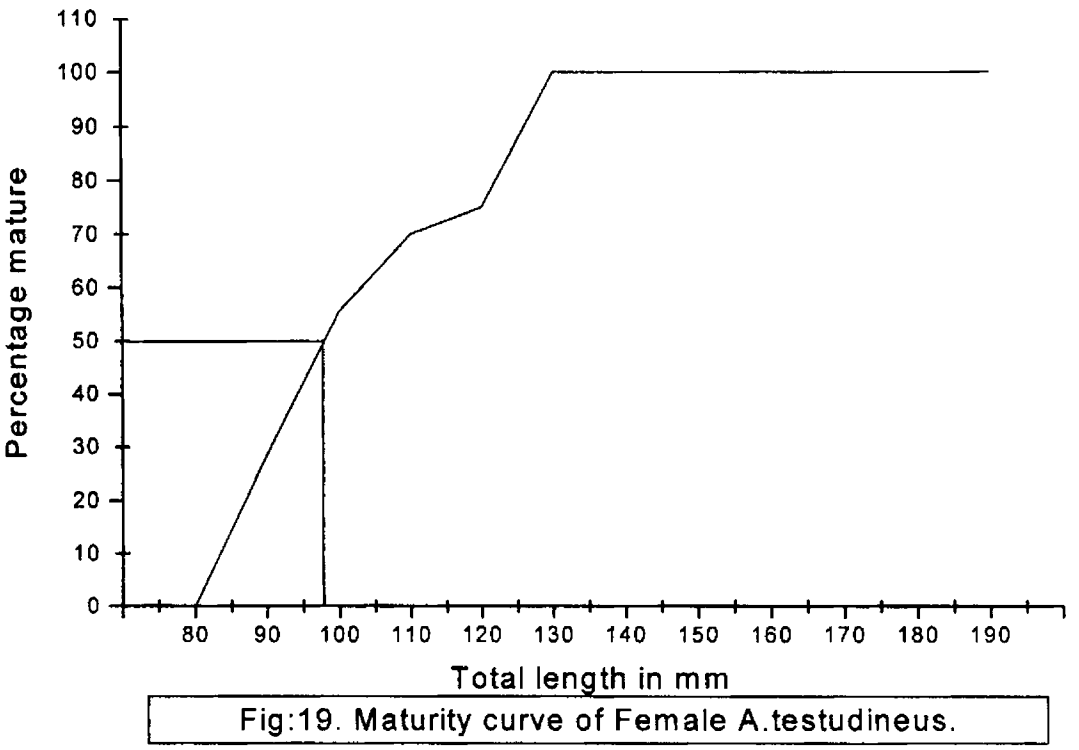



Fig:19. Maturity curve of Female A.testudineus.

Plate. 2

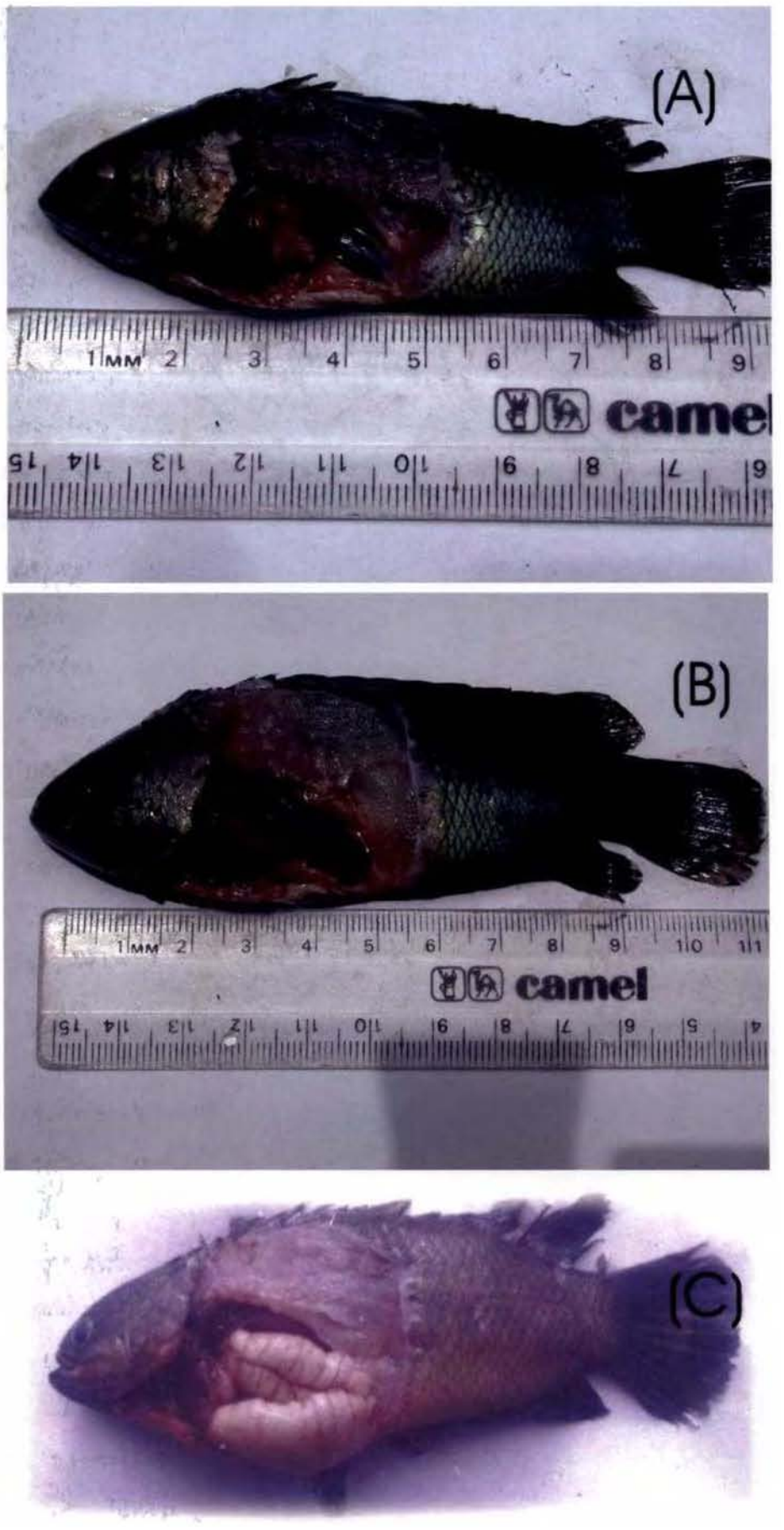



Fig. Fish (A. testudineus) dissected to show the appearance of ovary. A- Immature, B- Recovering spent, C- mature.

## 2. 4. DISCUSSION

*Anabas testudineus* (Bloch) is bisexual, but marked sexual dimorphism was not observed in the present study. Mookerjee and Mazumdar(1946b) and Dehadrai *et al.*, (1973 b) observed sexual differences in body and fin colourations in the species from West Bengal and Bihar respectively. Banerjee and Prasad (1974), however, did not observe any such colour difference in any of the specimens examined from Bihar waters, as in the present study. Externally, differentiation of the sexes was found difficult, except in ripe fish. Secondary sexual characters were not apparent and the genital papilla in either sex does not appear to show much distinction to the naked eye. While it is tempting to suggest that male fish generally have a more slender body, differentiation based on this criterion was found to be unsatisfactory.

The maximum length of female fish recorded in the present study was 19.0 cm as against 25.0 cm recorded by Chacko (1955) in Madras. Mookerjee and Mazumdar (1946), Alikunhi(1957), Misra (1959), Hora and Pillay (1962), Rao and Seshagiri (1971) and Banerji and Prasad (1974) had reported maximum lengths of 17.7 cm, 22.5 cm, 20.3 cm, 23.0 cm, 15.0 cm and 19.0 cm respectively in the species from different states of India.

As is true of most teleosts, ovaries of *A. testudineus* are paired and of the 'cystovarian' type, which has coelom- derived ovarian lumen (ovocoel) continuous with the oviduct. In oviparous teleosts, the ovarian cavity has been regarded as merely a "stock room" in which ovulated eggs are kept temporarily until they are spawned (Nagahama, 1983).

There are numerous reports on size discrepancies between the left and right ovarian lobes in teleosts. El Maghraby *et al.*, (1974) , Brusle (1981a), Muthukaruppan (1987) and Gopalakrishnan (1992), observed that the right ovarian lobe of *Mugil cephalus* was 11- 15% longer. In the present study, initially the ovaries were suspected to be of unequal lengths; however, this possibility was excluded through a Student's 't-test' (Table. 2. 2). Since the calculated 't- value' for the differences between the mean lengths of left and right gonads was found to be less than the tabled value, the observed difference was found to be not significant.

Different criteria, such as size of gonad in relation to body cavity, ova diameter, fat content and gonadosomatic index (GSI) were used by various workers for quantifying maturity condition in fish. Eight maturity stages were described in herring (Hjort, 1910), which were further modified by the International council for exploration of the seas (ICES) in 1962. However, this classification was developed primarily for the temperate water fishes, which have a definite spawning season within which the stages of maturity are fairly uniform throughout the population at any one time. Majority of the tropical fishes have prolonged breeding season. In these species, almost all maturity stages may be available through out the year (Clark, 1934., June, 1953., Yuen, 1955, Yuen and June, 1957., James and Baragi, 1980) and hence according to Quasim (1973), any classification of maturity based on the models of temperate forms will not give a correct picture of the breeding season. In such fishes, the maturity scale should be based on the modal positions of different batches of ova (James and Baragi, 1980).

Considering the overall implications of the problems related to quantification of maturity, Qasim (1973) suggested that in tropical and sub tropical fishes, the maturity classification should be limited to five stages. Accordingly in the present study, the maturity of the female *A. testudineus* has been classified into the following five stages: *viz*; I) Immature II) maturing virgins and recovering spent III) mature IV) Ripe and V) spent.

In synchronous spawners, the weight of the gonad may represent as much as 40 % of the overall body weight of the fish. (Tyler and Sumpter,1996). The heaviest gonad weighing 14.0 g was found in a female fish of 17.3 cm total length and 97.7 g body weight in the present study. Gonadosomatic index (GSI) or the maturity coefficient, first used in fish by Mein (1927), has been used widely to monitor seasonal or experimental fluctuations in gonadal state of a variety of teleost species. Reproductive cycles are characterized by pronounced variations in gonadal size, which is dependent on body size. The gonadosomatic index (GSI) eliminates the effect of body size of fish on gonadal weight (De Vlaming *et al.*, 1982: Erickson *et al.*, 1985).

In the present study, the mean GSI value was found to be 2.95 % to 9.84% in mature fishes as compared to 9.6 to 15.5 % reported (Shashi and Akela, 1996) in the fish from North India. It is observed that the higher the stage of development, the bigger is the GSI value. The highest monthly mean value of 8.67 in the present study was recorded in the month of May. The spent stage, marked by a lower GSI mean value of 2.03, was recorded in June, which indicated that spawning occurred in June. Among the ripe (stage IV) fishes, most were found in April and May while, most of the spent (Stage V) fishes occurred in June, and a

few in May. A single seasonal peak in mean GSI value in the month of May (Fig. 2. 3) and a similar peak in ovary weight in maturity stage IV (Fig. 2. 2) and the occurrence of many spent fishes in june, indicated a single spawning season of May-June, in *A. testudineus* in the present study. As in the present study, all the earlier reports on this species indicated a single spawning season (Day, 1889; De, 1910; Willey, 1910; Raj, 1916; Mookerjee and Mazumdar, 1946; Alikunhi, 1957; Hora and Pillay, 1962; Chaudhuri, 1969; Banerjee and Prasad, 1974).

A close relation was found between surface water temperature and GSI values (Fig. 2. 13) in the present study. Consequent to an increase in temperature beginning with February, the mean GSI value also rose and increased steadily to reach the peak in May.

Htun-Han (1978) explained that the increase in GSI during the period of gonad maturation is mainly due to the deposition of large amounts of protein and lipids in the developing eggs. Munkittrick and Leatherland (1984) found a statistically linear relationship between GSI and stage (IV) oocytes in the feral gold fish, *Carassius auratus* L., and suggested that in this species the GSI accurately reflected the gonadal state. Similar correlations were also found by Gupta (1975) in *Cyprinus carpio* L. However, the mathematical assumptions on which the GSI are based, have been questioned (Delahunty and de Vlaming, 1980; de Vlaming *et al.*, 1982).

The coefficient of condition or the condition factor or the ponderal index 'K' has been used in fishery science to express the relative robustness of the fish, in age and growth studies, to measure the effect of environmental improvement and the attainment of sexual maturity and

spawning (Luther, 1963). The fluctuations observed in the condition factor of fishes have been attributed to changes in ecological (absence or lack of food) or physiological conditions (sexual maturity) (Blanco-Racedo, 1983). The ponderal index also varies depending on the amount of food in the gut and the fatness of body (Rangaswamy, 1972). Changes in condition factor in several fishes have been ascribed to a depletion of body reserves during gonad maturation (Htun-Han, 1978).

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

In the present study, the values of condition factor 'K' remained relatively high and constant (1.80 ± 0.08) in the different maturity stages (Fig. 2. 5). Similarly, minor fluctuations (1.80±0.19) only were seen in the monthly values of 'K' (Table. 2. 4). The same trend was observed with the values of relative condition 'Kn' (Fig. 2. 6). These observations indicate that the fish remains in good condition throughout the spawning period. Low variations in the 'K' values show that the condition factor 'K' is least influenced by the breeding cycle. However, the 'Kn' values in different size groups showed a marked upward trend in 61-70 mm

length group, reaching a peak in the 71-80 mm size group (Fig. 2. 9), indicating the building of reproductive materials prior to spawning. The 'Kn' value then dropped to the lowest figure of 0.96, which occurred from 91 mm onwards, indicating the first attainment of sexual maturity. This observation is in agreement with the calculation of size at maturity from the maturity curve (Fig. 2. 19). Cone (1989) had suggested that condition factor may also give an indication of size at first maturity in fishes. Chan and Chua, (1980) based on fluctuations in 'Kn' values in different size groups, had estimated size at maturity in grey mullet *Liza subviridis* (Val.). Similarly, Chanchal *et al.*, (1978) had estimated the attainment of first sexual maturity in female *A. testudineus* from Bihar and found it to be at a length of 8.2 cm and at a total body weight of 12.23 g.

Zahnd (1959), in a study of two ovoviviparous fishes, *Xiphophorous helleri* and *Lebistes reticulatus*, showed that the liver weight decreased as the ovary weight increased during vitellogenesis. such an inverse correlation was found (Fig. 2. 2) in the present study also, as the HSI dropped to a value of 0.84 in stage III (mature) which then further rose to 1.16 in stage IV (Ripe) and declined to 0.86, in stage V (Spent). The monthly values of HSI showed regular fluctuations (Fig. 2. 10) with a marked decrease in liver index during February-April (corresponding to prespawning phase), when most of the fishes were in stage III (mature). HSI increased in the next couple of months when most of the fishes were in ripe stage (corresponding to spawning phase); this was followed by a decline in the values in the next couple of months, corresponding to the post-spawning period. In *Channa punctatus*, Jaba *et al.*, (1989) had observed three separate 'peaks' in vitellogenin content of liver during preparatory I, II and spawning

periods and two 'valleys', during the prespawning and post spawning periods.

Larson (1974) in a study of liver weights of brook trout, *Salvelinus fontinalis*, suggested that the decrease in liver weight during the prespawning season might have been due to the passage of materials from the liver to the gonads and concluded that the weight changes in liver plays an important role in gonad maturation. On the contrary, Wingfield and Grimm (1977) showed that in the Irish seasonal plaice, *Pleuronectes platessa*, HSI was highest in the prespawning period and lowest in the postspawning period. However, Htun-Han (1978) remarked that changes in liver weight are difficult to interpret and found no significant fluctuation in liver index in the dab *Limanda limanda.*

Fishes exhibit different spawning periodicities. Some species may spawn only once, others twice, while still others may spawn several times during a year (Saidapur, 1989). Hickling and Rutenberg (1936), and De jong (1939) had observed different types of spawning habits in teleosts. Based on these observations, Prabhu (1956) classified fishes, according to their spawning pattern, into the following four major groups:

Group I. Fishes with a short spawning season. The mature ovaries contain two separate batches of ova, immature and mature.
Group II. Fishes that spawn once, but over a long period. The sizes of mature eggs will be approximately half the total range of the entire egg compliment.
Group III. Fishes, which spawn twice a year. The ovaries contain mature eggs and a batch of maturing eggs.

Group IV. Fishes that spawn intermittently over a prolonged period. The ovaries may contain batches of eggs, which cannot be differentiated from each other.

Qasim and Qayyum (1961) have classified the fishes into three categories based on their spawning habits:

Category I. Fishes having a short breeding season, with a single batch of maturing eggs.
Category II. Fishes having a longer breeding season, with more than one batch of maturing eggs.
Category III. Fishes having non- seasonal breeding and without well defined batch of eggs.

A wide range in GSI values, a single seasonal peak in mean GSI value in the month of May (Fig. 2. 3) and a similar peak in ovary weight in maturity stage IV (Fig. 2. 2), and from the occurrence of only one distinct of batch of ripe eggs as may be clear from the oocyte size frequency profiles (Fig. 2. 12), it may be concluded that *A. testudineus* belongs to Group I of Prabhu's (1965) classification or category I of Qasim and Qayyum (1961).

According to Weber (1974), the spawning season of tropical fishes could be correlated with some hydrographical factors. Of these, temperature and salinity have been found to exert a profound influence on the fish throughout its life cycle (Pradhan and Reddy, 1964). The single and short spawning season of May to June, in *A. testudineus* in the present study coincided with the onset of southwest monsoon in Kerala. While examining the seasonal fluctuations of surface water temperatures

in the study area, depressed temperatures were observed (Fig. 2. 13) over the same periods. However, near zero salinity conditions prevailed in the sampling area throughout the year and were not observed to be of any importance in the onset of spawning.

There is a large variability in egg size in teleosts, with the largest known measuring up to 8.0 cm in diameter. The ripe eggs in *A. testudineus* were found to be 700 µm in diameter. Mookerjee and Mazumdar (1946 b) found the average diameter of fully swollen egg as 0.7 mm in the same species in Bengal waters. According to Tyler and Sumpter (1990), with the limits of variance set by genetic constraints, egg size may vary between populations of the same species.

Fecundity is a measure of the reproductive capacity of a fish and its knowledge forms an important tool in successful management of fishery resources. In the present study, fecundity ranged from 1000 to 59,022 in fish size range of 8.9-18.7 cm/12.74-125.4 g. The fecundity of fish varies with age, length, weight *etc.* Correlation with body length has been most widely used, as length is reckoned a better measure of size since a fish will not shrink, but can loose weight (Chan and Chua, 1980). The relationship between fecundity and total length was found to be curvilinear, as in most of the fishes. The exponential value 4.3283 in the present case is within the accepted range of 2.34-5.28 as is true for most species of fishes (Chan and Chua, 1980) and indicates that fecundity increases at a rate greater than the cube of the length. Many earlier workers were of the opinion that the fecundity of a fish increased in proportion to the square of its length (Somavanshi, 1985). However, Prabhu (1955) and Palekar and Bal (1961) had observed the fecundity relationship as greater than the fourth power of the fish length, to which

the results of the present study agreed. Correlation of the variables- body length, body weight, ovary length, ovary diameter and ovary weight, with fecundity was also found to be positive. The highest degree of correlation was found between fecundity and ovary weight. This is in agreement with the observations of Chaturvedi (1976) in *Tor tor*, Joshi and Khanna (1980) in *L. gonius*, Qadri *et al.* (1983) in *Schizothorax richardsonii*, Sunder (1986) in *S. longipinnis,* and Kurup (1994) in *L. dussumieri.* It is well known that the weight of ovaries of a fish is mainly influenced by the ova contained in them. The 'r' value between fecundity and body length and fecundity and body weight exhibited a fair correlation between the variables, but body weight was more closely related to fecundity than body length, as observed in *L. dussumieri* by Kurup (1994).

In Port Canning, Khan and Mukhopadhyay (1972) found the fecundity of 'Koi' to be from 10,002 to 36,477 in the fish length range 99 to 169 mm in W. Bengal and worked out fecundity- fish size relationships as i) Log F= 0.1707+1.9386 log Length (r = 0.7242) and ii) Log F= 3.3517+ 0.5877 log weight (r = 0.6856). The fecundity of the population of the fish of Bihar region mentioned by Banerjee and Prasad (1974) as 4,588 to 34,993 in the fish size range of 73.0-18.2 cm/8.4-100.2 g almost agrees with the observations of Khan and Mukhopadhyay (1972 b). Chanchal *et al.*, (1978) found the fecundity in the fish from Bihar as 3481 to 42, 564 in the fish weight range of 9.0 to 53.1 g.

From the maturity curve (Fig. 2. 19), the size at first maturity of *A. testudineus* was found to be 9.8 cm in total length. This is in agreement with the results of studies on changes of relative condition with respect to length (Fig. 2. 9), which indicated that first sexual

maturity begins from 9.1 cm onwards. Chanchal *et al.*, (1978), however, estimated the attainment of first sexual maturity in female *A. testudineus* ftrm water to be at a lower length of 8.2 cm. According to Hora and Pillay (1962), *A. testudineus* matures in about six months of age when about 8.0 cm long.

Sex ratio indicates the proportion of males and females in the population. This is expected to be 1:1 in nature. In the present study, when all the samples were pooled, a ratio of 1.0 male to 0.94 female was obtained and the low chi-square ($\chi 2$) value indicated that the ratio did not differ significantly from the hypothetical 1:1 ratio. This is in sharp contrast to a sex ratio of 1 male to 1.5 female reported by Chanchal et al., (1974) in *A. testudineus* from Bihar region. However, during the months of February and April, higher $\chi^2$ values, with sex ratio favouring males, were observed. This may be due to the differential behaviour of sexes, as in burrowing and migration. It is also possible that the sexes may be segregated seasonally or on grounds of age or size and this may account, at least in part, for the observed differences in the sex ratio (Smith, 1956).

To conclude, *A. testudineus* in the present study exhibited a single batch of maturing eggs and a single short spawning season (May-June). Some of the observed differences in reproductive parameters like size at maturity, egg size, fecundity and sex ratio reported by earlier workers in this fish from north Indian waters, may be attributed to genetic variation and environmental influences existing in these geographically separated populations. The present study indicates that while GSI values accurately reflected the stage of maturity, HSI and 'K' values were poor indicators. The first attainment of sexual maturity as

derived from the maturity curve was, however, corroborated well by the 'Kn' values. The length at maturity would be a useful index for determining the size of the exploitable stock. The statistically significant coefficients of correlations derived between fecundity and various body parameters such as body length and body weight, may be of immense help in enumerating the fecundity of fish, without having to sacrifice them.

# Chapter 3

# *Histology of the ovary*

# CHAPTER III

# HISTOLOGY OF OVARY

## 3. 1. INTRODUCTION

Spawning in teleosts occurs during a particular phase of the reproductive cycle. Some breed once annually, while some others breed at regular intervals throughout the year, and in some such as pacific salmon, death follows spawning. This implies that a sound knowledge of the natural reproductive cycle of a species is essential for designing experimental investigations on their reproduction (Mollah, 1986). Determining annual reproductive cycles, spawning frequency and timing of spawning are challenges in fish reproductive biology. Many facets of gonadal structure are not well understood; the germinal epithelium in the teleost ovary has not been well described and the origin of oocytes has been a matter of speculation (Tyler & Sumpter, 1996).

As a female fish grows, there is proliferation of oogonial cells in the ovary that eventually develop into the oocytes. An important step is the initiation of meiosis, which results in the reduction of chromosome numbers by half. The process of meiosis is interrupted at several stages in egg development and is only completed at fertilization. The following stages of reproduction result in oocytes being recruited into the next cohort of spawned eggs.

Stage-I of oocyte development involves the development of basic cellular structures, enlargement of the nucleus, appearance of multiple nucleoli and the various sub cellular organelles. There occurs a

substantial amount of protein synthesis in the developing oocyte, which is referred to as endogenous vitellogenesis. Two cell layers; the theca and the granulosa develop and surround the oocyte to form a follicle and support further growth. At the end of stage-I, there is a well-defined oocyte encased in its follicle.

Stage-II is vitellogenesis, which involves the synthesis and uptake of egg yolk protein that provide nutrients for the developing embryo. Vitellogenesis involves the interaction of the anterior pituitary, the follicle cells, the liver and the eggs. Vitellogenin is secreted into the blood and is taken up by the oocyte through specific receptors. Vitellogenin is further processed into smaller yolk proteins for storage until they are needed by the embryo.

Stage-III of oocyte development is maturation. It is the final stage of development and usually requires 24-72 hours. This step must be completed for viable eggs to be spawned. During this stage, the nucleus of the egg migrates from the center of the egg to the periphery and meiosis resumes but pauses again before completion. The membrane surrounding the nucleus disappears in a process called germinal vesicle breakdown. Depending on the species, uptake of water occurs during maturation. This often can be seen in females as the belly becomes even more distended and firm. When maturation is complete, the oocytes are ovulated from the follicle. In some species, the oocytes are retained in the ovary and in other species; the eggs are released into the peritoneal cavity until spawning.

Stage-IV is spawning, which should occur shortly after maturation, or the eggs will become overripe. In some species, the oil globules coalesce into a single large globule or oil drop when the oocyte

has completed maturation. Upon completion of hydration, the egg becomes translucent and hyaline. Spawning requires proper environmental factors like day length, water temperature and interactions between males and females. In some species, one sex will prepare a nest for spawning.

The ovarian morphology in teleosts has been reviewed by Hoar (1969), Dodd (1977), Jones (1978), Nagahama (1983), Dodd & Sumpter (1984), Guraya (1986) and Verma (1997). Wallace & Selman (1981) in their review of the cellular and dynamic aspects of oocyte growth in teleosts, recognized four principal stages of oocyte growth in teleosts. 1) The Gonadotropin-independent primary growth phase during which multiple nucleoli form as well as a yolk nucleus (Balbiani body) 2) the Gonadotropin-dependent secondary growth phase which involves the formation of yolk vesicles (lipid droplets) 3) true vitellogenesis and rapid oocyte growth and 4) maturation, accompanied in many teleosts by water uptake. Oogenesis described in detail in many teleosts has been reviewed by West (1990).

Studies concerning hormonal changes during vitellogenesis, maturation and ovulation have been reviewed by Kobayashi *et al.,* (1988) and Degani & Boker (1992 a). Le Menn *et al.,* (2000) in their review of the molecular aspects of vitellogenesis, recognized four stages in the development of fish ovarian follicle and considered irrelevant the traditional classification of vitellogenesis as endogenous or exogenous and asserted that vitellogenesis is totally exogenous.

Some other important works related to the present study are those of Najim *et al.,* (1979), Cyrus & Blaber (1984), Mollah (1986),

Thorogood (1986), Saat & Veersalu (1990), Palmer *et al.*, (1995), Degani *et al.*, (1997), Coward & Bromage (1998), Maddock & Burton (1999), Rideout *et al.*, (1999), Micale *et al.*, (1999), Merson *et al.*, (2000), Arocha (2002), Smith & Walker (2004).

Among the Indian works, Dutt & Govindan, (1967) distinguished six stages in oocyte growth in *Anabas scandens* and gave histochemical evidence for lipid inclusions in its oocytes. Chopra, (1958a, b and 1960) and Venugopalan, (1962) had conducted similar studies in some of the Indian freshwater teleosts. Saksena & Raizada, (1984) studied the spawning periodicity and post-ovulatory follicles in six freshwater teleosts and recognized four stages of follicular atresia. Some other related works are those of Anna Mercy *et al.*, (1982) Jayasankar & Alagar swamy (1994), Gopalakrishnan (1991) and Veena *et al.*, (1995).

Reproductive activities in most animals undergo cyclic rhythms. The patterns of these changes in the gonads are characteristic for each species. One of the convenient methods of studying the reproductive cycle including the spawning period and determining the degree of maturity of a fish, is gross examination of gonads, which lacks precision (Baglin, 1982). Macroscopic examination alone has its limitations, as it is difficult to distinguish macroscopically, the maturing virgins from recovering spents, presence of atretic follicles; and most important, the actual developmental stages of growing oocytes themselves may not be determined. Therefore, to understand the reproductive mechanism, to determine the stage of maturity of the gonad more correctly and for determining the developmental stages of the growing oocytes, in addition to securing the microscopic appearance of whole oocytes, a histological investigation was initiated.

## 3. 2. MATERIALS AND METHOD

### 3. 2. 1. Processing of tissue samples

Sample pieces of ovaries (5mm thick), belonging to the different maturity stages, taken from freshly killed specimens, were fixed in Bouin's fixative (15 part saturated aqueous picric acid: 5 part concentrated formalin : 1 part glacial acetic acid) for 24 hours, with the fixative : tissue volume maintained at a ratio of 5:1. The tissues were then washed under running tap water overnight and passed through a graded alcohol series following the standard procedure (Weesner, 1960). Ovaries with only early stage oocytes were embedded routinely in paraffin wax containing ceresin.

Yolk-laden tissues were, however, very brittle and difficult to section when embedded routinely. Therefore, a specific schedule was followed for their dehydration and wax infiltration. The protocol used was as given in Table: 3.1. The tissue samples in paraffin blocks were mounted on wooden blocks and sectioned at 5 to 7-μm using a rotary microtome. To aid section adhesion, glass slides were coated with a thin film of modified Mayer's albumen (Luna, 1968) containing equal parts of egg albumen and glycerol. Slides were dried overnight at $40^0$ c, using a slide warmer. The sections were deparaffinised, hydrated and stained in Harri's haematoxylin followed by Eosin as counter stain (Mc Manus & Maury, 1964) and mounted in DPX. The slides were examined under a Nikon photomicroscope and photographed using an attached Nikon digital camera. The more advanced oocytes were observed for the presence of multiple nucleoli, lipid droplets/yolk vesicles, yolk nucleus,

yolk globules, germinal vesicle breakdown, atretic follicles and post-ovulatory follicles.

### 3. 2. 2. Cell measurements and counts

The reproductive cycle was further defined by measurements of oocytes, whose nuclei were included in the sections. This procedure had been shown to be representative of the true oocyte diameter by Foucher & Beamish (1980). Discrete stages were measured using a binocular microscope fitted with a calibrated micrometer. If the cell was spherical, the diameter was measured; if oval, the mean of the longest and shortest axes were taken; if the cell was irregular in shape, the two longest and the two shortest axes were measured and the mean taken.

For each stage of development, the diameters of at least thirty sectioned oocytes from several different ovaries were measured. Diameter of nucleus, yolk vesicles and yolk nucleus was also measured. From the diameter measurements of nucleus and the oocyte, the ratio of nucleoplasm to cytoplasm (nucleocytoplasmic index) was calculated. The number of nucleoli and yolk vesicles was also noted. Ovarian maturation was assessed by evaluating the relative occurrence of the different oocyte stages in the ovary, the size and stage of the most advanced oocyte (West, 1990) and the presence or absence of post-ovulatory follicles and atretic yolked oocytes (West, 1990; Fowler *et al.*, 1999). Atretic oocytes were classified according to Lambert (1970a) and Hunter & Macevicz (1985) and post-ovulatory follicles were described and assigned the terms recent or late etc, following Elorduy & Ramirez, (1994).

Oogonia were not measured or counted since these cells were observed rarely as they are immersed in the stroma (Pl: 3.1, Fig. a) and this tissue was generally lost during the processing. Oocyte stages such as migratory nucleus stage and ripe oocyte stage, which are undergoing maturational events, were rarely observed because of their shorter duration and hence were not considered for counts and measurements.

To investigate the dynamics of ovarian growth, 500 oocytes from different sections in each stage were counted under the microscope and their percentage composition in the ovary was determined and plotted. From the measurements and counts, a histological classification scheme was developed to identify discrete stages of oocyte development in the different ovarian maturity stages.

The terminology used for describing oocyte stages based on their histological appearance was taken from Palmer *et al.,* (1995), which was adapted from Yamamoto & Yamazaki, (1961), Forberg (1982) and Mayer *et al.,* (1988). The inconsistencies in the use of the many stage definitions and the existence of somewhat confusing terminologies have made the cellular events of oogenesis difficult to understand. For example, the 'vacuolated oocyte' stage, described in several reports corresponds to the 'yolk vesicle' stage in Yamamoto's classification. These two terms exist because of the difference in the initiation of yolk formation in different fish species (Jayasankar, 1994). Some of The terminologies and abbreviations used in the present study are given (Table. 3. 2) along with their analogous terms.

## 3. 3. RESULTS

### 3. 3. 1. Histological structure

The ovaries were of the cystovarian type. A thin and transparent peritoneal layer beneath which lies a thick elastic tunica albuginea comprising connective tissue, smooth muscles and blood vessels constituted the ovarian wall (Plate: 3. 1a) that covered each lobe of the ovary. Inner to the ovarian wall lies the germinal epithelium which projects into the lumen of the ovary (ovocoel) along with the tunica albuginea to form finger like ovigerous folds or ovigerous lamellae (Plate 3. 1e). Oogonia developed on these lamellae. In ripe ovary, the ovarian wall became thin and highly stretched.

### 3. 3. 2. Histology of oocyte development

Eight developmental stages were distinguished and described under two phases of growth, apart from the atretic follicles and post-ovulatory follicles.

#### *3. 3. 2. 1. First Growth Phase (FGP)*

*Chromatin Nucleolus Stage (Pl: 3. 2 a)*

The round or oval oocytes measured 10.0 - 40.0 µm (mean = 21.90 µm) in diameter. Oocytes of this stage had a weakly basophilic cytoplasm and a more basophilic, large nucleus of 8.0 - 21.0 µm diameter range. Two or more darkly staining nucleoli were observed

adhering to the nuclear wall. The nucleus-to-cell ratio or nucleocytoplasmic index was about 0.42 (Table. 3. 4).

*Early Perinucleolus Stage (Pl: 3. 1a)*

This stage was characterized by the first significant increase in size of the oocytes, caused by enlargement of the nucleus as well as the cytoplasm. Rounded, oval, or polygonal oocytes often having a slightly eccentric nucleus, were 30.0 to 70.0 µm (mean = 44.2 µm) in diameter and had a basophilic cytoplasm. The nucleus, now referred to as germinal vesicle was less basophilic and contained 7 to 15 (mean= 10) basophilic nucleoli, arranged peripherally. The increase in number of nucleoli indicated increasing nuclear activity. The nucleus was in a diameter range of 17.0 – 36.0 µm. In the cytoplasm, a less basophilic yolk nucleus was visible close to the nucleus. Nucleocytoplasmic index decreased to 0.32 (Table. 3. 4).

*Late Perinucleolus Stage* (Pl: 3. 1a)

Oocytes at this stage appeared more regular in shape and were in the range of 60.0 to 140.0 µm (mean= 86.2 µm). At this last stage of primary growth phase, the ooplasm was less basophilic. The ooplasm became granular. Nucleus was enlarged and rounded and measured 34.0-57.0 µm in diameter. The number of nucleoli ranged from 12 to 30 (mean = 18). As the oocyte volume increased, the Nucleocytoplasmic index declined to 0.28. Oocytes at this stage were found in all females throughout the year and could be regarded as oocytes in the 'resting stage' (Forberg, 1982).

Yolk nucleus first appeared in the early perinucleolus stage, close to the outer periphery of nuclear envelope. It was also seen in late perinucleolus stage, which migrated to the periphery and disappeared subsequently. Yolk nucleus measured 6.38 - 12.75 µm (mean = 9.68 µm) in diameter. No trace of yolk nucleus was observed after the late perinucleolus stage.

### *3. 3. 2. 2. Second Growth Phase (SGP)*

*Yolk Vesicle Stage- I (Pl: 3. 2. b)*

Oocytes ranged from 90.0 - 150.0 µm (mean = 123.63 µm) in diameter. Ooplasm appeared less basophilic and yolk nucleus was no more visible. Nucleus measured 36.0-65.0 µm in diameter and started to become irregular in shape. Number of nucleoli ranged from 10-25 (mean = 15). During this stage of oocyte development, less than 100 yolk vesicles or lipid vesicles (Mayer *et al.,* 1988) appeared in the cytoplasm as unstained optically empty vacuoles. Nucleocytoplasmic index further declined to a value of 0.19 (Table. 3. 4).

*Yolk Vesicle Stage- II (Pl: 3, 2. b)*

This stage occurred from stage II ovaries onwards. Oocytes were 120.0 – 240.0 µm (mean = 172.7 µm) in diameter. Number of nucleoli ranged from 7- 17 (mean = 12). The cytoplasm, which had lost basophilia, was found flooded with lipid vesicles that numbered more than hundred and the latter moved centripetally forming a circular zone around the nucleus. The largest yolk vesicle measured 17.0 µm in diameter. The nucleus was in a diameter range of 47.0-77.0 µm. A thin

acidophilic layer, the future zona radiata (ZR), appeared at the periphery of the cytoplasm just beneath the follicle. Nucleocytoplasmic index further reduced to a value of 0.15 (Table. 3. 4).

*Primary Yolk Stage (Pl: 3. 2. d)*

This stage is easily distinguished by the appearance of acidophilic elongate yolk granules around the periphery of oocyte, which marks the phase of true vitellogenesis. Mayer *et al.,* (1988) had identified these bright pink structures as protein yolk granules. The yolk vesicles further enlarge up to a diameter of 33.0 µm. The oocytes measured 240.0 – 360.0 µm (mean = 265.53 µm) in diameter and the follicular layer became thicker. The number of nucleoli ranged from 7 to 21 (mean = 12). Nucleocytoplasmic index reached a low value of 0.08.

*Secondary Yolk Stage (Pl: 3. 2 e)*

Oocyte diameter ranged from 360.0 to 480.0 µm (mean= 413.11µm) and the protein yolk granules increased in number and migrated centripetally. The lipid vesicles have further increased in number and enlarged through coalescence. The ooplasm turned entirely eosinophilic. The nucleus became slightly acidophilic and formed niches along its wall in which the round nucleoli were located. Number of nucleoli ranged from 6 - 13 (mean = 11). Nucleocytoplasmic index further reduced to reach a value of 0.04.

*Tertiary Yolk Stage (Pl: 3. 2 f)*

Tertiary yolk stage oocytes were found in early stage IV (Ripe) ovaries. Oocyte diameter ranged from 480.0 to 540.0 µm (mean = 509.0 µm). The nucleus was stellate (Pl: 3.3, Fig. b) with a few remaining nucleoli appearing small and more basophilic. Nucleocytoplasmic index further declined to the lowest value of 0.02. The oocytes had densely packed yolk granules and yolk globules ranging from 4.0 µm to 32.0 µm in diameter and had large masses of yolk vesicles. The oocyte at this stage was enveloped by two layers, the squamous granulosa and theca (Pl: 3. 3a).

The tertiary yolk stage oocytes undergo maturation. This can be observed histologically in the form of germinal vesicle migration (Pl: 3. 3f), coalescence of the lipid vesicles with the subsequent disappearance of the nucleus, hydration and finally ovulation. This last process leaves behind a post-ovulatory follicle (Pl: 3. 3d), that later regresses.

***3. 3. 2. 3. Corpus Atreticum (Pl: 3. 3e)***

The oocytes that were subjected to early atresia were the tertiary yolk stage oocytes. These atretic oocytes are often termed corpora atretica in contrast to similar structures called corpora lutea, which are formed from follicular remnants after ovulation. The oocyte atresia were distinguished under the following four stages:

*α stage atresia*: In this stage, the nucleus and zona radiata deteriorates. The yolk starts to liquefy on the periphery. The lipid vesicles often

coalesced into a large central lumen. Zona radiata underwent hypertrophy and yolk globules joined to form bigger spherical masses. The zona radiata (Pl: 3. 3 c) often gets deflected off from the zona granulosa and the entire oocyte shows signs of degeneration.

*β stage atresia*: The nucleus disappears, zona radiata undergoes further breakdown and hypertrophy of granulosa cells occurs. The degenerated yolk materials form an amorphous mass.

*γ stage atresia*: Hypertrophy of the granulosa cells continues, zona radiata disappears completely and the oocyte becomes irregular in shape. Cytoplasm is rapidly phagocytized by the invading granulosa cells. Formation of orange and yellow lutein pigments among the cells were often observed. (Pl: 3. 3d).

*δ stage atresia:* Almost all the remnants of the oocyte were completely resorbed in this last stage, and all that remained was the evacuated outlines of the oocyte framed by the granulosa and thecal cells. Ovaries, in which all of the yolk stage oocytes were affected to varying degrees of atresia, were often observed.

### *3. 3. 2. 4. Corpus luteum (Pl: 3. 3d)*

During ovulation, the ripe oocytes are separated from the surrounding follicular layer, which subsequently undergo atrophy. The post-ovulatory follicular layer is defined here as the corpus luteum without implying similarity of function or homology to the structure in the mammalian ovary. Post-ovulatory follicles are direct evidence of recent ovulation as the follicle, which supported the developing oocyte,

is now broken down and resorbed. Two stages of corpus luteum were distinguished in the ovary of *A. testudineus* as given below:-

*Stage I(early)*. Post-ovulatory follicles were characterized by a large follicular lumen (atrium) formerly occupied by the ripe oocyte. After ovulation, the follicular layer consisting of theca and granulosa cells got hypertrophied with more marked effect in the granulosa. At times, an ovulation pore was visible, but most post-ovulatory bodies showed no indication for the release of eggs.

*Stage II (late)*. In this stage, the cells of granulosa and theca invaded the lumen of the follicle and occupied most of the space, causing considerable reduction in its size. The shrinkage continues leaving behind a patch of tissue that is indistinguishable from the ovarian stroma in later course.

### 3. 3. 3. Classification scheme used to identify different ovarian maturity stages (Table. 3. 3)

The eight oocyte stages identified histologically in the ovaries of *A. testudineus* can be correlated to the five ovarian maturity stages in the following manner: -

Stage-I (Immature): Oocytes are small and tightly packed within ovarian matrix. The ovary contains oocytes of chromatin nucleolus stage and perinucleolus stages, the early perinucleolus stage being predominant. Yolk vesicle stages and oocytes of later stages do not occur. Largest oocyte diameter (LOD) = 121.0 μm.

Stage-II. (Maturing virgins and Recovering spent): Ovigerous lamellae are in rows; in which are found oocytes up to yolk vesicle stage- II. Late perinucleolus stage oocytes form the predominant group. Atretic oocytes are found in spent recovering ovaries. Largest oocyte diameter (LOD) = 175.0 µm.

Stage-III. (Mature): one of the prominent groups of oocytes in this stage is constituted by the Yolk vesicle stage- II oocytes. These ovaries also contain yolky oocyte stages marking the vitellogenic phase of growth of oocytes. LOD = 415.0 µm.

Stage-IV. (Ripe): Tertiary yolk stage and higher oocyte stages predominate. LOD = 590.0 µm.

Stage-V. (Spent): Perinucleolar oocytes dominate among post-ovulatory follicles, residual oocytes and atretic vitellogenic oocytes.

### 3. 3. 4. Oocyte percentage distribution in different ovarian maturity stages (Fig. 3. 1)

To understand the dynamics of ovarian growth, the percentage composition of oocyte types in each of the five ovarian maturity stages were estimated from histological sections. In stage-I (immature) ovary, early perinucleolus stage oocytes formed the bulk group (55%). In stage-II, the late perinucleolus stage dominated (50%), amidst a significant group of yolk vesicle stage oocytes and a prominent batch of early perinucleolus stage oocytes. Stage-III (mature) was marked by the presence of vitellogenic stages (15%) along with yolk vesicle stage oocytes (13%) and a supporting batch of late perinucleolus stage oocytes

(36%). Vitellogenic stages (42%) constituted the prominent group along with a supporting batch of yolk vesicle stage oocytes (23%) in stage- IV ovary. In stage-V (spent) ovary, majority of the oocytes belonged to early perinucleolus stage (51%), along with a small fraction of tertiary yolk stage oocytes (10%), residual oocytes and a few post-ovulatory follicles. In stage-IV (ripe) ovary, 35% of the oocytes belonged to the category of first growth phase oocytes, whereas in the rest of the maturity stages, they ranged between 73% (in stage-III) and 100% (stage-I ovary).

**Table: 3. 1.** Schedule adopted for the dehydration and wax infiltration of yolky oocytes in the present study.

| Dehydration/infiltration agent | duration of treatment (min) |
|---|---|
| 70% ethanol | 60 |
| 80% ethanol | 60 |
| 90% ethanol | 30 |
| 95% ethanol | 30 |
| 100% ethanol | 10 |
| 100% ethanol | 10 |
| 100% ethanol | 10 |
| Benzene | 15 |
| Benzene and wax | 10 |
| Wax I | 10 |
| Wax II | 10 |
| Wax III | 10 |

**Table: 3.2.** Some of the terms used to describe oogenesis in the present study and their analogous terms

| Terminology used and abbreviations | Analogous terminology |
|---|---|
| Yolk nucleus (YN) (Abraham et al., 1966) | Perinuclear body, Balbiani's vitelline body, Idiosome (Stenger, 1959; Raven, 1961; Cle'rot, 1976) |
| Yolk vesicles (YV) (Kuo et al.,1974a, Palmer et al., 1995, Micale et al., 1999) | Lipid droplets (Mayer et al., 1988), lipid vesicles/yolk precursors (Cyrus & Blaber, 1984) |
| Post ovulatory follicle (POF) or Corpus luteum (Guraya & Kaur, 1979) | Corpus luteum of ovulation (Khoo, 1975) |
| Atretic follicles (AF) or Corpus atreticum (Lambert, 1970a). | Corpus luteum of atresia or preovulatory corpus luteum (Guraya, 1986). |
| Yolk globules (YG) (Yamamoto, 1956a; Guraya, 1986) | Exogenous yolk (Wallace, 1985), Yolk granules (Mayer et al., 1988), Red staining/secondary yolk (Cyrus & Blaber, 1984) |

**Table: 3. 3.** Histological characteristics in different maturity stages of ovaries of *A. testudineus.*

| Stage of ovary | Histology |
|---|---|
| I. (Immature) | Oocytes are small and tightly packed within ovarian matrix. Yolk vesicle stages and oocytes of later stages do not occur. Largest oocyte diameter (LOD) = 121μm. |
| II (Maturing virgins and recovering spent) | Ovigerous lamellae are in rows. Oocytes up to yolk vesicle stage-II occur. Atretic oocytes occur in spent recovering ovaries. LOD = 175μm. |
| III (Mature) | Yolk vesicle stage- II prominent; primary and secondary yolk stages also numerous. LOD=415μm |
| IV (Ripe) | Tertiary yolk stage and higher oocyte stages predominate. LOD = 590μm. |
| V (Spent) | Perinucleolar oocytes dominate among postovulatory follicles, residual oocytes and atretic vitellogenic oocytes. |

**Table: 3. 4.** Some characteristics used to identify discrete stages of oocyte development in *A. testudineus.*

| Oocyte stage | Oocyte diameter μm | | Number of nucleoli | | Nucleocytoplasmic index (N/C) |
|---|---|---|---|---|---|
| | Range | Mean | Range | Mean | |
| First growth phase | | | | | |
| *Chromatin nucleolus stage* | 10-40 | 21.9 | 2-7 | 4 | 0.42 |
| Early perinucleolus stage | 30-70 | 44.2 | 7-15 | 10 | 0.32 |
| Late perinucleolus stage | 60-140 | 86.2 | 12-30 | 18 | 0.28 |
| ***Second growth phase*** | | | | | |
| Yolk vesicle stage -I | 90-150 | 123.6 | 10-25 | 15 | 0.19 |
| Yolk vesicle stage -II | 120-240 | 172.7 | 7-17 | 12 | 0.15 |
| Primary yolk stage | 240-360 | 265.5 | 7-21 | 12 | 0.08 |
| Secondary yolk stage | 360-480 | 413.1 | 6-13 | 11 | 0.04 |
| Tertiary yolk stage | 480-540 | 509.0 | 8-14 | 10 | 0.02 |

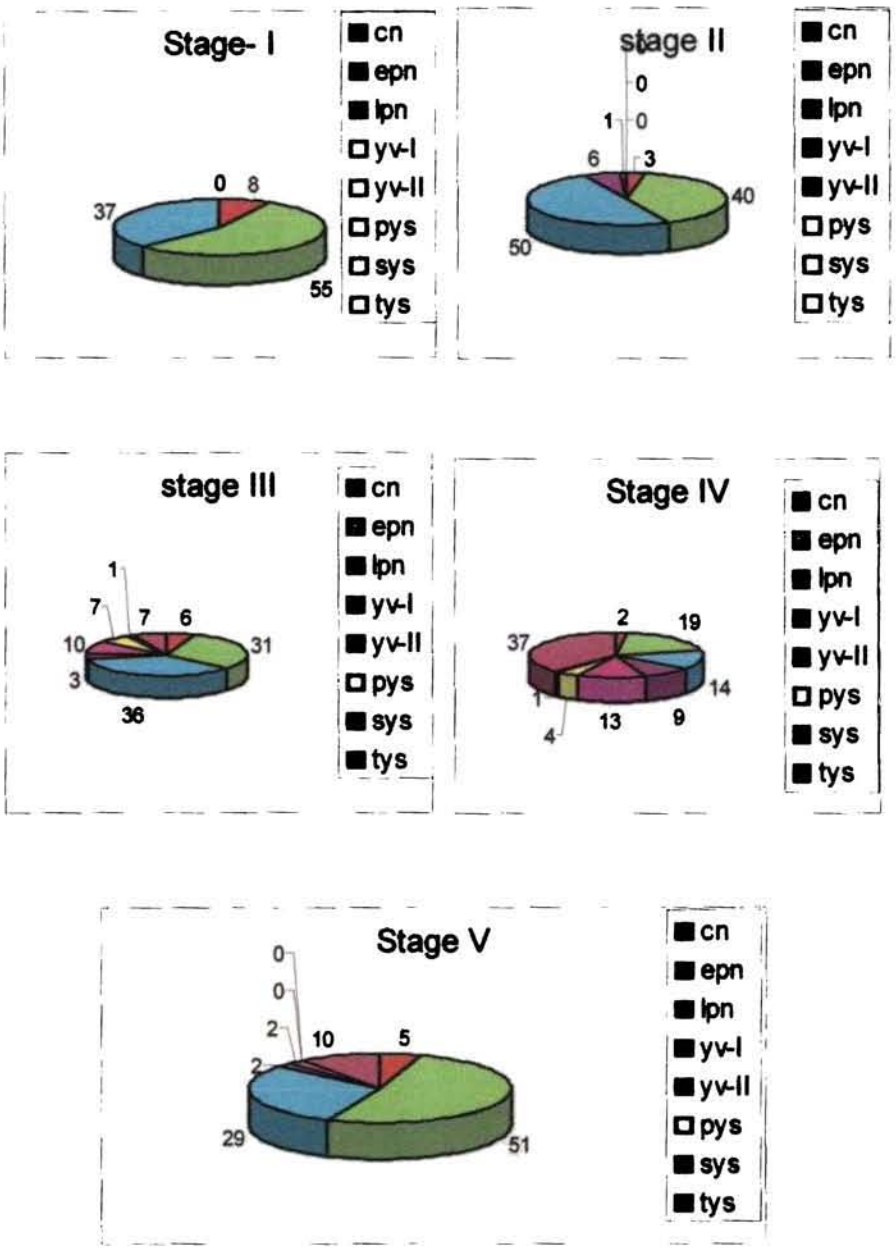



**Fig.3.1**. Maturity stage-wise distribution of oocyte types based on histological observations in *A. testudineus*. cn-chromatin nucleolus stage, epn -early perinucleolus stage, lpn-late perinucleolus stage, Yv- yolk vesicle stage, pys- primary yolk stage, sys-secondary yolk stage, tys- tertiary yolk stage.

# Plate 3.1

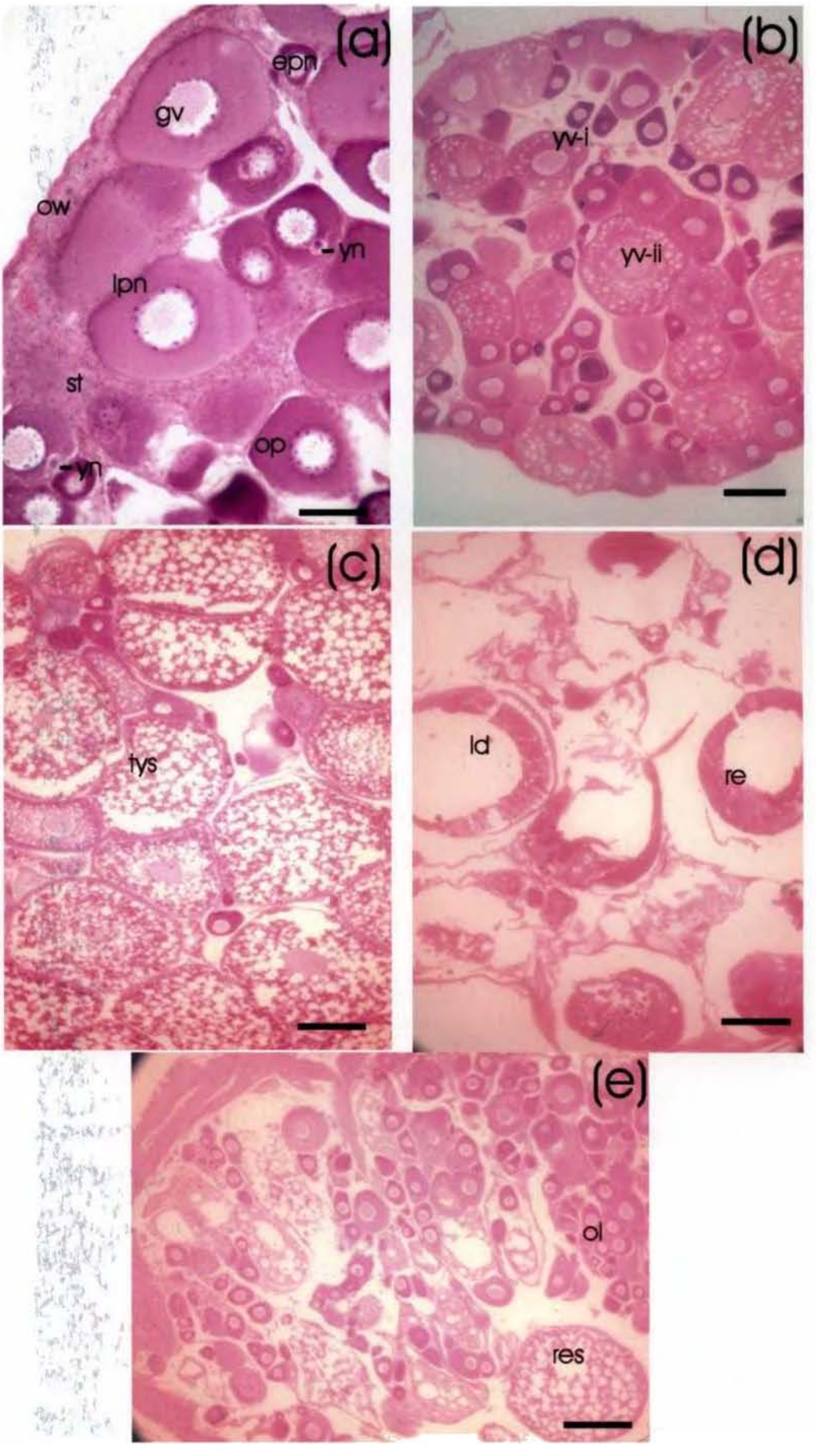


**Plate: 3.1**

**Histological appearance of ovaries of *Anabas testudineus* belonging to different maturity stages. Hematoxylin/Eosin.**

**a.** Immature ovary showing strong basophilic ooplasm (op). Early perinucleolus stage oocyte (epn), late perinucleolus stage oocyte (lpn), germinal vesicle (gv), ovarian wall (ow), and yolk nucleus (yn) are visible. Scale bar = 44μm.

**b.** Less basophilic maturing ovary with many yolk vesicle stage (yv) oocytes. Scale bar = 153μm.

**c.** Mature ovary with many tertiary yolk stage (tys) oocytes. Scale bar = 257μm.

**d.** Ripe ovary with ripe eggs (re) containing a large central lipid drop (ld). Scale bar = 207μm.

**e.** Spent ovary showing ovigerous lamellae (ol) and residual egg (res). Scale bar = 257μm.

Plate 3.2

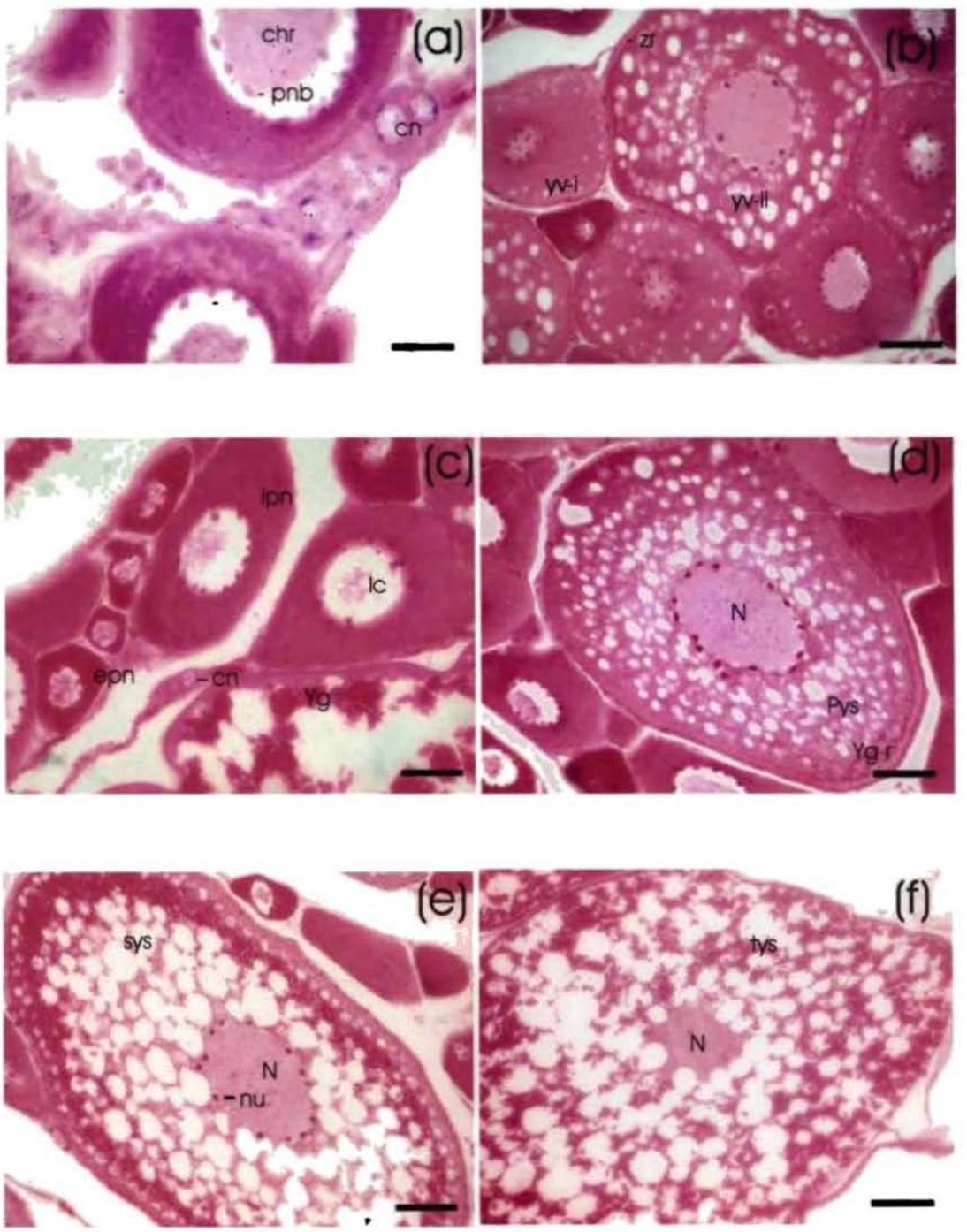

(a)
chr
pnb
cn
(b)
zf
yv-i
yv-ii
(c)
ipn
lc
epn
cn
Yg
(d)
N
Pys
Yg r
(e)
sys
N
nu
(f)
tys
N

**Plate: 3. 2**

**Photomicrographs of different oocyte stages in the ovaries of *Anabas testudineus*. Hematoxylin/Eosin.**

**a.** Chromatin nucleolus stage (cn) oocyte with a large central nucleus and one or two nucleoli. Perinucleolus stage oocyte with chromatin mass (Chr) and perinucleolar bodies (pnb) also visible. Scale bar = 19μm.

**b.** Early and late yolk vesicle (yv) stage oocytes containing many vacuole like yolk vesicles and developing zona radiata (zr). Scale bar = 44μm.

**c.** Early perinucleolus (epn) and late perinucleolus (lpn) oocytes showing differenes in number and disposition of nucleoli in their nuclei and lampbrush chromosome (lc). Chromatin nucleolus (cn) stage oocyte and yolk globules (yg) also visible. Scale bar = 35μm.

**d.** Primary yolk stage (pys) oocyte showing a large nucleus (N) and origin of yolk granules (ygr). Scale bar = 35μm.

**e.** Secondary yolk stage (sys) oocyte showing centripetal movement of yolk granules. Nucleus (N) and nucleoli (nu). Scale bar = 44μm.

**f.** Tertiary yolk stage (tys) oocyte with a stellate nucleus (N). Scale bar =86μm.

Plate 3.3

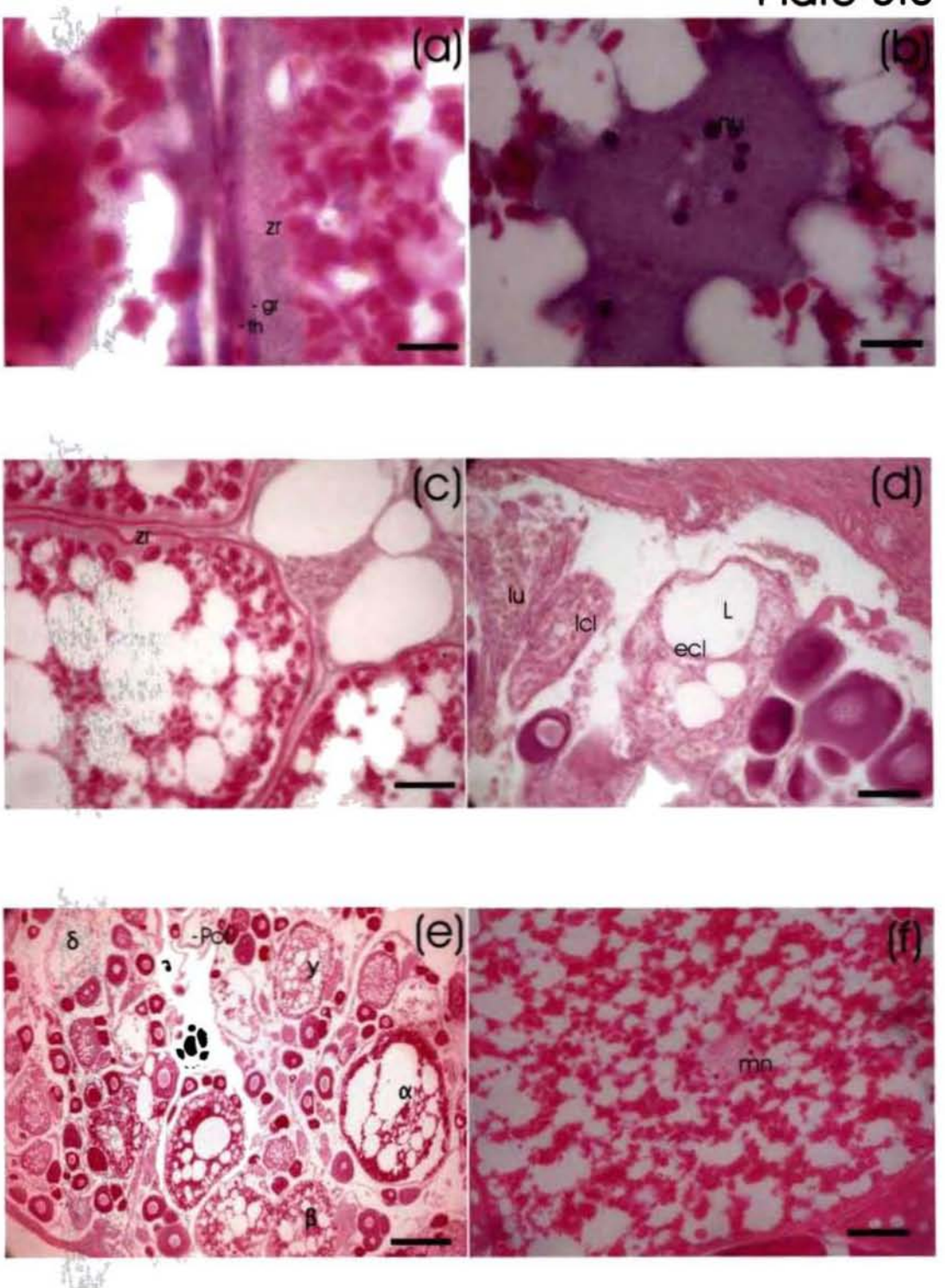


**Plate: 3. 3**

**Histological appearance of advanced stage oocytes, atresia and corpus luteum. Hematoxylin/Eosin.**

**a.** Tertiary yolk stage oocyte showing the theca (th), granulosa (gr) and zona radiata (zr) layers. Scale bar = 19μm.

**b.** Highly notched nucleus of a tertiary yolk stage oocyte containing a few nucleoli (nu). Scale bar = 23μm.

**c.** Partially hydrated oocyte with infolded zona radiata (zr). Scale bar = 44μm.

**d.** Spent ovary containing a lacuna (L) in early corpus luteum (ecl) and the late corpus luteum (lcl). Lutein (lu) pigments from atretic follicle appear in yellow shade. Scale bar = 68μm.

**e.** Spent ovary containing α, β, γ, δ, and * stages of atresia and post-ovulatory follicle (pof). Scale bar = 207μm.

**f.** Advanced stage oocyte with a migratory nucleus (mn) in which the nucleoli are seen interdigitating with the surrounding ooplasm. Scale bar = 44μm.

## 3. 4. DISCUSSION

For various reasons the fish ovary has attracted the attention of the research workers during the past many decades. Female reproductive organs and their functional mechanisms in *A. testudineus* was found to have a typical teleostean pattern, like in many other fishes such as *Colisa fasciata* (Bhatti & Javaid (1973) and *Barbus luteus* (Al-Daham & Bhatti, 1979). Most of the teleostean fishes undergo regular gonadal cycles. In some species this may be completed in weeks as in the case of *Poecilia reticulata* (Lambert, 1971), while in others, it may be completed in a year, as in the present case.

Patterns of oocyte growth generally are similar among teleosts, although subtle variations occur in the character of each developmental stage. As a more precise and objective method of determining the stage of maturity of the gonad, histological sections were employed in the present work. Histological techniques are more reliable (Booth & Weyl, 2000) and according to West, (1990) Should be based on the most advanced oocyte present, regardless of their number. West (1990) stated that calculating proportions of eggs at different developmental stages is too complex and time consuming, and is no more effective than predictions based on the most advanced oocyte. The classification scheme used in the present study following West (1990) is simple and maturity stages of the ovaries could easily be distinguished (Table.3. 3).

Once the oocytes are ovulated and spawned, many post-ovulatory follicles are observed in *A. testudineus* ovary. This signals that the female has spawned. According to West, (1990), spent stages are common in species where a large number of eggs are spawned in a short

period. There was always only a small number of vitellogenic oocytes left in the spent ovary of *A. testudineus*. Thus, the appearance of the spent *A. testudineus* ovary indicated a single spawning over a short period. The histological observations of ovaries of various maturity stages observed in different seasons supported the existence of a single spawning season in *A. testudineus*. Once the spawning season ends, the vitellogenic oocytes are resorbed as is clear from the absence of vitellogenic oocytes from most of the recovering spent ovaries.

The histomorphological details of the ovarian tissues of almost all the fishes are similar (Iwamatsu *et al.*, 1988; Merve, *et al.*, 1988). However, due to diverse internal (hormonal) and external (environmental) factors, the oocytes grow through different stages exhibiting their cytoplasmic and nuclear peculiarities.

Different authors have divided the oocyte types into different number of stages, based on morphological characteristics of developing oocytes. Maddock & Burton (1999) and Arocha (2002) distinguished only four stages. Wallace & Selman (1979), Thorogood (1986), Elorduy & Ramirez, (1994) and Coward & Bromage (1998) recognized six stages. Mercy *et al.*, (1982) proposed nine stages while, Saat & Veersaalu (1990) distinguished 11 oocyte stages. Gopalakrishnan (1991) divided oocytes into thirteen stages. In the present work, oogenesis has been described in only eight stages with the descriptions based on easily recognizable anatomical and size differences.

Widely varying views exist on the source of origin of oocytes in teleosts (Rai & Rai, 1981). Germinal epithelium in the ovigerous lamellae, however, is considered the most common site for the

production of new crops of oocytes. The pre-existing residual oogonia have also been considered as the source of a new generation of oocytes of *Channa gachua* (Khanna and Sanwal, 1971), *Schizothorax richardsonii* (Bisht and Joshi, 1975), *Channa marulius* (Srivastava, 1980). Sahu, (1983) has observed the transformation of follicular epithelial cells of an atretic follicle into an oocyte in *Channa punctatus*. Aravindan and Padmanabhan (1972) reported that new crops of oocytes in *Tilapia mossambica* arise from nests of cells of the germinal epithelium as well as from the epithelial strands ramifying into the ovocoel. Fishelson (1977) is of the view that the oocytes in *Dendrochirus brachypterus* originate from the stromal tissue. Dutt & Govindan (1969) reported that in *Anabas scandens*, new germ cells arise from the remnants of the follicular cells of germinal epithelium as well as from the remnants of follicular cells of the postovulatory corpus luteum. However, during the current investigation, no such instance of oogonia arising from follicular cells of postovulatory corpus luteum in *A. testudineus* ovary was evident.

From such varying information on the possible origin of oocytes, it appears that during the ovarian growth, some of the cells separate from the germinal epithelium and extends to the ovocoel to lie close to the stromal tissue, blood capillaries, thecal tissue, granulosa cells of normal or atretic oocytes or post-ovulatory follicle, in course of time. These migrating cells later differentiate into oocytes, misleading perhaps the observers to consider the different sources as the sites of oocyte differentiation (review, Verma, 1997).

Oocyte growth follows a similar general pattern in most teleosts. Oogonia give rise to immature oocytes with multiple peripheral nucleoli.

These perinucleolar oocytes then undergo primary vitellogenesis. The oocyte enlarges and its zona radiata thickens as vitellogenic yolk is deposited (N'da & Deneil, 1993). The nucleus migrates to the animal pole prior to the breakdown of the nuclear membrane (Yamamoto, 1956). Hydration precedes ovulation and the appearance of these hyaline oocytes is an indication of imminent spawning (West, 1990). The follicle collapses after the oocyte has been released to form structures called post-ovulatory follicles (POFs) which are indications of recent spawning and are not thought to persist for a long time (Maddock & Burton, 1999).

The differentiating young oocyte possessed a spherical large nucleus (germinal vesicle) almost filled with chromatin mass scattered among which a number of lampbrush chromosomes could be be seen ((Pl: 3. 2c). The presence of lampbrush chromosomes indicates a high rate of protein synthesis in the oocytes. According to Wallace *et al.,* (1987) the chromosomes of the late chromatin nucleolus stage oocytes become arrested in late diplotene of first meiotic prophase and shortly after this their ribosomal genes are amplified. The appearance of multiple nucleoli in the perinucleolus stage oocytes (Pl: 3.1a, Table. 3. 4) can be attributed to this gene amplification.

In course of time, small spherical bodies originated in the vicinity of the chromosomes. These bodies are the perinucleolar bodies (PNB) (Pl: 3.2a). Later a large number of nucleoli could be seen originating in the chromatin mass, which subsequently arranged themselves along the inner periphery of the nuclear membrane (Pl: 3. 2 a - e). Different views exist with respect to the origin, chemistry and function of the nucleoli in the teleostean oocytes. Dutt & Govindan, (1975) found in *Anabas scandens* that the differentiating oogonium has a single large primary

nucleolus (PNU), which persisted until the oogonial cell grew into immature oocyte and finally disappeared. They, and Sahu (1983) in *Heteropneustes fossilis,* suggested that the perinucleolar bodies, which occur in close association with the lampbrush chromosomes, give rise to numerous small nucleoli. These in course of time move out and get attached to the nuclear membrane as secondary nucleoli (SNU). Gregor (1972) and Bara (1960) believed that the nucleoli originated from certain heterochromatic regions of the chromosomes known as the nucleolar organizers. On the other hand, Chouinard (1963) reported that in *Salvelinus fontinalis,* no part of a lampbrush chromosome was involved in the formation of the SNU. Rai (1967) in *Tor tor,* Srivastava & Swarup (1979) in *Channa striatus*, and Srivastava (1980) in *Channa marulius,* were of the view that the SNU are formed by division or fragmentation of the PNU. Khanna & Sanwal (1971) in *Channa gachua*, Rita Kumari & Padmanabhan (1976) in *Etroplus suratensis*, and Verma *et al.,* (1981a) in *Tilapia mossambica,* found no signs of fragmentation of the nucleolus, and concluded that the SNU arose from the chromatin mass and then migrated to the periphery. In the present study also, no such fragmentation of the nucleolus was apparent.

Many workers have noticed a mass of cytoplasmic structure situated beside the nucleus in the early oocytes of vertebrates, and various terms like yolk nucleus (YN), Balbiani body, 'archoplasm', 'centrophore' and 'corps vitelline' have been used by them. Its presence in teleostean oocytes was first reported by Hubbard (1894). Coward & Bromage (1998) did not find the Balbiani body in the oocytes of *Tilapia zilli.* There exists no unanimity among the investigators regarding the origin, chemistry and function of yolk nucleus in teleosts. According to Dutt (1964) and Sobhana & Nair (1977), the yolk nucleus is nuclear in

origin. Rita Kumari & Padmanabhan (1976) and Mercy *et al.,* (1982) believed it to have originated *de novo* in the cytoplasm. In *A. testudineus* in the present study, the YN was first observed in the early perinucleolus stage closely apposed to the wall of the germinal vesicle, which later migrated to the periphery. During this stage of growth of the oocyte, an increase in number of nucleoli could also be observed. Therefore, it appears that nucleoli take part in the formation of YN. This is in agreement with observations of Reihl (1976), who found the YN to have nucleolar substance, mitochondria and dictyosomes and reported that the nucleolar material left the nucleus through the pores of the nuclear membrane and got into the juxtanuclear cytoplasm.

While most of the workers find the YN of fish oocyte to be composed of RNA and protein (Guraya, 1979), Livni (1971) detected a few enzymes in the YN of a number of fishes. Beams & Kessel (1973) noted that the YN of *Salmo gairdneri* constituted an essential precursor (RNA), which was necessary for oocyte growth and vitellogenesis. Guraya, (1965) believed that YN is associated with the formation of both protein and lipid yolk. Dutt & Govindan, (1969) also believed that YN is indirectly involved in vitellogenesis. However, it is to be noted that, in *A. testudineus* in the present study the yolk nucleus disappeared far before the onset of vitellogenesis and hence apparently it has no role in vitellogenesis.

By the end of the primary oocyte growth (previtellogenesis) in *A. testudineus,* the oocyte has grown from a diameter of 10-20 μm to a diameter of about 90-110 μm. Thus, approximately a thousand-fold increase in volume had taken place, primarily due to the elaboration of the normal cytoplasmic organelles and accumulation of large amounts of

cellular products. However, it is to be noted that the ovaries containing only primary growth stage oocytes are still relatively small and are generally observed as immature.

The germinal vesicle (GV) of the oocyte is the nucleus proper. During the early stages in the growth of the oocyte, the GV occupies a major part of the cell space. However, during the subsequent growth stages the nucleus fails to keep pace with the growth of the cell and hence becomes proportionately smaller as revealed by the decline in nucleocytoplasmic index from a value of 0.42 in chromatin nucleolus stage to 0.02 in the tertiary yolk stage.

The occurrence of two types of yolk inclusions (yolk vesicles and yolk granules) as observed in *A. testudineus* has been found in many other teleosts (Yamamoto, 1956a, Khoo, 1979, Mollah, 1986). Yolk vesicles are distinguishable from yolk granules by their larger size and staining characteristics. These two types of yolk were found to form sequentially with the yolk vesicles deposited before yolk granules. However, the occurrence of two types of yolk is not universal among teleosts (Khoo, 1979). In the species like *Hypomesus japonicus* (Yamamoto, 1956b) and *Channa marulius* (Guraya, 1965), three types of yolk inclusions *viz*; carbohydrate yolk, protein yolk and fatty yolk, have been observed.

Yolk vesicles were the first type of inclusions appearing in the cytoplasm of second growth phase (SGP) oocytes. These vesicles were likely to contain lipid, as they appeared as empty non-staining vacuoles on paraffin sections, suggesting that their contents were dissolved during routine histological procedures. The yolk vesicles in *A. testudineus,* in

the present study, have been found to follow the same centripetal sequence of development as observable in most of the teleosts such as *Tandanus tandanus* (Davis, 1977), *Carassius auratus* (Khoo, 1979), *Clarias macrocephalus* (Mollah, 1986). Dutt & Govindan, (1967) however, reported that the lipid vesicles in *Anabas scandens* moved to the peripheral ooplasm from the centre. In the present study, during oocyte maturation, the lipid vesicles were found to coalesce and formed a single large lipid drop, which might help the eggs to keep buoyant.

In histological sections, yolk appeared as granules first and globules later, within and between the lipid droplets. In the swordfish *Xiphis gladius*, the yolk granules concentrated in the inner cytoplasm, were found to multiply and spread outwards (Arocha, 2002), just the opposite way as found in the present study. Yolk deposition was presumed to stop and the next phase occurred when the nucleus migrated to the animal pole and meiosis resumed (indicated by the disappearance and dissolution of the nucleus) that was arrested when the oocyte was in the late previtellogenic stage (Nagahama, 1983 and Arocha, 2002). Based on histological examination, the early stages of vitellogenesis (or previtellogenesis) in A. *testudineus* were found in oocytes ranging from 120.0 to 240.0 µm. Vitellogenesis started when the oocytes reached a diameter of 240.0 µm.

If perinucleolus stage oocytes were to be constantly recruited to the maturing oocytes, an overlap should be observed in percentage distribution of oocytes at different developmental stages. In *A. testudineus* in the present study, the percentage distribution of oocytes broadly overlapped in each of the different stages of development. In immature fish, only up to perinucleolus stage oocytes were found (Fig. 3.

1). As vacuolization in perinucleolus stage oocytes started, previtellogenic oocytes developed and both stages overlapped in stage-II ovary. Once vitellogenesis started and vitellogenic oocytes appeared in the distributions, ovaries that had undergone vitellogenesis showed a continuous distribution and no gap could be distinguished between the different developmental stages. Thus, new perinucleolar stage oocytes were not found recruited after the onset of vitellogenesis.

Developmental events that occur in the oocytes of *A. testudineus* are similar to those described for other species with synchronous development. Chromatin nucleolus stage and perinucleolus stage oocytes were found in all ovaries all the year and are considered part of the initial growth phase (Fig. 3.1). The second growth phase (Forberg, 1982) in *A. testudineus* started with the emergence of previtellogenic oocytes, when it showed signs of lipid inclusions in the periphery of the cytoplasm, which ended when yolk accumulation was complete in the vitellogenic oocyte. Once yolk accumulation is complete, the final maturation process is initiated by migration of the nucleus (De Vlaming, 1983; Redding & Patino, 1993). This maturation process ends when the oocyte hydrates and is ovulated. However, some authors (Hunter *et al.*, 1986; Schaefer, 1987; Davis & West, 1993) used a ripe or spawning stage that included migratory nucleus and hydrated oocyte stages, which are of very short duration. Saat & Veersalu (1990) reported that germinal vesicle migration in *Cyprinus carpio* occured in 4 hours.

It is during vitellogenesis that, in oviparous vertebrates, the oocyte accumulates the egg nutritional reserves, which in fish mainly originate from the incorporation of vitellogenin (VTG) and lipids. The yolk is the result of exogenous material processed within the ooplasm for

use by the embryo and the larvae during their development. Numerous ultrastructural observations show clearly that endocytosis occurs simultaneously for the different constituents of the yolk. Nevertheless there are two successive phases: I) a type I vitellogenesis when lipid accumulation predominates over incorporation of VTG, which later is only detectable by electron microscopy, and when zona radiata externa is deposited ii) and a type II vitellogenesis when endocytosis of VTG is largely predominant over lipid uptake, and when zona radiata interna is deposited. Recent studies underline the importance of cellular tools that are being setup during vitellogenesis to prepare the endocytotic activity of the oocyte during vitellogenesis (Le Menn *et al.*, 2000).

Vitellogenin is believed to be manufactured in the liver from stored reserves (Norberg & Kjesbu, 1991) and is translocated to the ovary where it is incorporated as yolk granules in the vitellogenic oocytes (Bromely *et al.*, 2000). At the end of vitellogenesis, the oocyte resumes its progress through division I of meiosis, and maturational processes occur with the oocyte nuclear envelope breakdown: the GVBD (germinal vesicle breakdown). This first meiotic division gives rise to two cells differing greatly in size: a very small first polar body and a large secondary oocyte. In fish, as in most vertebrates, the second meiotic division proceeds to metaphase and pauses until fertilization by a spermatozoa. Therefore, the oocyte, separating from its follicular envelopes and dropping into the ovarian lumen at ovulation, is a secondary oocyte (Bromely *et al.*, 2000).

Yolk differs widely in proportion and total amount of its chemical components during different reproductive phases of the fish. The true yolk of most of the teleostean oocytes is either an admixture of proteins

and lipids (lipoprotein yolk) or composed of protein and lipid separately (review, Verma, 1997). The teleostean yolk has been given different nomenclature depending on its morphology, origin and chemistry. The terms like endogenous yolk and exogenous yolk are based on the notion that the former arises from ooplasmic organelles and the latter from vitellogenin synthesized in the extra ovarian tissue (liver). The fabrication of yolk precursors in liver and their subsequent sequestration within the ooplasm by the process of micropinocytosis in the formation of protein yolk have been shown with the help of autoradiography and electron microscopy (Peute *et al.,* 1978). Vitellogenin has been isolated and characterized from the plasma or serum of several teleosts (Campbell & Idler, 1980; Selman & Wallace, 1982). In addition, studies have shown that vitellogenic proteins are transferred from the blood to oocytes (Boheman *et al.,* 1981, Mukherjee *et al.,* 1989) and that teleost gonadotropins enhance this transfer (Idler & Campbell, 1980, Nath & Sundararaj, 1981b) by stimulating micropinocytotic activity at the oocyte surface (Upadhyaya *et al.,* 1978).

The oocyte maturation in *A. testudineus* is initiated with the migration of the germinal vesicle to the periphery of the oocyte (Pl: 3. 3, Fig. f) and its subsequent breakdown (GVBD). These changes in the oocytes are indications of resumption of meiosis-I and the liberation of fist polar body (Guraya, 1986). The remaining haploid set of chromosomes enters second meiotic metaphase, the meiosis is arrested once again, and the egg becomes "mature" (Wallace *et al.*, 1987). Another noticeable change during this period is the increase in size of the oocyte due to hydration (Pl: 3.3, Fig. c). This process is accompanied by the coalescence of protein yolk granules to form a uniform mass, giving the ripe eggs a translucent appearance.

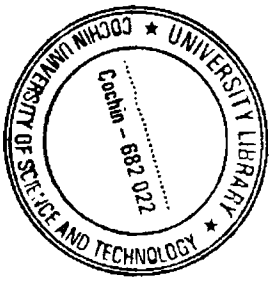
Histology revealed the resorption of the tertiary yolk stage oocytes and the other SGP oocytes in some spent ovaries (Pl: 3. 3, Fig. e), though not observed in most of the spent recovering ovaries of *A. testudineus* in the present study. Absence of any fresh primary yolk stage or secondary yolk stage oocytes in spent ovaries indicated that a second batch is not recruited to vitellogenesis. Some yolk vesicle stage-II oocytes were found resorbed, but yolk vesicles stage-I oocytes were perhaps not resorbed. It is also difficult to determine whether the observed atresia represented premature resorption of oocytes that could have been released later or it was part of the natural tissue resorption process after cessation of spawning. The true cause of follicular atresia is not known. The general belief is that it is probably caused by insufficient gonadotropic stimulation, particularly in the case of vitellogenic ovaries (Guraya, 1986).

The post-ovulatory follicles did not last long in the gonads and in the last stages were very difficult to distinguish from the late atretic structures. The duration of the post-ovulatory follicles within the gonads was not established, but a distinction between 'recent' and 'late' structures based on the overall size of the follicle and the relative size of the lumen could be made. Post-ovulatory follicles with large lumen are direct evidence of recent ovulation as the follicle that supported the developing oocyte is now broken down and resorbed (Pl: 3. 3, Fig. d). Residual eggs (Pl: 3.1, Fig. e) in the lumen of the ovary are also an indication of a recent ovulation episode.

Post-ovulatory follicles (POF) degenerate rapidly, making it difficult to assess the percentage of oocytes spawned unless samples are collected daily (Hunter & Macewicz, 1985). Khoo (1975), working on

gold fish ovaries, reported that post-ovulatory follicles have a definite pattern of histogenesis. According to Lam *et al.,* (1978) and Smith & Haley, (1987), the POFs are steroidogenic, but their precise role and potential significance to ensuing spawning cycle in any teleosts still remains unclear (Coward & Bromage, 1998).

In conclusion, the oocyte development in *A. testudineus* in the present study was found to follow the typical pattern as that of group synchronous spawners with a single spawning over a short period. The classification schemes developed, based on histology, may be helpful in identifying discrete stages of oocyte development and in identifying the different ovarian maturity stages with precision. Yolk nucleus disappeared far before the onset of vitellogenesis and hence apparently it had no role in vitellogenesis. The development of oogonia from follicular cells of corpus luteum as reported by Dutt & Govindan (1969) in *Anabas scandens* was not observable in the present study. Similarly, the present study also disagrees with the report of Dutt & Govindan (1969) in *Anabas scandens* regarding the direction of migration of the yolk vesicles.



Chapter 4

# *Induction of precocious ovarian recrudescence*

# CHAPTER IV

# INDUCTION OF PRECOCIOUS OVARIAN RECRUDESCENCE

## 4. 1. INTRODUCTION

Majority of fish breed during a restricted period of the year, a fact that indicates the significance of the role played by nature in reproduction. While studying the reproductive cycles of Kashmir fishes, Malhotra (1965) pointed out the importance of timed breeding. He stated that a proper breeding season not only offers better environmental conditions for hatching but also ensures sufficient food for the growth of the young. An extended period of favourable environmental conditions is imperative to protect developing young against hostile environment during tender embryonic life. A favourable environment also helps the spent fishes in recovery following spawning exhaustion. Hartridge (1936) first introduced the term "exteroceptive factors" for external factors, which mediate their effects upon regulation of reproductive cycles.

Indian freshwaters range from high altitude snow fed to warm subtropical and tropical habitats. These freshwaters thus provide diverse ecological conditions influencing fish reproduction in ways more than one. In temperate regions, four different seasons (spring, autumn, summer and winter) are well marked, but in tropics and subtropics of India, spring and summer are compressed in time to be ecologically indistinct. Further, an extended period of monsoon with much

precipitation is prominent, during which period, factor interaction is maximum, ensuring propitious period for growth of biota (Odum, 1971). There are many environmental factors that affect different phases of gonadal cycles. Baker (1938) while discussing the evolution of breeding season recognized them as the 'proximate' and 'ultimate' factors. While proximate factors (such as light, temperature and some physical factors) regulate the development of reproductive organs and processes in breeding adult, ultimate factors (like abundant food and favourable growing condition) effect the survival of young (Baker, 1938; Liley, 1969; Sadlier, 1973).

One of the environmental factors affecting reproductive cycle is light. In most of the fishes from Indian subtropical regions, long photoperiod (14L-10D) stimulated gonadal maturation as evidenced in *Cirrhina reba* (Verghese, 1967, 1970 & 1975) and *Heteropneustes fossilis* (Sundararaj & Vasal, 1976). In contrast, short photoperiod (10L-14D) accelerated gonadal maturation in *Channa gachua* (Sanwal & Khanna, 1972). Htun-Han (1977) generalized that in temperate species, long photoperiod stimulates gonadal maturation in spring spawners and short photoperiod influences maturation in autumn spawners. However, in temperate Kashmir fishes, light does not play any direct role in the pre-spawning maturation of gonads (Malhotra, 1965,1966).

Another important environmental factor, *viz*; temperature is considered the most important exteroceptive factor controlling sexual cycle in temperate fishes of Kashmir Valley (Malhotra, 1965, 1966; Jyothi, 1973). These authors observed a winter diapause (dormancy) in the ovaries either in immature stage (stage I) or in completely mature stage (stage III), and regarded this as an adaptation against acute winter

conditions. This inhibition is broken only when the temperature starts rising during March/April (spring). Often temperature is not accounted for in photoperiodic studies. Dependence of photoperiod on temperature was revealed by the studies of Sundararaj and Vasal (1976). They demonstrated that a long photoperiod (14L) for six weeks stimulated ovarian recrudescence, but this response was more effective at higher temperatures achieving the same effect in shorter duration; vitellogenesis continued unabated at 30° C regardless of photoperiod (12L-14D, continuous light or continuous dark). Similarly, Swara (1974) and de Vlaming (1975) observed that long photoperiod stimulated gonadal maturation only if it was acting in combination with warmer temperature. Malhotra (1965) also indicated the effect of light to be subordinate to that of temperature in the case of Kashmir fishes.

Impact of environmental factors on gonadal development in teleosts have been amply highlighted in literature (Goldberg & Herring, 1981; Xie & Xiang, 1990; Soto *et al.*, 1992, Santos, 1995). Sudha (1993) found that low temperature and salinity caused body and ovary lipid deposition in *Mystus vittatus*. Singh and Towheed (1993) observed that low temperature in winter phase caused depletion in lipid levels in ovary, muscle and liver in the female fresh water swamp eel *Monopterus cuchia* (Ham). Popma and Lovshin (1996) were of the view that, usually, low temperatures are harmful for growth and reproduction of fishes. Sarma *et al.* (2003) studied the changes in surface structure and histological features of gonads of *Labeo rohita* (Ham.) at mid altitude and at low temperature conditions in North Eastern state of Meghalaya. The role of environmental temperature on sexual maturation and breeding of fish had been studied by investigators like Hora (1945) and Chaudhuri (1960). Meske (1969) and Gupta (1975) found that carps

maintained at warm water aquaria showed very rapid rate of growth with unusually early maturation and spawning.

Sundararaj and Vasal (1976) reported that in *Heteropneustes fossilis*, ovarian recrudescence, which normally occured during the prespawning period, could be advanced by photothermal manipulation. They found that during the postspawning period, the tempo of ovarian recrudescence is much faster at $30^0$C than at other temperatures (ambient 25 or $34^0$C) regardless of photoperiod. Information available from the published literature suggests that studies on the effects of temperature and photoperiod, on ovarian cycle in fishes, have not been so far taken up in Kerala. The present investigation is an attempt to obtain an insight, into the effects of environmental factors such as temperature and photoperiod, on the ovarian cycle during the post spawning (July-November) period, in the ovary of *A. testudineus* and to determine whether combinations of a long photoperiod (14L-10D) and altered temperature would lead to precocious ovarian recrudescence (Alok *et al.*, 1994) during this period.

## 4. 2. MATERIALS AND METHOD

The present experiment was conducted during August-November 2001 in the Department of Marine Biology, Microbiology and Biochemistry, CUSAT, Kochi. Fishes were collected in August from Trichur '*Kole lands*' ($10^0$ 34' N and $10^0$ 36' N and $76^0$ 6' E and $76^0$ 9 ' E) and adjoining freshwater bodies. 78 fish, which ranged in size from 7.3 cm to 14.8 cm in length and 6.05 g to 60.76 g in weight, were divided into two equal groups and stocked in two rectangular fibre tanks. The fish, maintained at a stocking density of 1.0 Kg 100 $L^{-1}$, were acclimated for two weeks before the experiment. Everyday, the fishes were fed *adlibitum* alternately, with goat liver and a formulated feed having the following proximate composition: crude protein-38%, crude fat-8%, crude fibre-8%, ash-17%, moisture-8% and nitrogen free extract-21%. Remnants of feed and excreta were siphoned out everyday and 75 % of water was changed on alternate days.

### 4. 2. 1. The experimental system (Fig: 4. 1)

The experimental system was indigenously designed. To maintain a temperature of 30-31$^0$C (T1) in the tank, a waterbath fitted with refrigeration facility and a heating element, was used. The temperature sensor of the waterbath was immersed in the experimental tank. A ½ H.P. motor pump was split at its suction and delivery points and the connecting tubes were put in the tanks of the waterbath and the experimental tank in such a way that as the system worked, the water in the tank of the waterbath and that of experimental tank freely mixed, maintaining the same temperature in the experimental tank, as set in the waterbath. Working of the pump was regulated using a timer device to

prevent any overflows due to unequal suction and delivery. As an additional safe guard, siphon tubes were connected with the tanks so that any change in water level would be self adjusted during the pause of the pump. Using tube lights and a timer device, a photoperiod of 14L-10D was maintained. The second group of fish was maintained at a temperature of 26-27$^0$C (T2) under similar laboratory conditions and 14L-10D photoperiodicity. The experiment was terminated at the end of the third month. As a control, fish were collected from the original collection site during respective periods, analyzed and compared with the experimental groups. Surface water (0.5 m depth) temperature at the collection site was also noted during the term of the experiment.

### 4. 2. 2. Analysis of ovary

At the beginning of the experiment and at 1-month intervals, random samples of 4 fish each were sacrificed; the stage of maturity was determined and gonadosomatic index (GSI= Gonad weight × 100/ total weight) was calculated. The rest of the fishes were sacrificed on day 90 at the end of the experiment. Representative samples of ovaries were fixed in Bouin's fixative, processed and embedded in paraffin wax, sectioned and stained with hematoxylin and Eosin, following the standard histological procedure. From the histological sections, largest oocyte diameter (L O D) was measured; sections were examined for any histological change and for assessing the relative occurrence of the various oocyte stages, and photographed.

## 4. 3. RESULTS

### 4. 3. 1. Gonado Somatic Indices (Tab. 4. 1; Fig. 4. 2)

From an initial value of 0.42 ± 0.18, the mean GSI steadily declined to 0.23 ± 0.06, 0.21 ± 0.05 and 0.19 ± 0.07 in the treatment group T1 (30-31$^0$C) fishes, whereas in group T2 (26-27$^0$C), the values remained fairly stable for the first two months (0.42 ± 0.18, 0.41 ± 0.08), but showed a marked increase to reach a value of 1.39 ± 0.38 at the end of the third month. Among the control group C (28-29$^0$C) fishes, the GSI values steadily declined from 0.42 ± 0.18 to reach a final value of 0.23 ± 0.07 by the third month. Ovaries of all the Group T1 and the control group C fishes remained in the Stage II of maturity till the end of the experimental period. Among the fishes of T2 group, all remained in stage II maturity till the end of first month, but by the end of the second month, while 75 % of the ovaries remained in maturity Stage II, 25% reached stage III and by the end of the third month, 75% of the ovaries reached Stage III maturity, while the rest had stage IV level of maturity.

### 4. 3. 2. Histology of the ovary (Pl: 4. 1-2; Tab. 4. 2)

The spent recovering ovarian samples at the beginning of the experiment presented many second growth phase (SGP) oocytes (12.8%) amidst the perinucleolar oocytes (80.8%). Vitellogenic oocytes were undergoing atresia and largest oocyte diameter (LOD) was 460 µm. In the T1 group fishes, after one month, only perinucleolar oocytes (LOD = 128 µm) were observed and there were also a few atretic yolk vesicle stage oocytes; but by the second month, perinucleolar oocytes alone were visible and the LOD was 132 µm and towards the end of the third

month, massive atresia of the perinucleolar oocytes was observed in all the ovaries examined.

Among the T2 group fishes, after one month of the treatment, up to Yolk vesicle stage II oocytes (2.9%) were observed and the LOD was 189 µm; atresia was not observed and by the second month, the LOD marginally increased to a value of 197 µm and there were relatively a larger number of yolk vesicle stage II oocytes (6.2%). However, by the end of the third month, many vitellogenic oocytes (17.5%) appeared among Yolk vesicle stage II oocytes, and the LOD rose to 652 µm.

Among the group C controls, by the end of the first month, vitellogenic oocytes were not observable and there were a small percentage (2.9%) of yolk vesicle stage oocytes amidst the perinucleolar oocytes and the LOD was 142 µm. By the end of the second month, the number of yolk vesicle stage oocytes reduced marginally to 2.7% and the LOD was 138 µm. Towards the end of the third month, no significant histological change was observed and the LOD was 136 µm.

**Table. 4. 1.** Changes in reproductive cycle illustrated by mean GSI and LOD values in *A. testudineus* females maintained at a photoperiod of 14 L–10D at different temperatures, and compared with the controls (C) initially, and after a period of 3 months.

| Treatment group | Temperature ($^0$ C) | Gonadosomatic index (GSI) Mean ± S. D. | | Largest oocyte diameter (LOD; µm) | |
|---|---|---|---|---|---|
| | | Initial | Final | Initial | Final |
| T1 | 30-31 | 0.42 ± 0.18 | 0.19 ± 0.05 | 460 | 124 |
| T2 | 26-27 | 0.42 ± 0.18 | 1.39 ± 0.38 | 460 | 652 |
| C | 28-29 | 0.42 ± 0.18 | 0.23 ± 0.07 | 460 | 136 |

**Table. 4. 2.** Histological observations in the ovaries of *A. testudineus* maintained at a photoperiod of 14 L–10D at different temperatures, and compared with the controls (C) at one month intervals; PN- perinucleolar, YV- yolk vesicle stage, LOD- largest oocyte diameter.

| reat ıent :oup | Temper ature ($^0$ C) | Period | Histological observations |
|---|---|---|---|
| T1 | 30-31 | 1 month | PN oocytes (LOD = 128 μm) and a few atretic YV oocytes; all ovaries in stage II maturity. |
| | | 2 month | Only PN oocytes (LOD = 132 μm); all ovaries in stage II maturity. |
| | | 3 month | Massive atresia of PN oocytes in all ovaries; all ovaries in stage II maturity. |
| T2 | 26-27 | 1 month | PN oocytes (97.1 %),YV oocytes(2.9%); LOD = 189 μm); no atretic oocytes; all ovaries in stage II maturity. |
| | | 2 month | PN oocytes (93.8 %),YV oocytes (6.2%); LOD = 197 μm); no atretic oocytes; 75 % ovaries in stage II, 25% in stage III. |
| | | 3 month | Vitellogenic oocytes (17.5 %); LOD = 652 μm; 75 % of ovaries in stage III, 25% in stage IV. |
| C | 28-29 | 1 month | PN oocytes (97.1 %); YV oocytes (2.9 %); LOD = 142 μm; all ovaries in stage II maturity. |
| | | 2 month | PN oocytes (97.3 %); YV oocytes (2.7 %); LOD = 138 μm; all ovaries in stage II maturity. |
| | | 3 month | PN oocytes (97.0 %); YV oocytes (3.0 %); LOD = 136 μm; all ovaries in stage II maturity. |

Fig. 4. 1

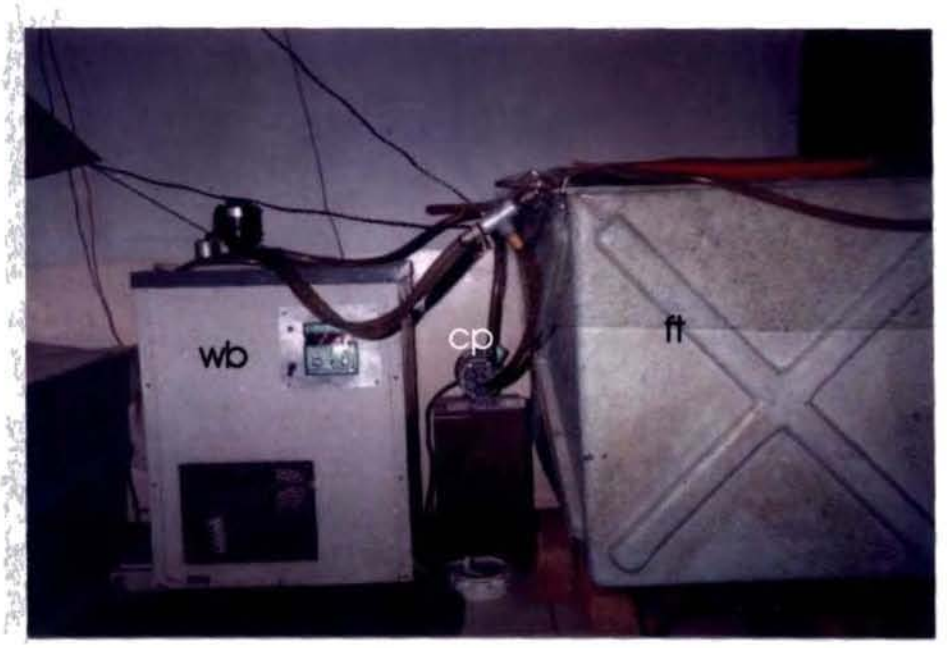



Fig. 4. 1.

Experimental setup used for maintaining controlled temperature conditions wb- water bath fitted with compressor, ft- Fibre tank, cp- Circulation splitpump.

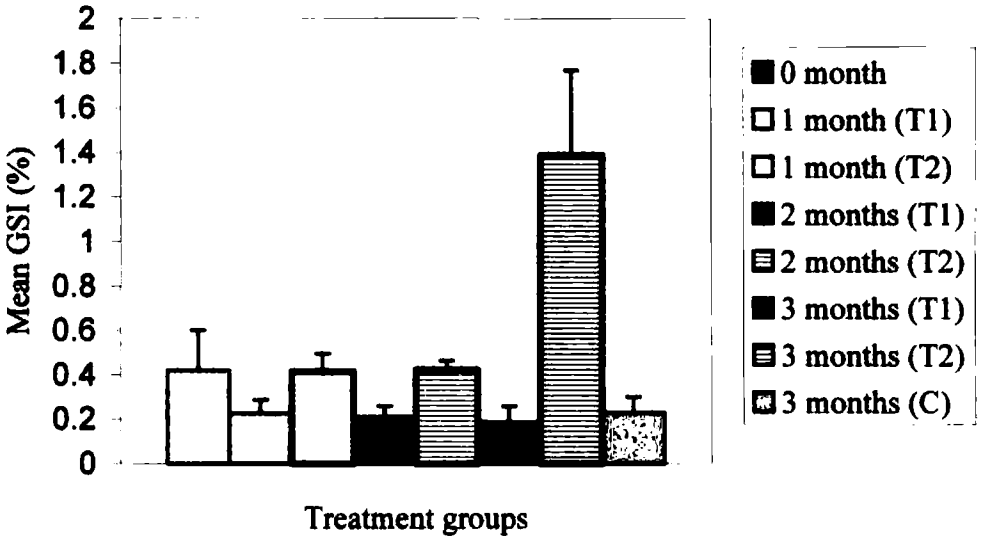
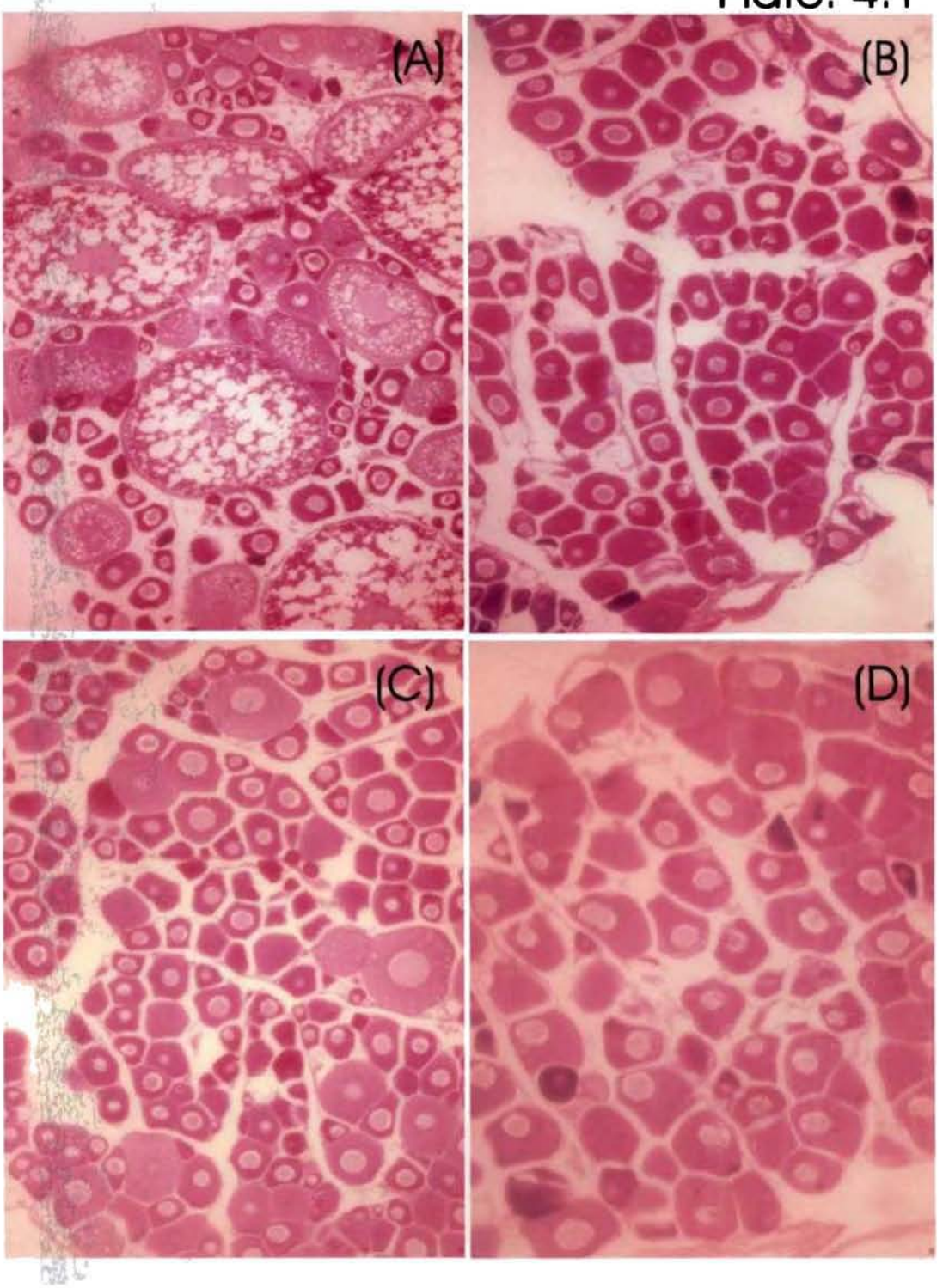



**Fig. 4. 2.** Variations in Gonadosomatic index (GSI) values following photothermal treatment, upto 3 months during postspawning season, in females of *A. testudineus*.

Plate: 4.1



## PLATE: 4. 1

**Photomicrographs of double stained (Hematoxylin/Eosin ) sections of ovaries of *A. testudineus* undergone photothermal treatment.**

**Fig. (A).** View of a spent recovering ovary at the beginning of the experiment.

**Fig. (B).** Histological appearance of the ovary of a T1 group (30-31$^0$C) fish showing the absence of second growth phase oocytes at the end of the first month of photothermal treatment.

**Fig. (C).** Histological details of group T2 (26-27$^0$C) ovary at the end of the first month of photothermal treatment elucidating the retention of yolk vesicle stage oocytes.

**Fig. (D).** Histological view of the ovary of a T1 group (30-31$^0$C) fish showing the complete absence of yolk vesicle stage oocytes by the end of the second month of photothermal treatment.

Plate: 4.2

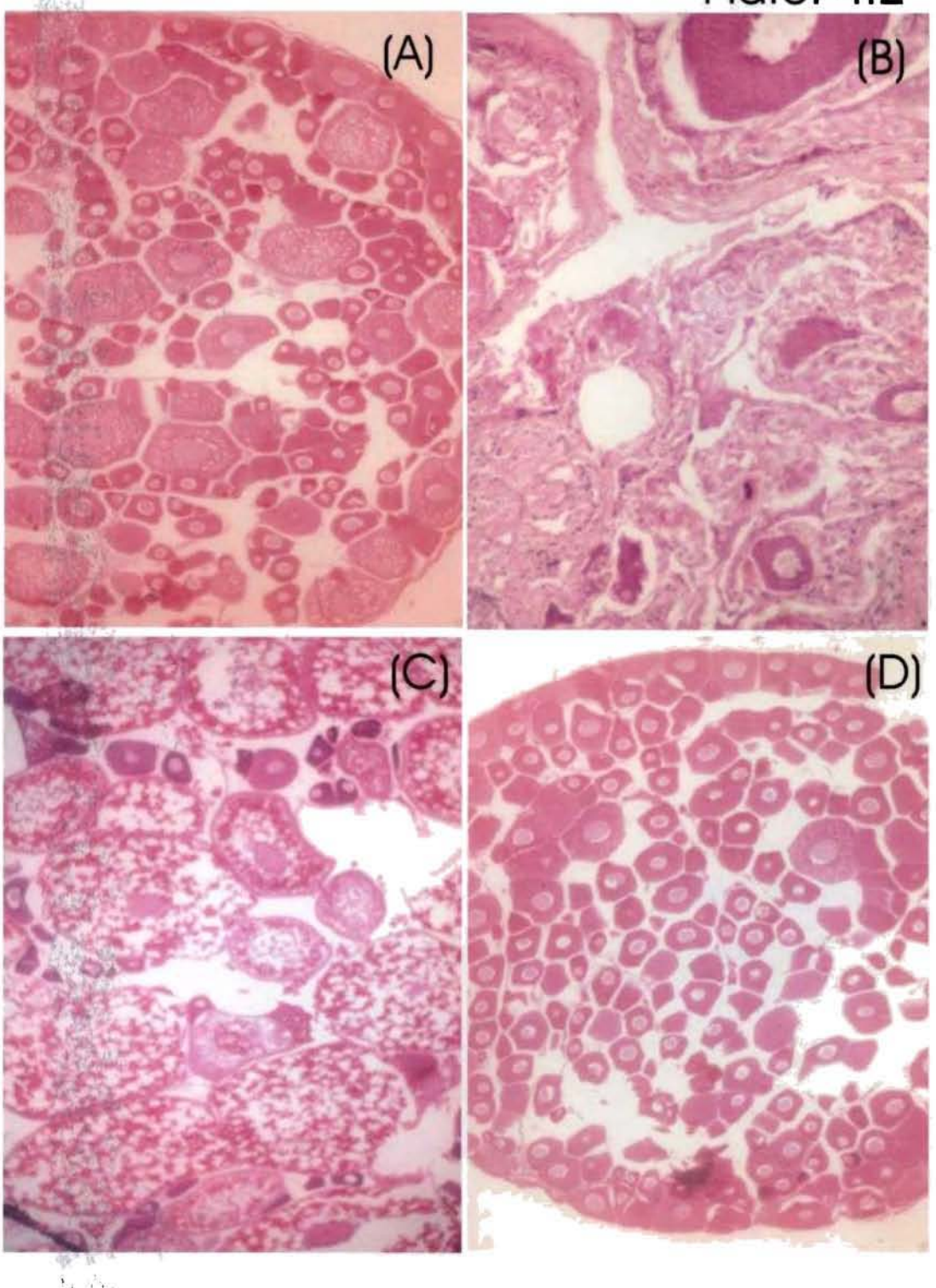


**PLATE: 4. 2**

**Photomicrographs of double stained (Hematoxylin/Eosin ) sections of ovaries of *A. testudineus* undergone photothermal treatment.**

**Fig. (A).** The histological condition of the ovary of a group T2 (26-27$^0$C) fish by the end of the second month of treatment elucidating the retention and relative abundance of yolk vesicle stage oocytes.

**Fig. (B).** View of massive atresia by the third month of treatment in the ovary of T1 group (30-31$^0$C) fish.

**Fig. (C).** Histological view of ovarian recrudescence as evidenced by the appearance of vitellogenic oocytes in the ovary of a T2 group (26-27$^0$C) fish by the third month of photothermal treatment.

**Fig. (D).** Histological view of the resting ovary in a control group (28-29$^0$C) fish at the end of the experiment.

## 4. 4. DISCUSSION

The seasonal reproductive cycle involving gonadal recrudescence, ovulation, spermiation and spawning is the most frequently cited example of an annual endocrine cycle in teleosts. Two major environmental factors involved in cueing reproductive activity are temperature and photoperiod. In order to spawn at a specific time of the year, fishes should use these environmental cues to initiate gonadal recrudescence so that gametes are mature in time for spawning (Bhattacharya and Banerjee, 1988).

The increasing trend observed in the gonadosomatic indices of female fish maintained at 26-27$^0$C with a photoperiod of 14L-10D, in the present experiment, could be attributed to the advancement of ovarian maturation due to accumulation of yolk-laden mature oocytes, in response to the combined effect of temperature and light. Sundararaj and Sehgal (1970) and Sundararaj and Vasal (1973) had stated that photothermal treatment can accelerate vitellogenesis and that the pathway of action, at least in part, is via the hypothalamo-hypophyseal axis.

There was a rapid increase in yolk formation causing marked enlargement of ova and ovaries and an increase in GSI values by the third month of treatment in the group T2 (26-27$^0$C) fishes. Among the group T1 (30-31$^0$C) and group C controls (28-29$^0$C), mean GSI values steadily declined while in the group T1 fishes, prolonged exposure to higher temperature produced massive atresia of the oocytes. de Vlaming (1972c) also had reported that warm temperature caused gonadal regression in *Gillichthyes mirabilis*.

Since sexual cycle is found to undergo considerable modifications under experimental conditions of light and temperature, it will be of great interest to study their effects on gonadal development of a species. The results discussed here highlight the feasibility of advancing maturation in *A. testudineus* by photothermal treatment.

The present study showed that ovarian recrudescence in *A. testudineus* is sensitive to temperature and photoperiodic conditions. The experimental results presented here indicated that *A. testudineus* is able to build up gonadal activity under the given conditions. Photothermal treatment for a period of three months, at a photoperiodicity of 14L/10D and temperature of 26-27$^0$C, led to an increase in GSI, LOD values, vitellogenesis and a progression from stage II to stage IV level of ovarian maturity as evidenced by the progression in the proportion of different oocyte stages. All these clearly indicated that ovarian recrudescence, which normally occurs during the prespawning period, can be advanced by about three months by photothermal treatment during the postpawning period in *Anabas testudineus*. Alok *et al.* (1993) had found that *Heteropneustes fossilis* maintained at 30$^0$C and a photoperiod of 14L-10D for 60 days between post spawning season and preparatory season attained gonadal maturity 4 months earlier compared to the natural spawning season.

Influence of light and/or temperature, on the reproductive cycle of many species of fishes, has been subjected to repeated investigations and these studies have shown that the requirements of photoperiods for the activation and regulation of the cycle vary from species to species and from place to place, as the length of day and temperature range differ, depending on the latitude of the locality. Day-length longer than natural,

accelerated the process of maturation in its later phase and short photoperiods retarded the process thereby delaying functional maturity (Verghese, 1975). In nature, the breeding season of many fishes including *A. testudineus* is directly related to the monsoon cycle; the fish attaining maturity towards the advent of the monsoon months. Fish integrate their reproductive activities with seasonal environmental cycles. Certain environmental factors such as temperature, photoperiod and rainfall act as cues for the approaching favorable season for reproduction. Environmental cues and endogenous physiological cycles input to the neuroendocrine system, which in turn regulates pituitary and gonadal functions (Lin and Peter, 1996).

In the light of the present observations, it is apparent that photo thermal treatment involving a temperature range of 26-27$^0$C and a long photoperiod of 14 hours, for a period of three months during the post spawning season, led to the retention of viable yolk vesicle stage oocytes, beyond the natural spawning season, resulting in ovarian recrudescence. Further, a high temperature of 30-31$^0$C was found detrimental to oogenesis as evidenced by massive atresia of the oocytes, including those of the perinucleolar oocytes.

# Chapter 5

# *Induced breeding*

# CHAPTER V

# INDUCED BREEDING

## 5. 1. INTRODUCTION

Aquaculture is an evolving technology for maximizing the survival and growth of captive fish, based upon stringent control of rearing conditions such as photoperiod, water temperature, salinities and feeding schedules. However, because the seasonal reproductive cycles of fishes are dependent on combinations of key environmental cues, e.g., fluctuations in food availability, light and temperature (Bye, 1990), failure of gonadal recrudescence, ovulation or spontaneous spawning may occur in cultured fish, thus decreasing the fecundity and gamete quality of domesticated broodstock (Crim, 1991). In early attempts to overcome these difficulties, captive fish have been induced to spawn with various preparations of mammalian or fish pituitary hormones-a technique commonly practiced in many Asian countries even today.

Procurement of pure seed of cultivable fishes from a dependable source posed a problem. Adequate availability of seed is the basic requirement for taking up farming of any species. Availability of seed from the wild is usually seasonal and subject to the mercy of nature. The need to induce fishes to breed in confined waters to ensure a dependable source of quality fish seed has been greatly felt in the country since long. The first attempt in this direction was made by Khan (1938) who tried to induce spawning of *Cirrhina mrigala* by injecting hormones of anterior lobe of mammalian pituitary gland (hypophysation).

Brazil was the first country to develop a technique for hypophysation. The technique of hypophysation was successfully applied to Indian major carps during 1957-1959 at Cuttack substation of the Central Inland fisheries Research Istitute (CIFRI). Pickford & Atz (1957), Das & Khan (1962), Jhingran (1969), Tripathy and Bhimachar (1972), Sinha *et al.*, (1974), Moitra & Sarkar (1975, 1976, 1977a, b; 1978) have reviewed the history of hypophysation of fishes. Chaudhury (1955), Ramaswamy & Sundararaj (1956, 1957), Alikunhi (1963a) were successful in breeding of various Indian fresh water fishes. Chaudhuri (1960) and Alikunhi *et al.*, (1960) had observed that induced breeding experiment yielded better results when the donor and recipient fishes were of the same species and of the same age group. Pituitary glands from live fishes presented better results compared to those of the preserved or spent fishes. Further, pituitary glands of the spawned fishes when collected and used on the same day also proved effective (Anand, 1973).

Fish breeding in nature has been compared to a spontaneous symphony of several finely tuned natural factors played in the wild so that the fish farmer does not get the full benefit of it. He finds it necessary to make the fish breed at a place of his choice. He does this either by force (stripping) or by deception (by simulating natural conditions) or by the technique of providing some inducements (hormonal induction).

Induced spawning enables farmed broodstocks to ovulate under conditions of intensive culture and allows the production of eggs to be adjusted as required to suit procedures on the farm. Techniques of induced spawning would help culturists to increase hatchery production

and supplement insufficient natural production. Still, purified pituitary gonadotropic hormones (GtH) are expensive and crude pituitary hormone preparations may not be reliable due to problems with storage, potency estimation or perhaps, adverse immunological reactions to such treatments (Zohar, 1989). Thus, fish culturists felt a need for a substitute for pituitary. Newer substances other than pituitary extracts were later employed to induce ovulation.

### 5. 1. 1. Hormone preparations

Treatment with hormone preparations makes it possible to mimic the GtH rise associated with ovulation and thereby control the time of ovulation and spawning. Such preparations include extracts from the pituitary glands of donor fish (e.g. carp or salmon) and mammalian GtH. However, there are certain disadvantages to these procedures (Worthington and Mcfarlane, 1983). Fish gonadotropins often have a reduced potency when administered to fish of other species. GtH from Pacific salmon, *Oncorhyncus tshawytscha*, was less effective in stimulating adenylyl cyclase activity in gold fish ovaries *in vitro* than GtH from the more closely related carp (Fontaine *et al.*, 1972). Thus it is difficult to select an appropriate dose of hormone. Pituitary preparations also contain many hormones other than GtH, which may well disrupt the internal environment of the recipient fish. In addition, all forms of GtH are expensive. The use of pelleted hormones to induce gonadal development and to synchronize ovulation and spawning is gaining considerable popularity (Crim, 1985). An alterative approach is the use of steroid hormones targeted at the oocytes (Jhingran, 1991).

In teleosts, secretion of maturational gonadotropin (GtH-II) from the pituitary is controlled by a stimulatory brain mechanism (Tharakan and Joy, 1996) that is identified as the gonadotropin – releasing hormone (GnRH). Prior to the availability of fish GnRH, mammalian GnRH (m GnRH) or its super active analogues (m GnRH-a) have been used to investigate GtH release activity (Peter *et al.*, 1986, 1991). It has been further shown that circulating gonadal steroids and certain monoamines modulate GnRH activity at the levels of the brain or pituitary (Peter *et al.*, 1991).

In the last two decades, the use of GnRH or its super active analogues alone or in combination with dopamine antagonists, such as pimozide, domperidone, Org 5222, and Org 30067 have been used in favour of the traditional hypophysation technique to induce ovulation in a variety of teleosts: gold fish (Chang & Peter, 1983), African catfish (de Leeuw *et al.*, 1985, Goos *et al.*, 1987), the Asian catfish *Clarias batrachus L.* (Manickam & Joy, 1989), the Arctic char, *Salvelinus alpinus L.* (Janssen, 1993) and the yellow perch *Perca flavescens* Mitchill (Dabrowski *et al.*, 1994). The use of gonadotropic hormone-releasing hormone analogues (GnRH-a) has removed some of the difficulties associated with reproduction of cultured fish, leading to successful inductions of spawning and increased fecundity of domesticated broodstock (reviewed by Crim *et al.*, 1987). In many fresh water cultured fish, GnRH-a in combination with a dopamine antagonist (the Linpe method) is an effective technique (Peter *et al.*, 1993).

To induce spawning in many fresh water fish, such as the cyprinids, a dopamine antagonist (e.g., pimozide, metoclopramide, domperidone or haloperidol) in conjunction with GnRH analogues is

necessary. Neither mGnRHa nor pimozide alone evoked a response in catfish *Clarias batrachus*, but mGnRHa combined with pimozide produced high rates of ovulation, fertilization and hatching of normal larvae (Manickam and Joy, 1989).

The results of ovulation stimulation with ovopel [a complex of mammalian GnRHa analogue (D-Ala$^6$, pro$^9$ Net) and water soluble metaclopramide blocker of dopamine receptors] showed that this preparation applied in two doses induced spawning in a lower percentage of grass carp females in one group and an equal percentage in the other, compared with females after hypophysation (Klodzinska & Okoniewski, 1998).

Being an oligopeptide, which can be manufactured at very low cost, LH-RH is receiving great attention as a potential agent for controlled reproduction in fishes (Jhingran, 1991). The need for long acting slow formulation of LH-RH which would maximize the biological effectiveness of the peptide hormones administration led to synthesis of LH-RH analogues which have been shown to have potency 30-50 times higher than that of simple LH-RH (Coy *et al.*, 1974).

There are many synthetic substitutes for natural hormones. These compounds, developed for mammalian applications, are generally cheap and easy to obtain, and their efficacy is likely to be more consistent among different teleost species (Worthington and Mcfarlane, 1983). Examples of such drugs are the non-steroidal antioestrogens clomiphene and tamoxiphen (ICI 46474), which have been used in the treatment of anovulatory infertility (Greenblatt *et al.*, 1961) and breast cancer (Ward, 1973) respectively.

The synthetic compound clomiphene citrate [p (∃-diethylamino ethoxy)- phenyl-1, 2-diphenyl-2-chlorethylene citrate] used in the therapy of some human reproductive dysfunctions (Jacobson *et al.*, 1968; Kase, 1973) has also been used for inducing ovulation in teleosts (Pandey & Hoar, 1972; Singh & Singh, 1976; Kapoor & Toor, 1978). It has been reported that clomiphene may act at hypothalamo-hypophyseal level to stimulate gonadotropin secretion in *cyprinus carpio* (Breton *et al.*, 1975). Bhowmick *et al.*, (1979), however, could not induce spawning by using clomiphene citrate singly or in combination with a threshold dose of 4-5 mg $kg^{-1}$ of carp pituitary extract.

Synahorin, (a mixture of chorionic gonadotropin and mammalian pituitary extract) in combination with fish pituitary has also been found to be quite effective in inducing the fish to breed. Liu (1963) demonstrated that the pituitary of *Cyprinus carpio*, combined with a certain amount of synahorin gave better result in inducing spawning of the Chinese carps, than individual use of the pituitary extract or synahorin alone.

According to Brzuska & Adamek, (1999), in hatchery practice, it is impractical to treat fish with hypothalamic hormones and their analogues (which are applied at very low doses, most frequently 20-50mg $kg^{-1}$) or dopamine receptor antagonists (particularly those of pimozide which are insoluble in physiological salt solution). Hence, newly developed preparations contain the hormone and the dopamine antagonist in one solution, which is easily applied in one injection. Apart from such preparations like dagin (Drori *et al.*, 1994) and ovopel (Horváth *et al.*, 1997), ovaprim is a widely used ovulation stimulator.

### 5. 1. 2. Hormonal effects on ovulation

The process of maturation and ovulation in warm water fishes, in which spawning is synchronized among females, is relatively short and is associated with an abrupt GtH surge (Szabó, 2001). During induced ovulation, a circulating GtH surge is generated by injecting GtH from exogenous sources (hypophysation) or a synthetic analogue of gonadotropin releasing hormone (GnRHa) is used to stimulate the secretion of the endogenous GtH from the pituitary of the treated fish (Yaron, 1995). GnRH analogues act at a higher level on the reproductive axis in inducing not only the release from the pituitary of the fish's endogenous GtH, but also potentially regulating or indirectly affecting the release of other hormones necessary for the successful final oocyte maturation and ovulation (Szabó, 2002).

The action of pituitary gonadotropins on final oocyte maturation is known to be mediated through steroid hormones (Jalabert, 1976). *In vitro* ovulation has been induced effectively by cortisone in *Misgurnus fossilis* (Krishenblatt, 1958) and by deoxycorticosterone (DOCA) in *Heteropneustes fossilis* (Ramaswamy, 1962). The effects of steroid hormones on ovulation are seen primarily as germinal vesicle breakdown (GVBD). One or more steroids produced in the ovaries under gonadotropin stimulation normally control GVBD, but the timing of ovulation related to that of GVBD varies (Jhingran, 1991).

In captivity, many bony fishes undergo normal processes of gonadal activity, but spermiation, oocyte final maturation and ovulation do not occur due to an insufficient surge of GtH from the pituitary

(Tharakan and Joy, 1996). Therefore, for culture purposes these processes are often induced artificially (Donaldson & Hunter, 1983).

### 5. 1. 3. Environmental and hormonal aspects of breeding

Both sensory and endocrine pathways mediate the effects of seasonal environmental factors on reproduction. Sensory input *via* retinal or pineal routes induces the appropriate endocrine responses from the hypothalamus and pituitary. Thus, pituitary gonadotropin (GtH), released under environmental stimulation, triggers the production of gonadal steroids, and gametogenesis. In the female, oestrogen secreted by the developing ovary, apart from playing a direct role in gamete development, stimulates vitellogenin production in the liver and further, GtH synthesis in the pituitary. In addition, increasing titres of plasma oestrogens suppress GtH release from the pituitary. Prior to spawning, favourable environmental conditions cause a surge in plasma GtH that induces ovulation (Worthington and Mcfarlane, 1983). Relationships between environmental factors, hormones and reproduction in teleosts have been reviewed by Billard *et al.*, (1978).

Teleosts show seasonal regularity in the timing of reproductive development and spawning. Environmental factors such as photoperiod, temperature, salinity, food availability, presence of vegetation or substrate provide stimuli for these reproductive events. When captive broodfish are exposed to an environment different from their natural habitats, reproductive cycles are generally disrupted, and many of these species do not complete their gametogenic cycle. In captive fish, oocytes are arrested at the post vitellogenic stage that undergoes further

maturation and ovulation only after hormone administration (Fermin *et al.*, 1999).

The hormonal signals that initiate ovulation are naturally induced by specific environmental cues. In contrast, captive stocks, which may not be fully prepared for spawning, are induced to ovulate artificially by hormonal injections.

### 5. 1. 4. Some earlier attempts on induced breeding

Giridhar and Rao (1976) induced spawning in *Labeo fimbriatus* by injecting the female with two doses of copper sulphate solution (0.5 and 1.0 mg $kg^{-1}$ body weight) at an interval of 6 hours. Worthington and Mcfarlane (1983) induced spawning in roach *Rutilus rutilus* using antioestrogens clomiphene and tamoxiphen, and suggested that both drugs probably acted by indirect mechanisms, blocking sex steroid feedback inhibition of gonadotropin (GtH) secretion by the pituitary thereby inducing plasma GtH surge.

Meenakaran (1986) reported successful induced breeding of P*angasius Pangasius* using a mixture of carp pituitary gland extract (cPGE) and HCG in Indonesia. Shah *et al.*, (1990) reported induced breeding of major carps in Bangladesh. Nandesha *et al.*, (1990) were successful in induced spawning of Indian major carps through single application of ovaprim-C.

Rahman *et al.*, (1993) found both carp pituitary gland extract (cPGE) and human chorionic gonadotropin (HCG) to be equally effective in inducing spawners of *Pangasius sutchi* in Bangaladesh.

Kagawa *et al.*, (1995) showed that in the Japanese eel *Anguilla japonica*, 17,20 β-dihydroxy-4-pregnen-3-one (DHP) could induce final maturation of migratory nucleus stage oocyte over 700 :m in diameter. Pandey *et al.*, (1998) were successful in the induced breeding of the endangered golden mahseer *Tor putitora* using ovaprim (sGnRHa+domperidone; Syndel Laboratories, Vancouver, Canada) in a farm near Dehradun.

Brzuska, (1999) found that in the case of grass carp treated with LHRHa, the percentage of stripped females was greater than that recorded after hypophysation. Brzuska & Adamek, (1999), in the European cat fish *Silurus glanis*, found a higher percentage of ovulating females (producing eggs of sufficient quality) after the LHRHa and ovaprim treatments (100% and 80 %) compared to fish treated with the pituitary extract (60% and 66.67%). After 60 h of incubation, the best quality of eggs was found in the group treated with ovaprim (62.9% of live embryos) and the poorest in the two groups, which underwent hypophysation (50.41 % and 50.75 %).

Fermin *et al.*, (1999) found in catfish *Clarias macrocephalus* that the onset of final oocyte maturation and ovulation and levels of serum estradiol-17 β ($E_2$) did not vary with season in LHRHa+PIM-injected fish. Szabó, (2001) modified pituitary treatment in Northern pike *Esox lucius* by using sustained release vehicles, a hydrophilic biodegradable matrix of carboxymethylcellulose sodium (CMC-Na) and a carbopol resin, to produce a higher mean fecundity index and improved fertilization rate compared to pituitary injections in saline.

The air-breathing teleosts comprise a moderate catch fishery constituting nearly 15 % of the total marketable surplus of inland fisheries in India (Prasad *et al.*, 1993). The culture of air-breathing fish has been popularized to exploit vast swampy areas and derelict water bodies for immediate benefit to the people, without involving costly processes of their reclamation. Unlike water-breathing fish, the air-breathing fish can be easily stored and transported alive to the consumers. Owing to air-breathing habit and ability to live in water with low oxygen content, *A. testudineus,* is also ideal for waste water aquaculture. Because *A. testudineus* do not spawn in confinement, it needs to be induced artificially.

Banerji and Prasad (1974) found a low dose of 15 to 20 mg of pituitary gland of major carp administered in a single dose to be sufficient for a successful hypophysation in *Anabas testudineus* in Bihar. However, no attempt has been made to induce *A. testudineus* using ovaprim so far. For sustaining culture, it is imperative that the technology is standardized to artificially produce the seed of *A. testudineus* under local conditions. The present work was an attempt in this direction.

The objectives of the present work were: a) to induce spawning in *A. testudineus* by temperature manipulation and b) by hormonal intervention using ovaprim; c) to determine time taken for spawning and hatching; and d) to assess the efficacy of hormone dosage on ovulation index, fertilization rate, and hatching within 24hours of fertilization in *A. testudineus.*

## 5. 2. MATERIALS AND METHOD

### 5. 2. 1. 1.Trials on inducing breeding by temperature manipulation

Experiments on induced breeding by temperature manipulation were conducted during June-August (2002) in the wet lab of the Department of Marine biology, Microbiology and Biochemistry, Cochin university of Science and Technology, Kochi. The fishes were collected from Trichur '*Kole lands*' (Lat. $10^0$ 34' N and $10^0$ 36' N and Long. $76^0$ 06' E and $76^0$ 09' E) and adjoining freshwater bodies. The fishes were treated with potassium permanganate (1mg $l^{-1}$) for 2 minutes before being released into the stocking tanks. The brooders were maintained under ambient temperature of $26^0$C-$28^0$C and fed @ 3% of body weight on alternate days with goat liver and a formulated feed (Marvel breeder pellets) having the following proximate composition: Crude protein-38%, Crude fat-8%, Crude fibre-8%, Ash-17%, Moisture-8% and Nitrogen free extract-21%. Water was changed on alternate days to remove decomposed and unwanted food materials. They were acclimated for two weeks before starting the experiments. Males and females were segregated at least one week before starting the experiments.

Oocyte development was monitored by taking a small sample from the ovary by inserting a plastic surgical tubing via the genital pore into the gonad. The ovarian sample (of about 30-40 oocytes) was cleared in a solution of ethanol, formalin (40% formaldehyde) and glacial acetic acid (6:3:1; by volume) (Rothbard and Yaron, 1995). This solution enabled the position of the germinal vesicle to be seen under a dissecting microscope. By employing this method, only suitable females were

selected for spawning induction while others were returned to the stocking tank, until their oocytes showed further progress *viz;* initial migration of germinal vesicle.

Twenty-five breeding pairs were selected. The females weighed between 15.1 g and 33.2 g and the males ranged between 10.3 g and 27.3 g in body weight. The dissolved oxygen was in the range of 5-6 mg $L^{-1}$ and pH was 7 ± 0.3.The brooders were allocated to five experimental groups, each group consisting of five breeding pairs. Each breeding pair consisted of one female and two males. The last group formed the controls.

### 5. 2. 1. 2. The experimental set up

A steady temperature (± 0.1$^0$C) of 24$^0$C, 25$^0$C, 26$^0$C or 27$^0$C was maintained, in an experimental tank of 100 L capacity containing fresh water, by using a waterbath with refrigeration facility, split circulation pump and a timer device as detailed in chapter IV (section 4. 2. 1). Water was sprinkled from above to give a rain like effect using a perforated inlet tube connected with the circulation pump. The treatments were continued up to 72 hours. The amount of dissolved oxygen was estimated by Winkler's method, pH was measured using a pH meter. The control group was maintained at ambient temperature of 27-28$^0$C.

### 5. 2. 2. Induced breeding experiment using ovaprim

These experiments were conducted during June-July (2003) in the wet lab of the Department of Marine biology, Microbiology and Biochemistry, Cochin University of Science and Technology, Kochi.

The fishes were collected, maintained, fed and oocyte development monitored as detailed in section 5. 2. 1. 1. Thirty-six breeding pairs were selected for injection each consisting of one female and two males. Females ranged in total length from 9.6 cm to 15.2 cm, weighing between 13.6 g and 40.0 g. The males ranged in total length between 7.8 cm and 14.3cm and weighed between 9.6g and 31.0g. The brooders were allocated to six groups of equal numbers and similar size ranges and transferred to separate tanks of similar sizes. The last two groups formed the controls. Males and females were segregated at least one week before starting the experiments.

The water temperature remained at 27 ± $2^0$ C during the course of the experiment. The dissolved oxygen was in the range of 5-6 mg $L^{-1}$ and pH; 7 ± 0.3. Each breeding pair was given a single injection as detailed in table-5. 2. 1. Using a micropipette, 500µl of ovaprim (product # 94019, Syndel Laboratories Ltd, Vancouver, Canada) was pipetted into a 10 ml standard flask and made up to the mark with distilled water or 0.6 % saline, to give a final concentration of 50µl of the hormone in 1 ml of the solution. Fish of the group I and II were injected with hormone diluted in distilled water and 0.6 % saline respectively @ 0.5 ml $Kg^{-1}$ body weight as recommended by the manufacturer, whereas the males were given half the dose of the hormone as that received by the females. The volume of injection never exceeded 0.3 ml. The group III and IV females were given a single dose of undiluted hormone within ranges of 1-3 ml and 3-6 ml $Kg^{-1}$ body weight respectively, whereas the males were given a single dose ranging between 25 and 50µl depending on their body weight. The volume of injection received by group III and IV females was within a range of 25 to 200µl depending on their body weight. The fishes were not fed afterwards. The group V and group VI

fishes were injected with distilled water and 0.6 % saline vehicles respectively.

While injecting, the head of the fish was covered with a conical plastic cover to keep the fish calm. ovaprim was drawn into a 1ml insulin syringe having 40 divisions and slowly injected intramuscularly in caudal peduncle above the lateral line. The needle was withdrawn gradually so that the hormone found its way between the muscle fibres. The whole operation from catching the fish to the hormone administration was completed within 3 minutes, so as to reduce stress to the fish. The injected individual breeding pairs were carefully released in to separate round fibre tanks of 12-litre capacity containing 4 litres of fresh water. The medium was well aerated and closely examined for the egg release. The control groups were injected with a saline vehicle or distilled water.

Once complete ovulation was observed, the time taken for ovulation was noted, the brooders were removed and excreta if any, was removed by careful siphoning. The number of ovulated eggs was determined from three counts of one litre random samples taken after thorough stirring. In order to determine the ovulation index, the females were sacrificed on day 2 post-injection, and the remaining eggs were counted. Spawning ratio (number of responsive females/number of females injected) and the ovulation index [Number of ovulated eggs/ (number of ovulated eggs + number of eggs remaining in the ovary)×100] were calculated. The eggs belonging to each individual breeding pair were transferred to separate well-aerated incubation tanks of 50 L capacity having flow through facility (Plate. 5. 1. C). The outlet

of the incubation tank was covered with a nylon mesh of 7μm mesh size to prevent the escape of eggs.

Fertilization rates were calculated within 12 hour of fertilization of the eggs. A sample of about 100 eggs were placed in a petri dish and the number of developing eggs were counted under a magnifying glass. Three such measurements were carried out and the mean percentage was calculated. Unfertilized dull looking dead eggs, were separated from transparent living ones. The time of hatching was noted and the percentage of hatching was calculated from three counts of one litre random samples taken after thorough stirring.

The significance of differences between the groups for traits such as, ovulation index, percentage of fertilization and percentage of hatching were analyzed by Student's t-test (Parker, 1986) with the level of significance in different groups set at $p = 0.05$.

## 5. 3. RESULTS

### 5. 3. 1. Trials on inducing breeding by temperature manipulation (Table. 5. 1)

The females extruded eggs upon gentle pressure on their abdomen whereas males readily milted. Since spawning was not observed in any of the four experimental groups and in the controls, the experiment was repeated with single breeding pairs in a 25 L tank containing 10 L water (Plate. 5. 1. A-B), to exclude adverse effect of aggregation if any, on breeding in artificial conditions. Breeding was not observed in these trials also. Therefore, it was decided to induce breeding, using ovaprim (sGnRHa + domperidone).

### 5. 3. 2. Induced breeding using ovaprim (Table. 5. 2. 1-2; Fig. 5. 1-4)

The fishes used in the present experiment were in pre-spawning condition. Most of the females had oocytes with a peripheral germinal vesicle. Milt was flowing freely from males following gentle abdominal pressure. Among the fishes of group I and II, which received hormone doses at the recommended rate of 0.5 ml $Kg^{-1}$ body weight, only 33 % of group I and 50% of group II fishes spawned. Fishes of both these groups released a few hundred eggs over a prolonged period of 12 to 24 hours. Therefore the reproductive parameters such as ovulation index, fertilization rate and hatching rate were not estimated in these groups. All of the fishes of group III and IV that received hormone doses ranging between 1-3 ml $Kg^{-1}$ body weight (1.2 -2.9 ml) and 3-6 ml $Kg^{-1}$ body weight (3.3-5.0 ml) respectively showed complete spawning response. Ovulation index remained above 95% in both these groups

except (79.17%) in one fish of group IV. The mean ovulation index was 97.9 in group III and 95.2 in group IV. Fertilization rate was above 90% except (nearly 85%) in two fishes, one each belonging to group III and IV. Mean fertilization rate was 95.0 in group III and 93.3 in group IV. Hatching rate was above 90% in all fishes belonging to group III and IV, except (88.9%) in one fish of group III. Mean hatching percentage was 95.4 in group III and 98.2 in group IV. Spawning was not observed in any of the fishes of the control groups. The results of the breeding experiment are summarized in tables 5. 2. 1-2. The Student's t-test showed no significant differences, between the averages of groups III and IV, for the traits- ovulation index, percentage of fertilization and percentage of hatching ($p < 0.05$).

The time taken for the spawning response was found to be 10-12 hours after the hormone injection in fishes of group III and 9-13 hours in the case of group IV fishes.The group III fishes spawned an average number of 9804 eggs (399 eggs $g^{-1}$ body weight) whereas group IV fishes spawned an average of 8792 eggs (375 $g^{-1}$ body weight). The fertilized floating eggs were found translucent and non-adhesive and appeared as hyaline spheres whereas unfertilized eggs gave a milky appearance (Plate. 5. 2 A). The eggs measured 920 ± 20$\mu$m in diameter. Within 9 hours of fertilization, the eggs appeared dark due to pigmentation (Plate. 5. 2. B). Hatching time was found to be uniformly between 19 and 20 hours, after fertilization in both these groups and the larvae (Plate. 5. 2. C) appeared normal.

**Table. 5. 1.** Results of trials on inducing breeding of *A. testudineus* by temperature manipulation.

| Group | No. of breeding pairs | Temperature ($^0$C) | Period of treatment | Response |
|---|---|---|---|---|
| 1 | 5 | 24 | 72 hours | Not ovulated |
| 2 | 5 | 25 | | Not ovulated |
| 3 | 5 | 26 | | Not ovulated |
| 4 | 5 | 27 | | Not ovulated |
| Controls | 5 | 27-28 | | Not ovulated |

**Table. 5. 2. 1.** Details of the hormone treatment in female groups of *A. testudineus* (1 ml of ovaprim = 20 :g of sGnRHa + 10 mg of Domperidone)

| Group | Treatment | Length (cm) | Body weight (g) | Hormone dose (ml $Kg^{-1}$B.W) | Response | Spawning ratio (Number spawned/ Number injected) |
|---|---|---|---|---|---|---|
| I | Ovaprim diluted in D.W | 9.8-13.9 | 16.2 - 26.0 | 0.5 | Partial spawning | 2/6 |
| II | Ovaprim diluted in saline | 10.0-14.1 | 17.4 -28.1 | 0.5 | Partial spawning | 3/6 |
| III | Ovaprim (1-3 ml) | 9.7-13.1 | 13.6-34.0 | 1.2-2.9 | Complete spawning | 6/6 |
| IV | Ovaprim (3-6 ml) | 9.9-13.5 | 15.4- 40.0 | 3.3-5.0 | Complete spawning | 6/6 |
| V | Distilled water only | 10.1-14.8 | 17.2 - 36.0 | nil | None spawned | 0/6 |
| VI | Saline only | 9.6-15.2 | 14.4 - 28.9 | nil | None spawned | 0/6 |

**Table. 5. 2. 2.** Effects on various reproductive parameters in *A. testudineus* following Ovaprim administration : ovulation index = (number of ovulated eggs/ number of ovulated eggs + remaining eggs in ovary) x 100.

| Group | Treatment | Ovulation index (%) (mean ± s.d) | Result of t-test (df = 5) | Fertilization rate (%) (mean ±s.d) | Result of t-test (df = 5) | Hatching (%) (mean ± s. d) | Result of t-test (df = 5) |
|---|---|---|---|---|---|---|---|
| III | Ovaprim (1-3 ml) | 97.9 ± 1.8 | t- value = 0.90 ($p < 0.05$)* | 95.0 ± 5.0 | t- value = 0.47 ($p < 0.05$)* | 95.4 ± 4.7 | t- value = 1.70 ($p < 0.05$)* |
| IV * | Ovaprim (3-6 ml) | 95.2 ±7.9 | | 93.31± 5.0 | | 98.2 ± 1.4 | |

* Not significant

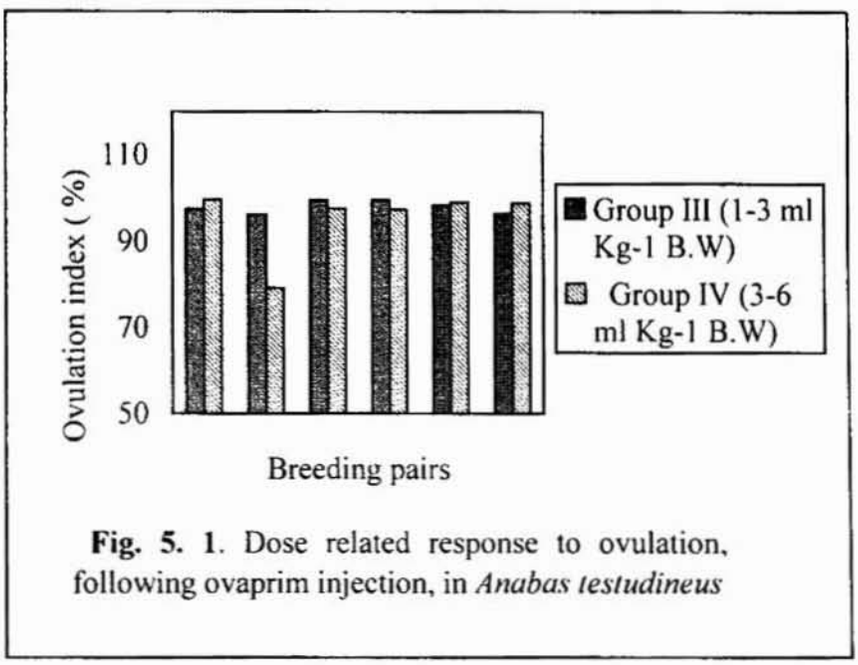



**Fig. 5. 1.** Dose related response to ovulation, following ovaprim injection, in *Anabas testudineus*

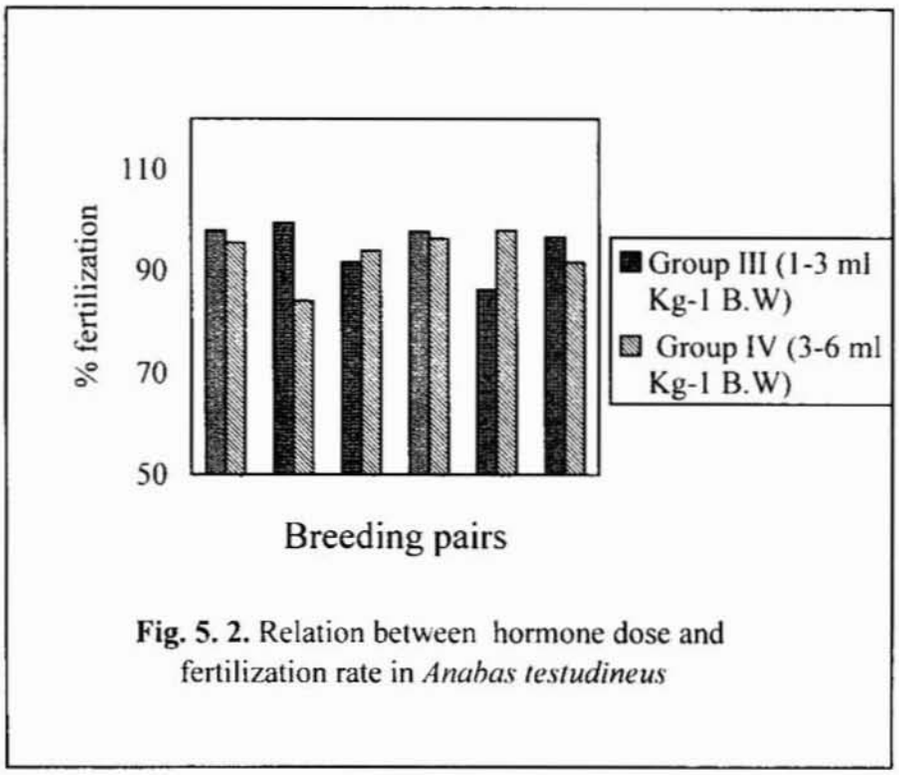



**Fig. 5. 2.** Relation between hormone dose and fertilization rate in *Anabas testudineus*

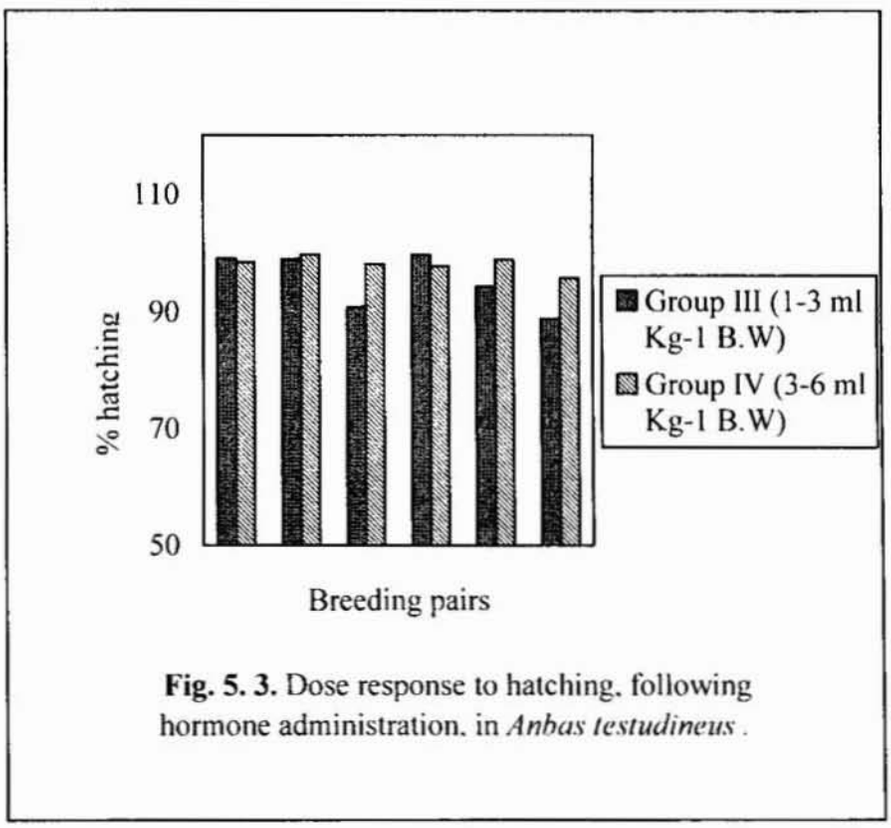



**Fig. 5. 3.** Dose response to hatching, following hormone administration, in *Anbas testudineus* .

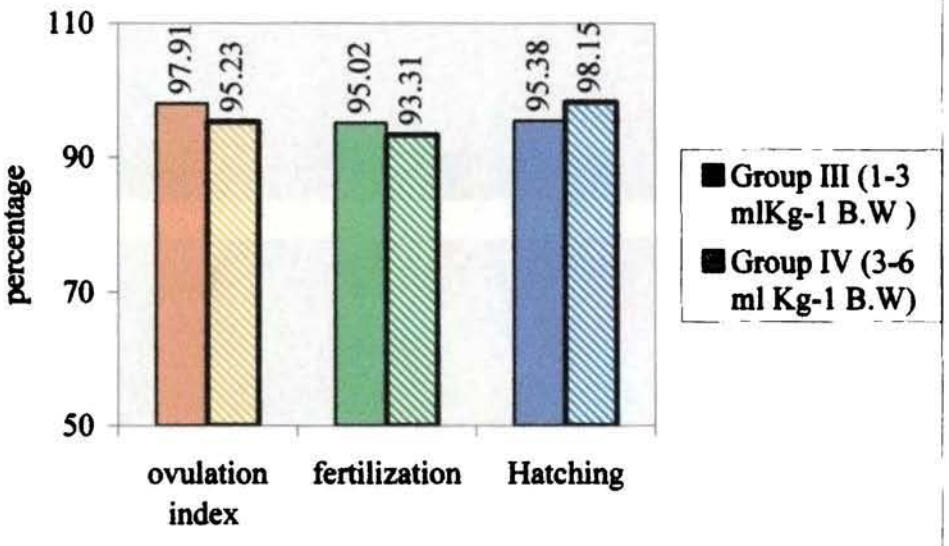



**Fig. 5. 4.** Dose response of *A. testudineus* to the three reproductive parameters, *viz;* ovulation, fertilization & hatching (Mean %).

# Plate. 5.1

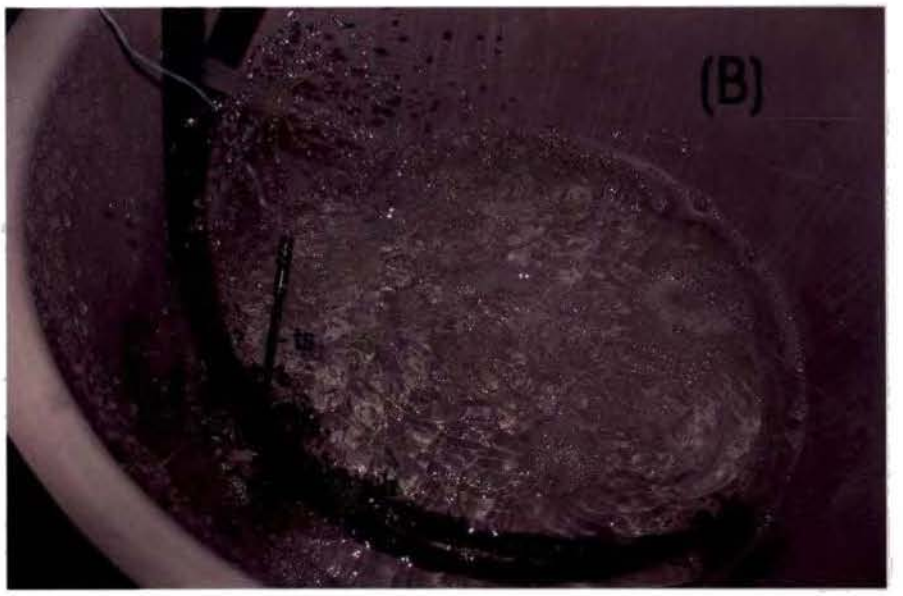




**Plate. 5.1**

**Fig. (A).** System setup used for trials on inducing breeding, in single breeding pair, by temperature manipulation. (rwb- Refrigerated waterbath)

**Fig. (B).** A view from above the water tank in Fig. (A). (ts- temperature sensor; sh- suction hose)

**Fig. (C).** Flow through tank used for larval rearing after ovaprim induced spawning. (ol- outlet covered with nylon mesh, at- aerator tube)

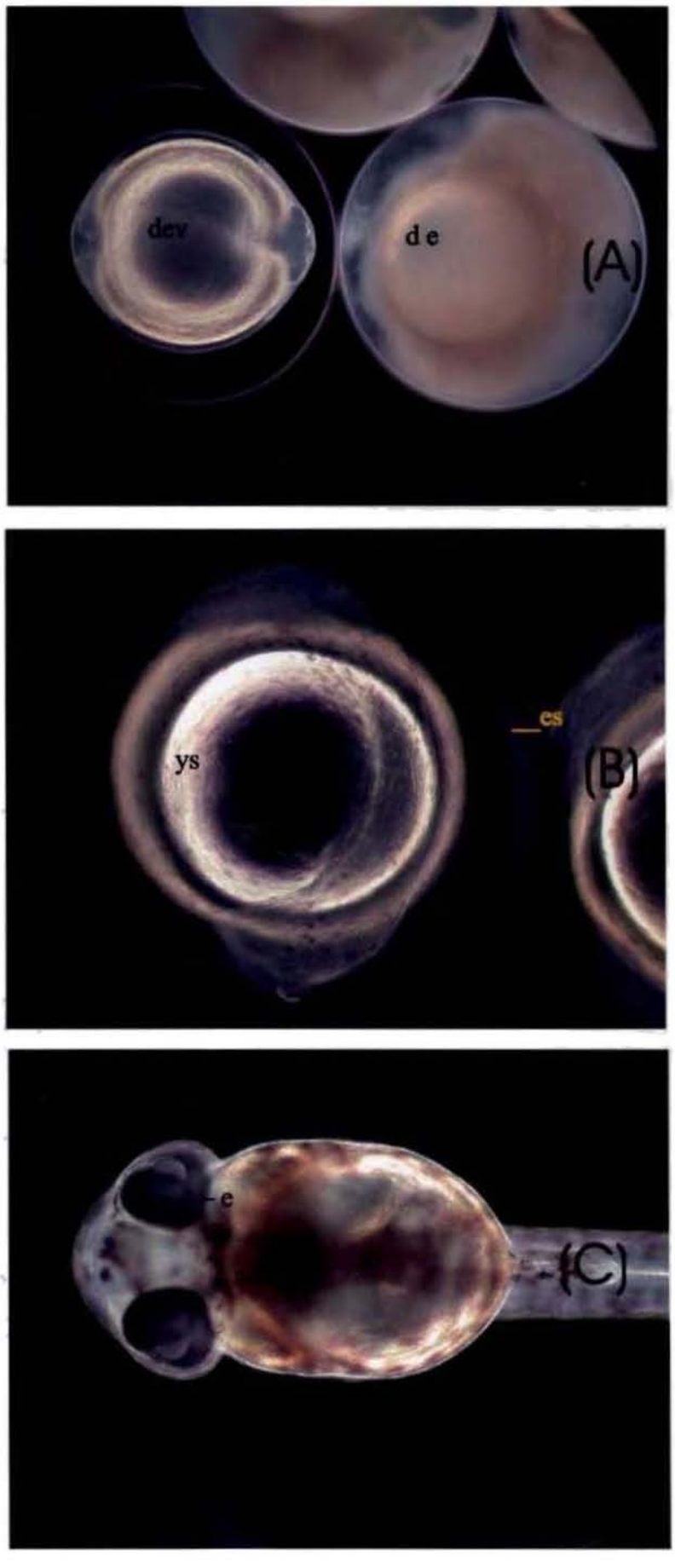
**Plate. 5.2**

**Fig. (A).** Photomicrograph of eggs of *A. testudineus* 6 hours after fertilization. (dev-developing egg; de-dead egg).

**Fig. (B).** Developing embryo, 9 hours after fertilization, showing pigmentation. (ys- yolk sac; es- egg shell).

**Fig. (C).** Larva of *A. testudineus,* 12 hours after hatching. (e- pigmented eye).

Plate. 5.2



## 5. 4. DISCUSSION

### 5. 4. 1. Inducing breeding by temperature manipulation

Spawning, the most critical and sensitive phase in gonadal cycle, is responsive to changes like sudden rise or fall in temperature (Sadlier, 1973) or rains and floods. Temperature is regarded as an important variable effecting spawning of temperate zone fishes (Malhotra, 1965, 1966; Jyoti, 1973). Rains and floods have long been held the responsible exteroceptive factors triggering spawning in Indian carps (Khanna, 1958 and Sinha, *et al.*, 1974). Hora (1938) and Sathyanesan (1960, 1961) believed that flood condition created by premonsoon and monsoon rains act as important exteroceptive factors responsible for spawning.

Mookerjee and Mazumdar (1946) bred *Anabas testudineus* as early as 1942 by keeping the fish under a constant flow of water maintained through a siphonic system. The water was supplied from a reservoir filled from a '*Jhil*,' which had rainwater in addition to its old water. They concluded that falling of rain or rushing of cold water stimulated the fish to spawn in the aquarium. Sinha *et al.*, (1974) succeeded in inducing spawning in Indian major carps though simulation of condition of flooding by increased pond water levels by refilling of sun dried ponds. They were however not sure about the factors of rainfall or floods that induced spawning. They suggested numerous possible factors *viz.*, lowering of water temperature, dilution of electrolytes, increase in oxygen content and change of pH to be the relevant factors. However, in the present study, the different temperatures maintained ranging between 24 and 27$^0$C and showers provided in the tanks with five breeding pairs each, or with a single breeding pair were found to be

ineffective in making the fish to spawn despite the fact that all fish were in ripe condition. From these results, it may be concluded that other than temperature and showers, some other factors of the aquatic medium might be involved in bringing about spawning in this fish.

### 5. 4. 2. Induced breeding using ovaprim

Control of reproduction by the hypothalamo-hypophyseal ovarian axis in female teleosts is well documented by a number of research workers. The axis, having several levels for external intervention, can be utilized to induce oocyte maturation and ovulation (Donaldson and Hunter, 1983). The maturation of oocytes and ovulation can be accelerated by intervening the hypothalamo-hypophyseal-ovarian axis at the hypothalamic level with antiestrogens (Donaldson *et al.,* 1981) or dopamine antagonists (Richter *et al.,* 1987), at the pituitary level with GnRH and its analogue (Peter, 1983; Richter *et al.,* 1987; Nayak *et al.,* 2000) and at the ovarian level with pituitary extracts (Lin *et al.,* 1985) or gonadotropin preparations (Henderson-Arzapalo and Colura, 1987), progestins (Richter *et al.,* 1987) or corticoids (Hogendoorn, 1979) or prostaglandins (Stacey and Goetz, 1982) and catecholamines (Jalabert, 1976).

In the past, acetone-dried pituitary was the most commonly used agent employed to induce ovulation (*i.e.*, hypophysation technique). The hypophysation involving exogenous administration of the pituitary extract had been the main practice used in quality production programme of different fishes before the availability of ovaprim on commercial level (Ram *et al.,* 2001). Gonadotropin is the active ingredient in pituitary extract preparation responsible for inducing spawning in fishes

(Donaldson and Hunter, 1983; Goetz, 1983; Nagahama *et al.,* 1994; Peter and Yu, 1997). A surge of gonadotropin (GtH-II) associated with ovulation and its induction of final oocyte maturation by stimulating the synthesis of maturation inducing steroids (MIS) by the follicular cells (Goetz, 1983, Kime, 1993; Nagahama *et al.,* 1994) has been observed in several teleosts. On the basis of dual neurohormonal regulation of gonadotropin secretion by gonadotropin releasing hormone (GnRH) and gonadotropin release inhibitory factor (GnRIF; possibly dopamine, DA), a highly effective technique known as '*Linpe method*' was developed (Peter *et al.,* 1986, 1988) for induced breeding of teleosts. In this technique, the combination of a super active analogue of the mammalian hormone (LHRHa) which lacks species specificity, and a dopamine receptor antagonist (dompridone) was effectively used for inducing ovulation (Peter *et al.,* 1988). The wonder spawning inducing agent ovaprim embodies the principles of the above technique and contains a super active analogue of salmon gonadotropin releasing hormone with D-Arg$^{6}$-Pro$^{9}$-Net chemical configuration (sGnRH-a) and a more effective dopamine receptor antagonist, domperidone. In a number of field trials, better quantity and quality of fertilized eggs has been reportedly produced by using ovaprim, compared with pituitary extract.

The use of GnRH analogues in aquaculture serves several purposes; like advancing the spawning season, initiating the process of final oocyte maturation of gametes, and synchronizing the spawning of broodstock. Under captive conditions, fish often develop mature gonads (and gametes) but fail to undergo the process of final oocyte maturation and spawning, due to an insufficient surge of GtH from the pituitary. Thus, females may develop vitellogenic oocytes, yet remain incapable of ovulating (Crim and Bettles, 1997).

In the present experiments, ovaprim diluted in distilled water or 0.6 % saline at the recommended dose of 0.5 ml $Kg^{-1}$ body weight was ineffective in inducing successful ovulation in this species, Marx and Kumar (2004) also found ovaprim injection of 0.5 ml $Kg^{-1}$ body weight to be ineffective in producing an ovulation response in *Channa striatus*.

Among the group III fishes, complete spawning occurred in one female when undiluted hormone was given as a single injection at a small dose of 1.2 ml $Kg^{-1}$ body weight. The mean ovulation index was slightly higher in group III (1-3 ml $Kg^{-1}$) fish (97.9 ± 1.8) compared to group IV (3-6 ml $Kg^{-1}$) fish (95.2 ± 7.9). Banerji and Prasad (1974) had observed only 78-85 % spawning of eggs in *A. testudineus* in Bihar region. Hatching rate was above 95 % in both the III and IV groups, but mean hatching percentage was slightly higher among group IV fishes. The Student's t-test showed no significant differences between the averages of groups III and IV for the traits- ovulation index, percentage of fertilization and percentage of hatching ($p < 0.05$). These results indicate that an undiluted hormone dose of 1 to 3 ml $Kg^{-1}$ body weight is an effective dosage capable of inducing spawning, in *A. testudineus* under the given conditions.

Banerji and Prasad (1974) found that the spawners of *Anabas testudineus* in Bihar bred, 6 to 14 hrs after a single injection of 10-20 mg of carp pituitary gland extract. Fertilization was never less than 80 % and practically all the fertilized eggs had hatched within 19 to 20 hours after spawning. Kharbuli *et al.*, (2004) found in *Danio aequipinnatus,* that spawning took place after 36 hours of ovaprim injection.

In the present experiments, the time taken for spawning response was found to be within a narrower range of 10-12 hours among group III fishes and 9-13 hours among group IV fishes. However, hatching time of 19 to 20 hours is comparable with the results of earlier hypophysation trials in the species. Since a higher dose of 3-6 ml $Kg^{-1}$ body weight was found to be of no obvious advantage during the present study, a dosage of 1-3 ml $kg^{-1}$ body weight of ovaprim may be used as a standard, in future breeding of *A. testudineus*.

Mookerjee and Mazumdar (1946) reported that the fertilized eggs of *A. testudineus* measured 700$\mu$m in diameter, which is lesser than the current estimate of 920 ± 20$\mu$m. The fishes spawned an average number of 387 eggs $g^{-1}$ body weight during the present investigation.

The use of dopamine antagonists with GnRHa (Linpe method) for spawning captive fish has several advantages (Peter *et al.*, 1993): 1) It provides complete or consistently high rates of ovulation between groups of broodstock, 2) Latency periods (time to ovulation after treatment) are predictable, 3) High fertilization and viability rates are usually obtained, and 4) It has little or no effects on subsequent reproductive cycles. According to Zohar (1989), the advantages in using GnRHa are; a) GnRHa can be produced in pure form in large quantities, b) GnRHa is economical because only small doses are needed to induce spawning, c) GnRHa releases endogenous GtH supplies thus increasing its effectiveness and d) GnRHa is nonimmunogenic, and has a broad degree of species reactivity.

The use of GnRHa to induce spawning may affect gamete (primarily egg) quality, yet studies relating to this subject are sparse. In

brown trout *Salmo trutta*, injected with various doses of mGnRHa, significantly lower fertilization rates were observed in the group given the highest (10μg/Kg) dose (Mylonas *et al.*, 1992). However, reduced fertilization rates were not attributed to dose or treatment, but rather time to ovulation after initial injection. It was concluded that eggs induced to spawn early in the season had insufficient time to resume meiotic maturation before being ovulated and therefore were incapable of being fertilized. In a study on early maturing Atlantic salmon subjected to various doses of m GnRHa, Taranger *et al.*, (1992) found no difference in egg and larval survival at doses of 1 and 10 μg/Kg body weight. However, at doses of 100μg/Kg body weight, high mortalities until eyed-stage were observed.

Many advantages to the use of GnRHa for spawning induction have been shown, particularly given the diversity of reproductive strategies among the teleosts. The disadvantages of their use, such as possible losses in gamete quality, must be weighed against the advantages, such as synchronization of broodstock spawning, before it can be concluded that GnRHa is an efficient method for inducing spawning in captive fish (Crim and Bettles, 1997).

The success of induced spawning depends on a number of factors, which in most of the fishes are not clearly understood (Ram *et al.*, 2001). In teleosts, augmented water content in gonads during active reproductive phase is considered as one of the prime factors associated with the success of spawning (Idler and Bitners, 1960; Medford and Mackay, 1978; Lal *et al.*, 1995). Successful spawning in majority of fishes has been induced on cloudy and rainy days, especially after heavy showers (Chaudhury, 1969). During the present experiment, these

environmental variables were not manipulated and still successful breeding was observed. Clemens (1967) had stated that hormonal injection to some extent might bypass the environmental variables of temperature, light, and rain.

Ram *et al.*, (2001) reported that in *Labeo rohita* treated with pituitary extract, only fully-grown yolky oocytes were found to undergo maturational events. In ovaprim treated fish, the oocytes passing through the growth phase were also induced to enter the maturational phase in subsequent succession to fully-grown yolky oocytes. Also that, the pituitary preparation is capable of inducing terminal events in gametic maturation faster than ovaprim, whereas the activation of events related to sustainability in quality gametic production was of greater magnitude in response to ovaprim resulting in successful synchronization and breeding performance in *Labeo rohita.*

Hormonally induced ovulation permits the culturist to schedule propagation, but the variable amount of eggs produced, the quality of the ovulated eggs and the relatively low fertilization rates however puts restriction on large scale fish production. Therefore, it is necessary to know the amount of eggs produced and the fertilization rate before planning a culture programme (Szabó, 2001). Ako *et al.*, (1994) compared egg quality between hormonally induced and naturally spawned milkfish *Chanos chanos.* The hormonally induced spawners produced higher numbers of eggs, although they were smaller and had lower fertilization rates.

The current investigation was carried out in one spawning season and with a small number of fish. Nevertheless, the preliminary results

suggested that ovaprim might be recommended as a good ovulation stimulator for *A. testudineus*. In hatchery conditions, an important trait of ovaprim is its application to fish in the form of one injection. Further, the hormone tolerates storage at room temperature. A known drawback of applying GnRH analogues is that in various fish species, ovulation does not take place at the same time in all the treated females. However, if the 'Linpe method' (Peter *et al.*, 1986) is used, this time does not usually exceed 4 hours (Brzuska & Grzywaczewski, 1999). The present study also endorses this observation as spawning occurred in most cases within a period of 9-13 hours. On the basis of present findings, it can be concluded that ovaprim is an effective inducer of ovulation in *A. testudineus*.

The present study showed that *A. testudineus* neither bred naturally under captivity nor at altered temperatures. The attempt on induced breeding however was found effective when undiluted ovaprim was injected as a single dose, with the hormone dose (1-3 ml $Kg^{-1}$ body weight) slightly higher than that recommended by the manufacturer. Ovaprim administration produced high rates of ovulation, fertilization and hatching of normal larvae in *Anabas testudineus* in the present study. The positive response of both male and female to a single dose of ovaprim is significant from the point of commercial seed production as it helps save a considerable amount of time and the need for excessive handling of brood fish.

Chapter 6

# *Larval development and rearing*

# CHAPTER VI

# LARVAL DEVELOPMENT AND REARING

## 6. 1. INTRODUTION

Most species of fish are dioecious; while some are hermaphroditic. In some fishes, internal fertilization occurs usually leading to live bearing. Some fishes guard their eggs or young for varying periods of time. In most of the fishes, fertilization is external and the large number of eggs produced by each female is often left to develop, hatch and grow without parental care. The newly hatched young is a few millimeters long, usually in the form of a larva quite unlike the adult, with a yolk sac and relatively undeveloped body form. Once the yolk is resorbed, the larva must find food on its own. After a period of growth, the larva metamorphoses into the juvenile, immature adult form.

Fish eggs and larvae provide a relatively untapped source of biological material. Experimentally based information on these early stages is required for further progress and advancement of fish culture and fisheries research (Hoar and Randall, 1969). Identification of early developmental stages, assigning them to their species, determining their ages and morphic attainments at different developmental stages (periodization) is a taxing problem particularly concerning the biology of Indian freshwater fishes (Gupta and Sharma, 1996). The existing information on the developmental biology of Indian fishes is vividly insufficient. Thus a great deal of further work is required before any practical use could be made of the knowledge. Though a few workers

have generated information on the egg and larval forms of Indian freshwater fishes, none of them have tried to project a single character or group of characters that could be useful in larval identification of these species (Gupta and Sharma, 1996).

Fish populations are declining on a worldwide basis as a result of over-fishing and unscientific fishing practices, habitat degradation, and pollution of natural waters (Holt, 1998). Rapid growth of aquaculture could compensate for the decline in catches by supplying consumers with alternate sources of fish products. Underproduction of juveniles for grow-out is a major impediment for aquaculture practices. Nutrition is one of the principal factors influencing survival of larvae in culture. Successful production of juveniles depends on effective ingestion, digestion and assimilation of diets containing the required essential nutrients. There are two main approaches in feeding larvae: the first utilizes live food organisms such as rotifers and brine shrimp, while the second focuses on the development of microparticulate diets. Although many species are reported to be weaned only after a functional stomach has developed, there is evidence that this is not the general case (Holt, 1998). Development of a diet that can be digested and assimilated well by first feeding larvae remains as a challenge for fishery scientists even now.

### 6. 1. 1. Events in fertilization and development

Fish eggs are usually spherical. In some like *Stolephorus* spp. and *Amphiprion* spp., they are elliptical, but in lizardfish *Synodus indicus* and *S. gracilis,* they are honeycomb-like. Eggs of *Hemiramphus* sp. possess long and short filaments on the outer membrane *ie.* chorion of

the egg for attachment to seaweeds or some other substratum (Venkitaramanujam and Ramanathan, 1994). Most of the fish eggs are telolecithal with yolk concentrated at the vegetative pole and the cytoplasm at the animal pole, giving a polarity to the egg. Formation of a perivitelline space, with the swelling of eggs as a result of water uptake, signifies fertilization. The egg passes through a process of cleavage and morphogenesis as the cells divide, form layers and then organs. In lampreys, cleavage is holoblastic, but in hagfishes, elasmobranchs, and teleosts, it is meroblastic. The first cleavage is meriodinal, which leads to the formation of two equal blastomeres that flatten slightly. After the second cleavage, the protoplasm oozes out through the entire protoplasmic disc. Following the fourth cleavage, a small cavity appears between the blastodisc and the anterior yolk. This cavity is the beginning of the segmentation cavity or the blastocoel. The blastoderm now commences to thin and overgrow the yolk (epiboly) and at the same time invaginate at its periphery. With formation of the segmentation cavity, the embryo enters the blastula stage. The blastodisc is at first more or less uniformly thick throughout. But at the commencement of gastrulation, the entire edge appears opaque and begins to thicken. The thickened edge constitutes the germ ring. The neurula stage ensues with its future head and spinal cord, and body musculature becomes visible. The tail region later grows away from the neurula and coils round the perivitelline space. The optic cups and the auditory organs develop, the heart starts beating and the embryo wriggles and rotates within the chorion.

The chorion or egg case is relatively tough with a funnel-shaped micropyle at the animal pole. Within the chorion, the vitelline membrane surrounds the yolk and cytoplasm (ovoplasm) of the egg. Fertilization is

normally monospermic in teleosts, the micropyle being too narrow to allow more than one sperm to pass at a time. The ovoplasm and chorion separate as the sperm activates the egg and a plug forms in the micropyle, further sperm entry being prevented. The chorion hardens thus protecting the embryo in the early more vulnerable stages. From fertilization to hatching, embryogenesis proceeds from a single egg cell to a highly organized larva. The process occurs within 12 hours in *Danio rerio*, while it takes more than 100 days in some salmonids (Bagenal and Braum, 1968).

### 6. 1. 2. Hatching

Most species of fish pass through a larval stage before assuming the adult form by metamorphosis. Hatching results from the softening of the chorion because of enzymic or other chemical substances, which are secreted from ectodermal glands usually on the anterior surface or from endodermal glands in the pharynx (Hoar and Randall, 1969). After several hours of development, the fertilized eggs hatch. The activity of the embryo assists in breaking through the chorion. Some workers report that the head of embryo comes out first, while others have observed the tail taking the lead. Venkitaramanujam and Ramanathan (1994) reported the existence of an aperture of hatching. The newly hatched fish is called a prolarva until the yolk is resorbed, and then a postlarva (or fry).

### 6. 1. 3. The larva

The larval phase begins with hatching and is a fundamental stage of early life history. At hatching, the larva is usually translucent and bears some pigment spots. Notochord and myotomes are clear with usually little development of cartilage and/or ossification in the skeleton.

A full complement of fins is rarely present, but a primordial fin fold is well developed in the sagittal plane. The mouth and jaws may not have yet appeared, and the gut is often a straight tube. Although the heart starts functioning much before hatching, the blood is colourless in majority of species and the circulatory and respiratory systems may be poorly developed (Hoar and Randall, 1969).

It is well documented that the larval stage is a critical period in the life history of fish. Fish larvae, whose organ systems are still under-developed, have to perform many biochemical and physiological activities in order to survive in the variable environments, as the adult fishes do. As the yolk is resorbed, the mouth begins to function, the gut and the eyes develop further, and the larva becomes fit for utilization of external food. One of the earlier systems to develop is that responsible for locomotion and support, the primordial fin being fairly soon replaced by median fins and the skeleton laid down. A clear change or metamorphosis from the larval to adult form is found in many species. The most obvious signs are the laying down of scales and pigmentation and often the first appearance of hemoglobin in the circulation. The time to reach metamorphosis may be a matter of days in tropical species to a few weeks or months in the majority of fish especially from temperate latitudes (Hoar and Randall, 1969).

### 6. 1. 4. Studies on larval development and survival

A study, which does not include estimates of the larval growth and mortality, is likely to miss a significant part of the production, like the population stability and year class fluctuations. Mortality is usually more during the early stages of life, and a small change in the daily or

weekly rate of mortality can add up to a severe total effect so that an year-class is 'weak' or even 'blank. Thus it is of great interest and importance to obtain information on the rate of survival for short time intervals during early life. Apart from lending themselves to the study of normal vertebrate development, studies on development assume importance as abnormal larvae are sometimes encountered during hatching so that it may serve as an indicator of possible teratogens in our diet and environment (Gilbert, 2003).

To cite a few studies on fish larval development, Bagarinao *et al.*, (1986) surveyed the early life history characteristics of 135 teleost fishes from freshwater, marine, tropical, temperate and boreal habitats, and found that egg diameters are positively correlated with larval lengths and weights at hatching. Kharbuli *et al.*, (2004) studied the embryonic and postembryonic development of the freshwater aquarium fish *Danio aquipinnatus,* following ovaprim induced spawning. Sahoo *et al.*, (2004) observed some abnormalities in the induced bred larva of *Clarias batrachus*. Dhanya *et al.*, (2005) studied the embryonic development in alligator pipefish *Syngnathoides biaculeatus.*

### 6. 1. 5. Larval rearing

In modern Aquaculture, feeding is a major component that contributes to the cost of production. Much attention has been paid towards devising measures to increase the efficiency of feeds so that fishes can be reared to desired size in a short period at a low cost (Jayaprakash and Sindhu, 1996). Sultana *et al.*, (1989) raised larvae of freshwater sweet fish 'Ayu' (*Plecoglossus altivelis*) on live planktonic feed (Rotifer) which yielded a much better growth and survival

compared with those raised on micro-capsulated artificial diet. Kenawy, (1993) recorded the differences between natural food and tested formulated diets, in promoting growth in mullet fry. Kerrigan, (1997) found that food availability and female body size significantly influenced size, eye diameter and levels of yolk reserves of larvae at hatching in *Pomacentrus amboinensis*. Kucharczyk *et al.*, (1998) studied the development of cannibalism of northern pike (*Esox lucius*) larvae under controlled conditions. Ojanguren *et al.*, (1999) found positive relationships between incubation temperature and body size in the less developed stages of Atlantic salmon. Haniffa *et al.*, (1999) observed that fry and fingerlings of *Channa striatus* fed on formulated diet showed significantly better survival but poor growth. Yufera, *et al.*, (2000) developed an inert diet for the first feeding larvae of gilthead seabream *Sparus aurata* by micro encapsulation by polymerization of dietary protein. Moteki *et al.*, (2001) have studied early growth, yolk and oil globule absorption, early morphological development and initial feeding in the Black Sea turbot *Psetta maxima* and found that oil globule remained for a long period, resulting in an extended mixed feeding period and that feeding rate was extremely high in larvae immediately following final absorption of the oil globule. Booth and Alquezar, (2002) found that supplementary feeding of the larvae of damselfish *Acanthochromis polyacanthus,* led to increased growth in length and enhanced condition relative to unfed control larvae, and suggested that variation in food supply may strongly influence persistence of larvae to juvenile stages, thus influencing cohort size.

Aquaculture in developing countries is affected by the lack of knowledge of the lipid and protein content of the live zooplankton supplied to the fish and crustacean larvae often causing mass mortality in

the first stage of larval development. Rosas *et al.*, (1998) bridged this gap by revealing the chemical composition in percentage (%) of dry matter, organic matter, inorganic matter, crude lipid, crude protein and carbohydrate of different zooplankters. Evidences suggest that hormones are passed on to eggs by broodfish prior to spawning. This store of maternal hormones may be an important determinant of egg/larval quality. There is also evidence to suggest that prolactin accelerates hatching in fish while thyroid hormones delay it (Lam *et al.*, 1991). Conceicao *et al.*, (1993) came up with a simulation model for the metabolism of yolk sac larvae of the African catfish, *Clarias gariepinnus*. Balakhnin and Savchenko, (1996) presented indirect evidence in favour of a close relationship between lectin (glycoprotein) titer and embryo survival in the coast rainbow trout (*Oncorhynchus mykiss*). Copeman *et al.*, (1999) were of the view that marine fish require the dietary polyunsaturated fatty acids (PUFA), docosahexaenoic acid (DHA, 22:6n-3), eicosapentaenoic acid (EPA, 20:5n-3) and arachidonic acid (AA, 20:4n-6) for normal growth and development.

Availability of the right type of food in right concentration in the environment is implicated as an important factor that influences larval survival (May, 1974). Therefore, knowledge about food selectivity in larval fish is of considerable relevance in aquaculture. Proper management of nursery ponds involves providing the growing fry and fingerlings with sufficient right kind of live food organisms at right time (Langer *et al.*, 2002). Since the young larvae have small mouths, it is essential that their food, such as copepod eggs and nauplii, are available when the larvae are very small. In fish culture practice, the production of fry and their rearing are prime requirements. A review of the literature on the subject indicated the existence of only preliminary and casual

observations on these aspects of air breathing fishes. The air-breathing teleosts comprise a moderate capture fishery constituting nearly 15% of the total marketable surplus of inland fisheries in India (Singh, 1993). While considering for utilization of a species for culture, it is necessary to know its embryonic and larval developmental stages. Although considerable investigations have been conducted on fishes during recent years, no reliable information exists regarding the identification of early life history stages of many Indian freshwater fishes (Gupta and Sharma, 1996). Considering the lack of information on the developmental aspects and rearing of *A. testudineus* from Kerala, a study on the embryonic and larval development of this species was taken up to gather essential information on the production of fry and their rearing.

## 6. 2. MATERIALS AND METHOD

### 6. 2. 1. Study on Development

The present study was conducted during June-August 2003. Ripe females treated with ovaprim at a dose of 1.0 - 1.5 ml $Kg^{-1}$ body weight, were stripped for their eggs when they started to extrude eggs. The eggs were then immediately mixed for 1-2 minutes with milt from ripe male fish, in a plastic bowl, with the help of a sterile bird-quill. After 10 minutes of mixing of eggs and the milt, filtered freshwater was added to the plastic bowl and the contents were stirred gently. Water was poured again and this process was repeated until the surplus milt was completely decanted off. Thereafter, a small quantity of water was added to the bowl so as to keep the eggs submerged. The eggs were reared in plastic basins covered with fine meshes having 0.7 :m mesh size and kept under aged running tap water.

The developing eggs in the different sets were examined under a microscope, photographed using a digital camera and the developmental stages were studied till hatching and thereafter at intervals of 6, 12, 24 and 48 hrs. Twenty eggs were measured from different samples at random to record the size of egg. Body measurements of 10 specimens each, belonging to different larval stages, were carried out. The length of the larvae recorded was total length, from the tip of the lower jaw to the tip of the caudal fin. Length and width of the head and tail, caudal fin height, diameter of yolk sac and eyecup were also recorded. The water temperature recorded during the study period was in the range of 26-28$^0$ C. Some well marked developmental stages such as the cleavage, blastula, appearance of the germ ring, establishment of embryonic axis,

pigmentation, separation of tail region from the yolk, development of the embryonic circulatory system, development of eye cups, lens, otic vesicles, gill, mouth, number of myomeres etc were selected, to study the development.

### 6. 2. 2. Larval Rearing

*A. testudineus* larvae used in this study were obtained from fish spawned upon treatment with ovaprim at a dose of 1.0 - 1.5 ml $Kg^{-1}$ body weight. Two hundred larvae each, at the age of 3 days post hatching, were introduced into rectangular fibre tanks containing 25 L water. Tap water kept aerated for 3 days (for removal of chlorine) was used for the rearing experiments. During the course of the experiment, water was well aerated and the larvae were fed *ad libitum* with the different diets; three times a day. Three replicates were tried for larvae with each diet, maintaining 50% water changes; twice a day. Larvae were bathed in 1-ppm malachite green solution for 20 minutes every alternate day, before changing water, to prevent fungal attacks. The experiment was conducted up to 15 days post hatching at an ambient temperature of 26-28$^0$ C. pH was in the range of 7.1 - 7.3 and dissolved oxygen recorded was between 7 and 8 mg $L^{-1}$ during the course of the experiment.

The larvae in each set were offered diet as follows:

Set I - Egg yolk alone

Set II - Artemia nauplii alone

Set III - Artemia nauplii + Egg yolk

Boiled chicken egg yolk was dried and filtered, after powdering. 0.750 g of cysts of the brine shrimp *Artemia franciscana* were activated by keeping under a 60 W incandescent bulb for about 45 minutes and then put in 1 L of filtered 30 ppt seawater under aeration in a 4 L cylindrical glass container for hatching. The cysts hatched within 22-24 hours and the nauplii (Pl: 6. 6. D) could be collected by covering surfaces of the glass container with black paper leaving a small area where a bright light source was provided. The nauplii (75000 $L^{-1}$) that aggregate at the well-lit zone were then siphoned out, added some freshwater and poured into the rearing tanks.

Percentage mortality among the larvae of *A. testudineus* was determined by counting the larvae on every third day. Survival rates as well as the daily gain in weight were also calculated. A sample of 20 larvae was taken on a glass slide, excess water was drained off and allowed to air dry for four minutes and then weighed in an electronic balance having 10 μg accuracy.

## 6. 3. OBSERVATIONS

### 6. 3. 1. Embryonic Development

Unfertilized eggs appeared opaque and milky, while the fertilized non-adhesive, floating, translucent eggs appeared as glass like spheres, having a large perivitelline space. The chorion [Plate: 6. 1 (C)], which swelled due to water absorption, lifted away from the surface of egg increasing the egg diameter by nearly 5.0 %. Diameter of the eggshell measured 930 to 980μm while that of the egg proper was 700 to 760μm and perivitelline space measured 130-150μm. The various stages of the embryonic development are shown in plates: 6. 1- 3 and in Table. 6. 1. Upon fertilization, the previously homogeneous contents reorganized, segregating the yolk-free cytoplasm to the animal pole forming a cap of clear cytoplasm called blastodisc [Plate: 6. 1 (A)].

The fertilized eggs passed through usual divisions. First cleavage commenced in about 35 minutes after fertilization, dividing the blastodisc into two blastomeres [Pl: 6. 1 (B)]. 4-celled stage [Pl: 6. 1 (C)] was reached within 15 min and after that the divisions were rapid. The blastula stage was attained within 2 hour post fertilization [Pl: 6. 1 (E)]. Soon the blastoderm started invading the yolk by spreading over the latter (epiboly) in the form of a thin layer. Epiboly continued and by about 4 hours, the embryo reached gastrula stage [Pl: 6. 1. (F)] and the germ ring soon appeared [Pl. 6. 1 (G)]. In successive stages of development, the spread of embryo over the yolk sac gradually increased. By about $6^{th}$ hour, the embryo axis was established [Pl. 6. 1 (H)]. Somites were differentiated between $8^{th}$ and $10^{th}$ hour of development, embryo took the shape of the English alphabet 'C' and the

head and tail ends of the embryo were distinguishable [Pl: 6. 2 (B)]. Pigmentation appeared by about 9$^{th}$ hour [Pl. 6. 2 (C)]. By 10$^{th}$ hour tail became rounded and distinct [Pl: 6. 2 (D)] and the micropyle plug [Pl: 6. 3 (A)] that prevent polyspermy, was still visible. About 12 somites were distinct by 12$^{th}$ hour [Pl: 6. 3 (B)] and the larva exhibited twitching property. At this stage the heart [Pl: 6. 3 (E)] started to pump blood, which was colourless. The tail remained undetached from the embryo disc. By 14$^{th}$ hour, the unpigmented eyes lacking lenses [Pl: 6. 3 (D)] appeared and the tail got detached [Pl: 6. 3 (C)] and the larva showed vigorous rolling movements within the chorion. Notochord became distinct as a tubular structure within the body of the embryo [Pl: 6. 3 (C)]. With the increase in the number of somites, the embryo elongated further and encircled about 2/3 of the oval yolk mass and occupied the entire area of the eggshell. The otic vesicles [Pl: 6. 3 (F)] appeared towards the end of embryonic life. Of the two concretions inside the oval otic vesicle, the anterior one was slightly larger. At about19 hours the embryo hatched out into a free-swimming larva. Three modes of hatching were observed; the head coming out first [Pl: 6. 3 (G)], tail coming out first [Pl: 6. 3 (H)] and the yolk sac coming out first [Pl: 6. 4 (A)].

### 6. 3. 2. Larval Development

The newly hatched larva had a translucent body with scattered stellate violet pigments [Pl: 6. 4 (B)]. The larva floated upside down with the large oval yolk sac directed upwards. Larva showed darting movements when disturbed. Mouth remained closed. The number of distinct myomeres increased to 21/22 though some were indistinct towards the tail. The various stages of the larval development are given

in plates: 6. 4 & 5; the respective developmental features in Table. 6. 2 and average body measurements in table 6. 3. At 6 hours post hatching, body pigmentation was intense but the eyes remained unpigmented. Lens placode and pectoral fin bud appeared meanwhile. By 12-hour post hatching, eyes got pigmented and gill rudiments were observable; but blood remained colourless. At 24 hours, the one-day-old larva further elongated, mouth however, still remained closed. Operculum, gill arches and gill filaments, caudal fin rays and scale marks were apparent. The 2-day-old larva had a well-formed mouth and operculum and pectoral fin bearing spines and rays. The larvae started to feed on algae, when offered. Towards the end of the second day, blood started to gain reddish tinge indicating the onset of hemoglobin synthesis. The 3-day-old larva had movable eyes complete with lens and readily accepted exogenous food, like chicken egg yolk and brine shrimp (*Artemia franciscana*) when offered. The blood became distinctly red coloured. The gut was complete which opened out by a striopore, the future anus of the post larva. The larvae started to swim actively and their endogenous yolk reserves had mostly diminished. But in many of the post larvae, which were fed with Artemia, some balance yolk remained even on $8^{th}$ day [Plate: 6. 6 (G)].

### 6. 3. 3. Abnormal Larvae

In one of the induced breeding attempts, on 29-06-03, a number of abnormal larvae were obtained and photographed [vide Pl: 6.6; A-C)]. These abnormal larvae exhibited various types of abnormalities like bent tail, malformed lower jaw etc. The larvae were very inactive and cent percent mortality occurred by day six post hatching. The newly hatched larvae were smaller in size and had an average length of 1.70 mm, with a 0.81mm long head and 0.91 mm long tail.

### 6. 3. 4. Larval rearing

Data on survival percentage, weight gain as well as daily growth rate of the larvae reared on different exogenous diets is presented in table. 6. 4. It is evident from the table that the diet II consisting of artemia alone, gave the best growth rate of 76.5 ±9.1$\mu$g per day per larva, compared to a lower value of 57.5 ± 7.3$\mu$g per day provided by the diet III comprising egg yolk and artemia. However, the best survival (55.5 ± 0.92 %) was shown by the sets provided with the diet III, followed by 49.4 ± 0.64 % in larvae fed on diet II. Diet I comprising egg yolk alone was found not suitable with lowest survival (3.2 ± 0.97 %) and daily growth rate (6.3 ± 0.56$\mu$g) respectively. Because of the transparency of the larvae, food items were visible through the skin and gut wall [Plate: 6. 6 (E & F)]. Cannibalism during rearing was observed in the present study on day 15 post hatching [Pl. 6. 6. (H)]. Surfacing for aerial respiration was not observed till 15$^{th}$ day post hatching which indicated that air breathing habit began only later in larval life.

**Table 6. 1.** Time course of embryonic development in *A. testudineus*

| Time after fertilization | progression in development |
|---|---|
| 0 h 00 minutes | Fertilized egg; chorion swells, blastodisc forms |
| 0 h 35 minutes | Cleavage; Two-celled stage |
| 0 h 50 minutes | Four-celled stage, rapid divisions |
| 2 h 00 minutes | Blastula stage; tiers of blastomeres added; blastoderm starts invading yolk (epiboly) |
| 4 h 00 minutes | Gastrula stage; Epiboly continues and the germ ring appears |
| 6 h 00 minutes | Embryo axis establishes |
| 8 h 00 minutes | Segmentation stage; First few somites appear |
| 9 h 00 minutes | Pigmentation stage; Pigments appear on yolk sac and body of the embryo which appears like the letter 'C' |
| 10 h 00 minutes | Tail bud stage; Tail with round end becomes distinct; 6-8 somites visible |
| 12 h 00 minutes | Organogenesis stage; Heart begins pumping; embryo exhibits twitching property; 12 somites counted |
| 14 h 00 minutes | Tail detaching stage; Eyes appear; tail detaches; larva occupies entire egg shell area and starts rolling within the egg shell |
| 16 h 00 minutes | Embryo elongates, 18 somites counted |
| 18 h 00 minutes | Otic vesicles appear |
| 19 h 00 minutes | Larva hatches out |

**Table 6.2.** Larval development stages in *Anbas testudineus*

| Time after Hatching | Length of larva (mm) | Developmental features |
|---|---|---|
| 0 h | 2.1 | Large oval yolk sac; 21 myotomes; larva darts if disturbed |
| 6 h | 2.3 | Body well pigmented; lens placode appears on unpigmented eye; pectoral fin bud visible |
| 12 h | 2.7 | blood still colourless; eye gets pigmented; gill rudiment appears |
| 24 h | 3.2 | operculum, scale marks and caudal fin rays appear; mouth not yet open |
| 2nd day | 3.4 | operculum distinct; gill arches and gill filaments appear; well developed open mouth; larva starts feeding; yolk depletion evident; reddish tinge to blood (Hemoglobin synthesis begins) |
| 3rd day | 3.5 | movable eyes with lens; active feeding and swimming; gut opens out by a striopore; yolk absorbed to a great extent, blood with distinct red colour; gill lamellae appear. |

**Table 6. 3.** Average body measurements of larval stages of *Anbas testudineus.*

| Measurement of Body part (μm) | Newly hatched (0-12 h) | Larval age in days | | |
|---|---|---|---|---|
| | | 1 day old | 2 day old | 3 day old |
| Total length | 2365±277 | 3052±378 | 3306±247 | 3513±190 |
| Head length | 1029±75 | 1072±78 | 1178±123 | 1270±129 |
| Tail length | 1388±224 | 1980±358 | 2128±138 | 2243±74 |
| Head width | 599±31 | 580±50 | 568±30 | 551±27 |
| Tail width | 137±30 | 138±25 | 155±21 | 155±18 |
| Caudal fin height | 301±37 | 362±98 | 413±18 | 517±78 |
| Yolk sac Diameter | 562±55 | 436±105 | 321±94 | 267±49 |
| Eye cup Diameter | 224±18 | 246±33 | 288±22 | 293±13 |

**Table. 6. 4.** Performance of *A. testudineus* larvae fed on different diets.

| Diet | Mean survival (%) | | | | | Average weight of larva (μg) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Days post hatching | | | | | Initial weight | Final weight | Weight gain | Daily growth |
| | 3 | 6 | 9 | 12 | 15 | | | | |
| I. Egg Yolk only | 100 | 86.6 ± 0.98 | 60.5 ± 3.65 | 30.6± 2.56 | 3.2± 0.97 | 360 | 435 | 75 | 6.3± 0.56 |
| II. Artemia only | 100 | 87.2 ± 1.17 | 83.9 ± 1.33 | 51.9± 2.15 | 49.4± 0.64 | 360 | 1278 | 918 | 76.5 ± 9.1 |
| III. Artemia + Egg yolk | 100 | 84.2 ± 2.16 | 67.7 ± 4.21 | 63.2± 1.47 | 55.5± 0.92 | 360 | 1050 | 690 | 57.5 ± 7.3 |

Plate. 6. 1

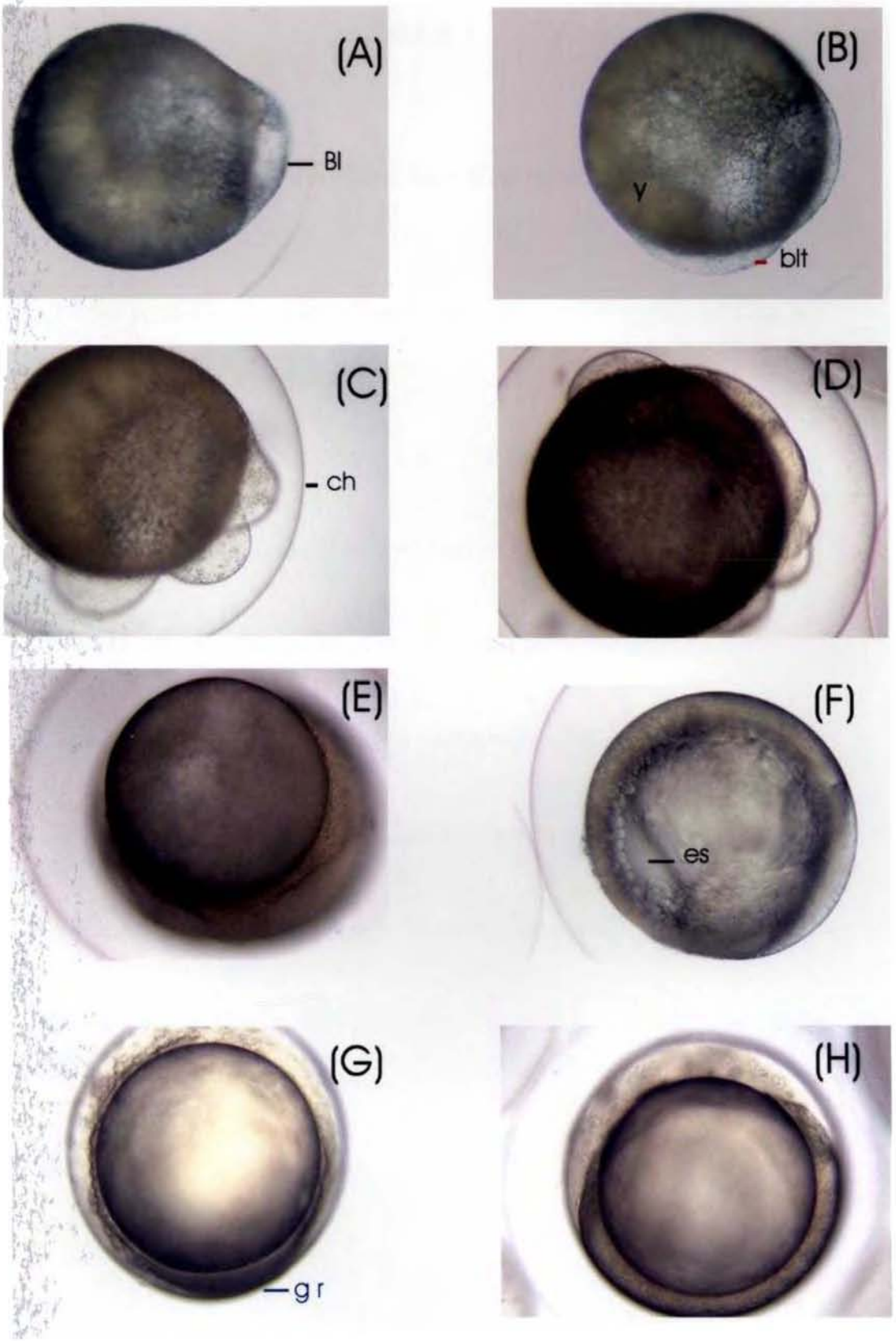


**Plate. 6. 1**

**Photomicrographs of developing eggs of *A. testudineus.***

**(A)** Eggs within minutes after fertilization; cytoplasm shifted to form the blastodisc (bl)

**(B)** An egg just after first cleavage; y = yolk.

**(C)** 4-celled stage in cleavage; ch = chorion.

**(D)** Multi-celled cleavage stage at 1 ¼ h post fertilization.

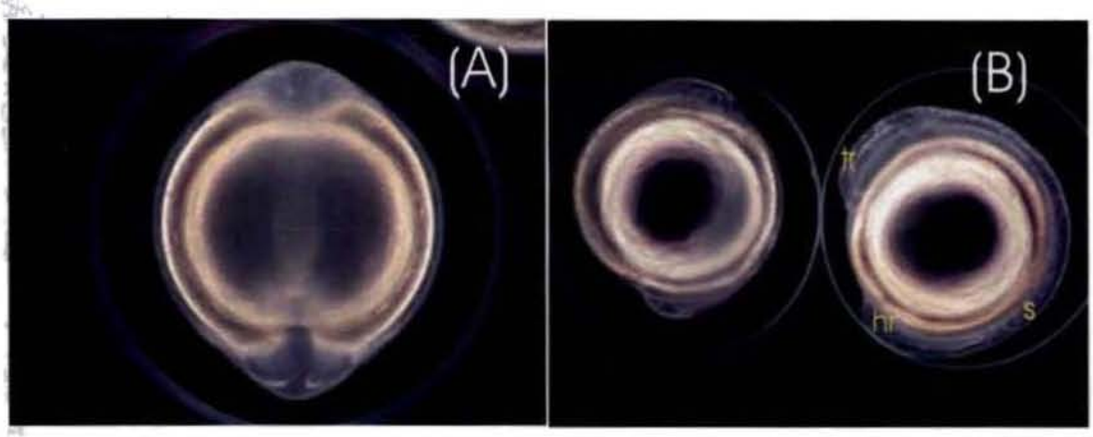
**(E)** Blastula 2 ½ h post fertilization undergoing epiboly.

**(F)** Epiboly continues in the gastrula; es = embryonic shield

**(G)** The germ ring (gr) appears in the gastrula 4 h post fertilization.

**(H)** Embryo 5 h post fertilization showing the establishment of the embryo axis.

Plate. 6. 2



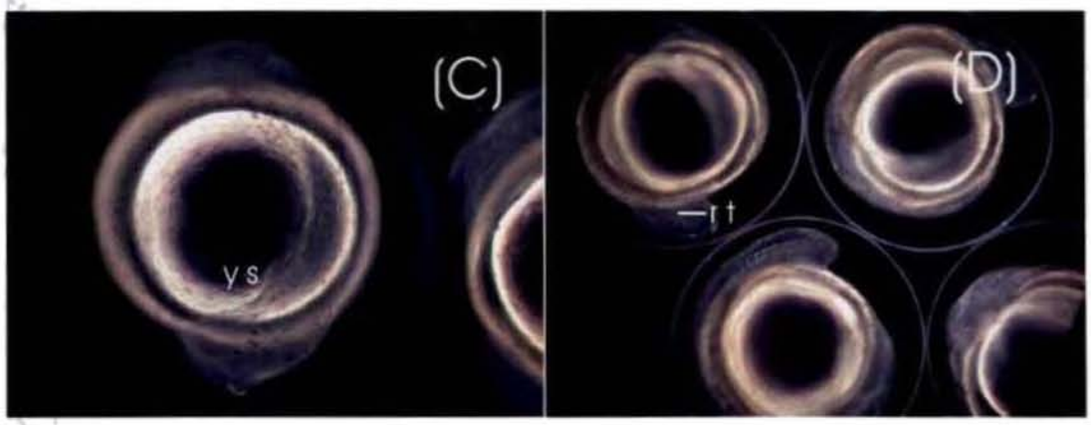



**Plate. 6. 2**

**Photomicrographs of developing eggs of *A. testudineus* as observed under a phase changing microscope.**

**(A)** View of an embryo 6 h post fertilization.

**(B)** C-shaped embryos 9 h post fertilization, with a distinct head region (hr) and tail region (tr). A few somites (s) are also visible.

**(C)** 9 hour old embryo adorning pigments on yolk sac (ys) and the body.

**(D)** 10 hour old embryos having many somites and a distinct round tail (rt).

Plate. 6. 3

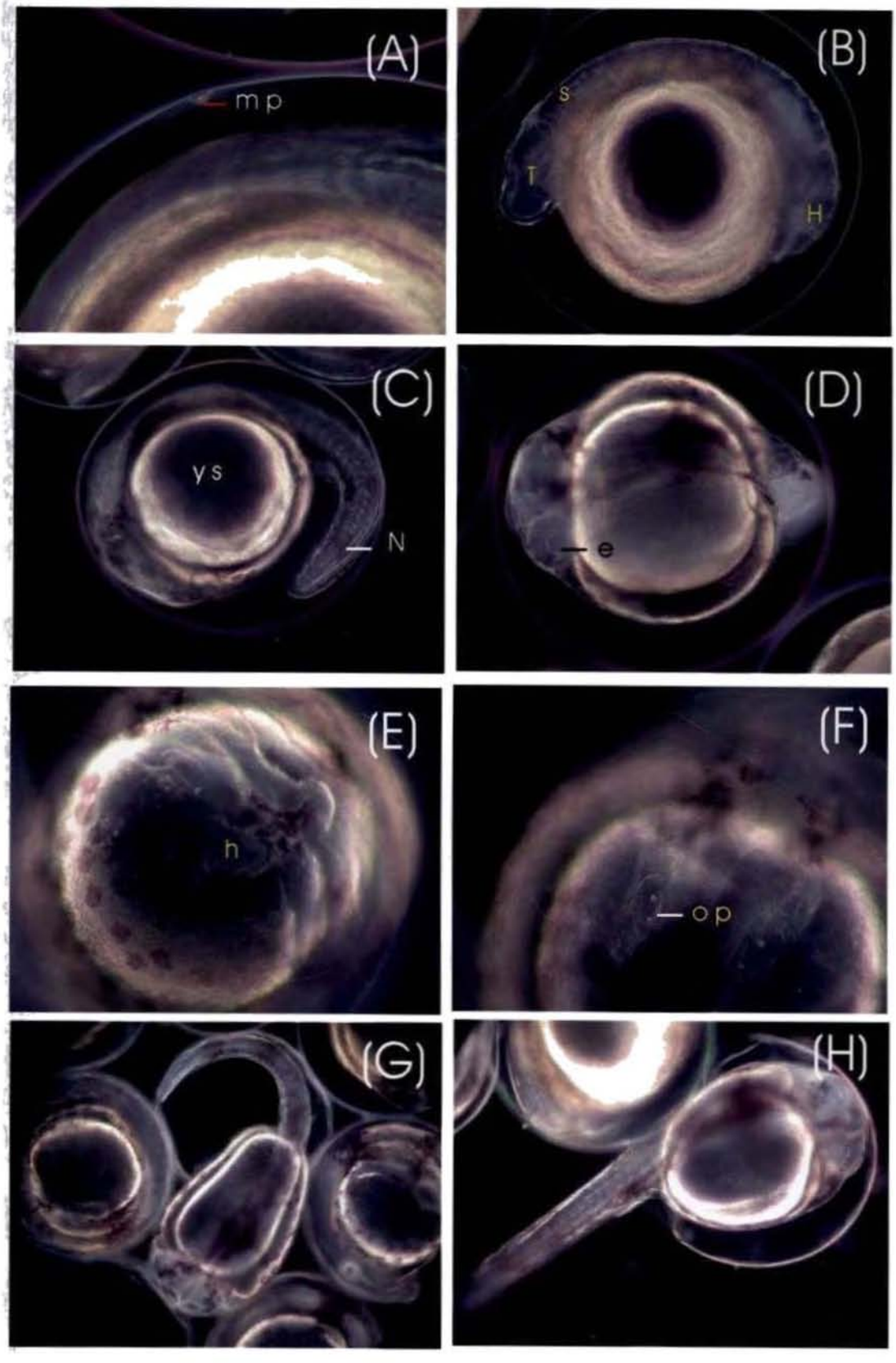


**Plate. 6. 3**

**Photomicrographs of advanced stages of developing embryos of *A. testudineus***

**(A)** Enlarged view of a developing embryo 10 h post fertilization; note the persisting micropyle plug (mp)

**(B)** An embryo 12 h post fertilization having a dozen somites (s); H=head, T=Tail

**(C)** 14 h embryo with a round yolk sac (ys) and a detached tail; the larva occupies the full area of the oval egg shell; note the notochord (N)

**(D)** 14 h embryo bearing unpigmented eyes lacking lens

**(E)** Photomicrograph of a 14 h embryo showing the heart rudiment (h)

**(F)** Otic placode (op) containing otoliths in an 18 h embryo

**(G)** Larva in the hatching process with the head coming out first; note the empty eggshell and the tail of the larva

**(H)** Another larva at the time of hatching; the tail comes out first

# Plate 6. 4

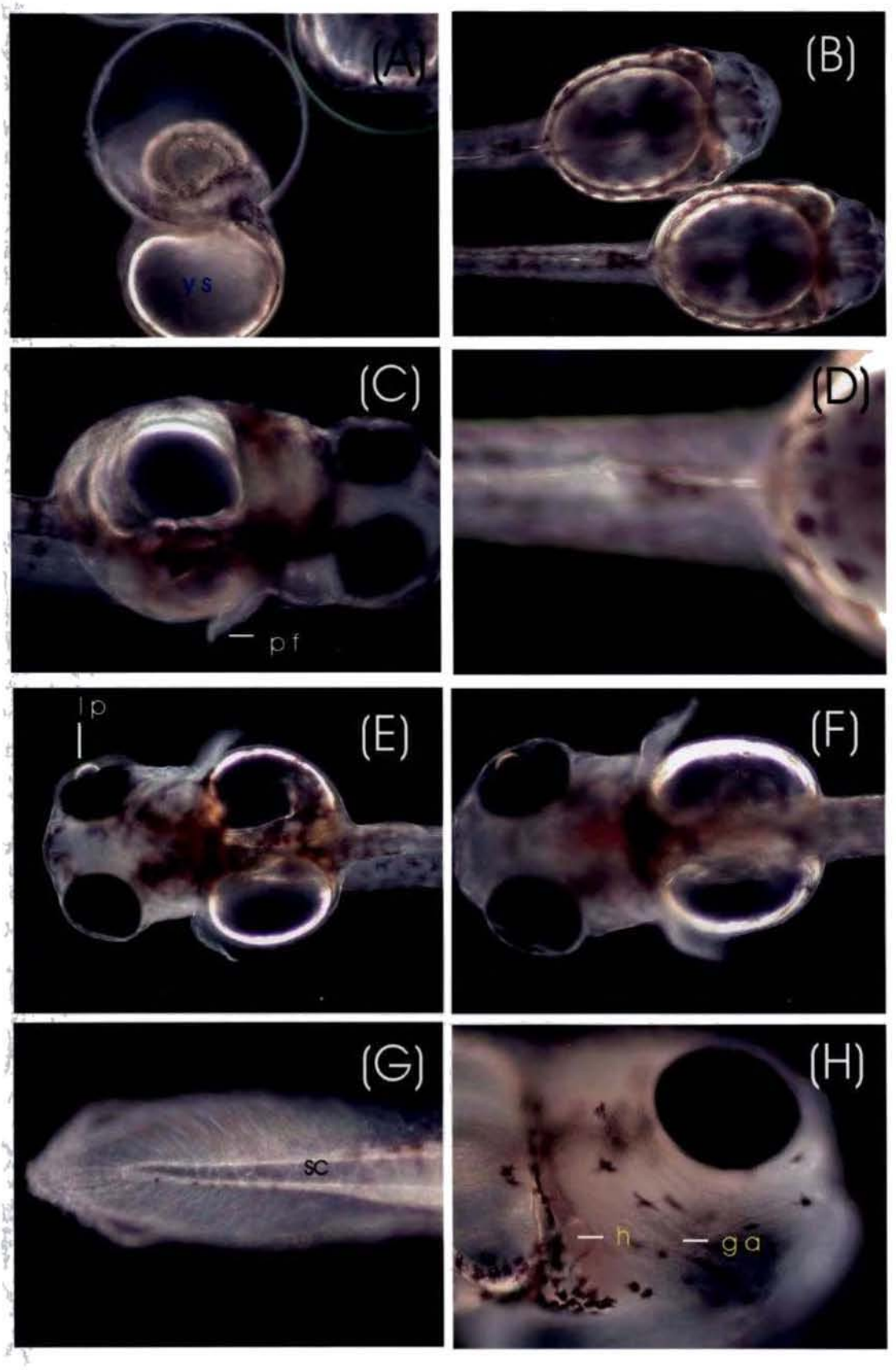


**Plate. 6. 4**

**Photomicrographs of early larval stages of *A. testudineus***

**(A)** Hatching of a larva with the yolk sac (ys) coming out first

**(B)** Larva 2 h post hatching having a large oval yolk sac.

**(C)** 6 h old larva with a pectoral fin bud (pf) and pigmented eyes lacking lenses

**(D)** Enlarged view of a larva 10 h post hatching, in which the first spine bud arise on the prospective dorsal fin

**(E)** Photograph of a 24 h old larva; note the lens placode (lp) of the eye

**(F)** A 2-day-old larva (33 h) with an open mouth.

**(G)** View of the developing caudal fin bearing rays in a 44 h (2 day old) larva; note the scale marks (sc)

**(H)** Larva in fig (G) bearing a prominent heart (H) and gill arches (ga); note the reddish tinge of blood

Plate. 6. 5

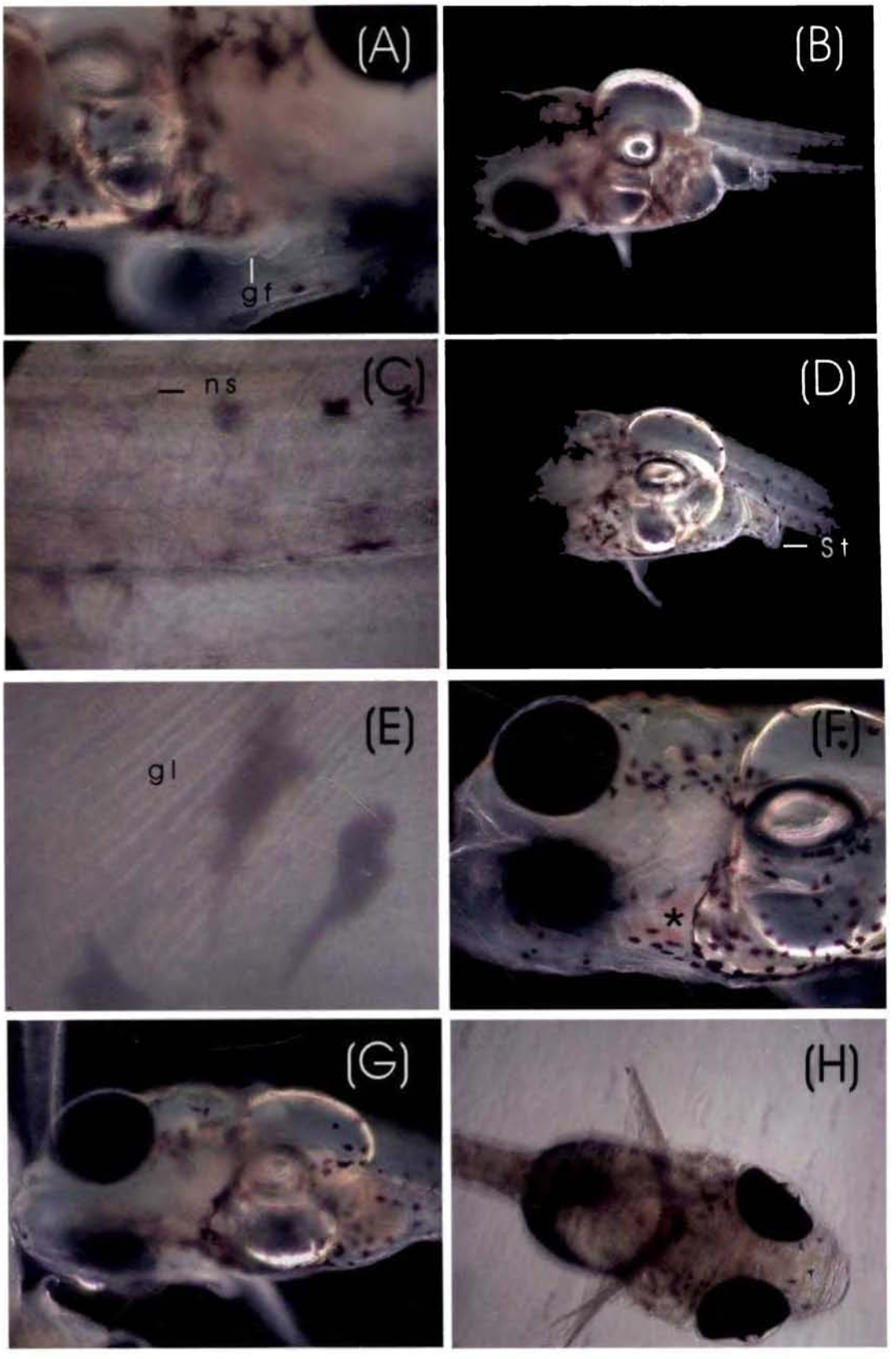


**Plate. 6. 5**

**Photomicrographs of later larval stages of *A. testudineus***

**(A)** View of two-day-old larva with developing gill filaments (gf).

**(B)** Two-day-old larva with well-formed mouth and fully pigmented eye; note that yolk is significantly reduced.

**(C)** Magnified view of tail portion of larva in fig. (B); note the neural spines (ns) and impression of the developing scales.

**(D)** Three-day-old larva having a gut complete with striopore (st).

**(E)** View of the larva cited above with the head region enlarged to show the development of fine gill lamellae (gl).

**(F)** Photograph of a larva 3-day post hatching to elucidate the coloured blood indicated with asterisks (*) mark.

**(G)** View of a five-day-old postlarva containing food (fd) in gut; note the complete lack of endogenous yolk in the postlarva.

**(H)** Photograph of a seven-day-old postlarva having a less translucent body.

Plate. 6. 6

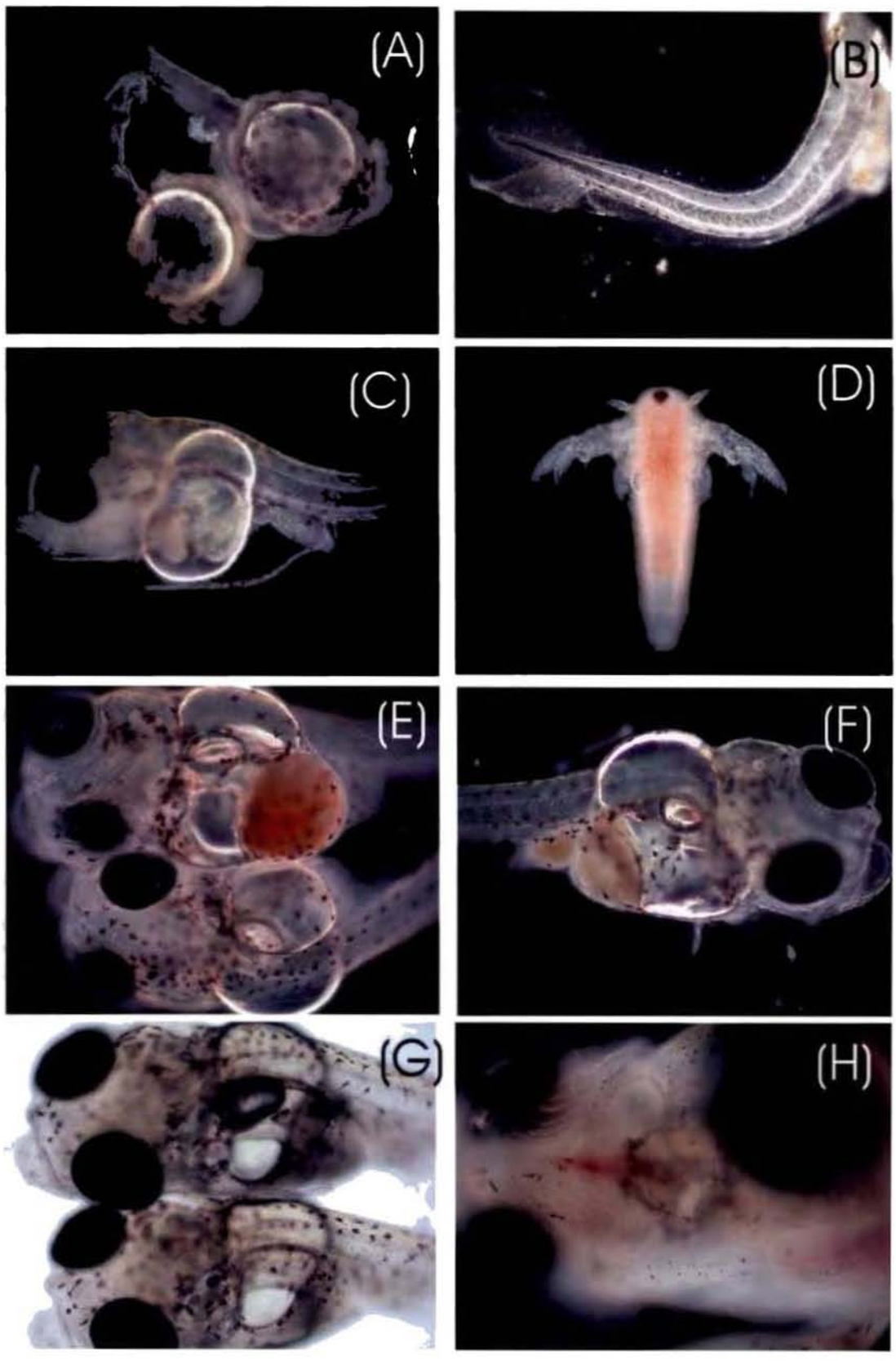


**Plate. 6. 6**

**Photomicrographs of deformed larvae of *A. testudineus* and larvae fed on different diets**

**(A)** Photomicrograph of newly hatched abnormal larvae; note the abnormally bent tail and the poorly developed head region.

**(B)** Photograph to illustrate the abnormally bent tail in a 4-day-old abnormal larva.

**(C)** Abnormal jaw, malformed gut and yellow pigments in a 5-day-old deformed larva.

**(D)** Photomicrograph of an *Artemia franciscana* nauplius with its orange gut.

**(E)** Transparency of larvae allows type of diet consumed to be distinguished: 4-day-old larva fed *Artemia franciscana* nauplius (above) and unfed larva (below).

**(F)** Photograph of a 4-day-old larva fed cooked egg yolk.

**(G)** 8-day old-larvae with traces of yolk remaining (above) and without any yolk (below).

**(H)** Photograph illustrating cannibalistic behaviour in a 15-day-old larva.

## 6. 4. DISCUSSION

For successful utilization of a fish species for culture, knowledge about its embryonic and larval development is an essential prerequisite. The present study was an attempt in this direction. Since eggs are released in *A. testudineus* over a fairly prolonged time during induced spawning, the eggs from a given set of spawners did not develop at the same time necessitating artificial fertilization by "dry stripping" reported here.

The various stages of initial development observed in *A. testudineus* in the present study matched well with those described in other teleosts. However, some variations in the details of embryonic and larval development were also recorded. Hoff *et al.*, (1978) reported that fertilized eggs might shrink as much as 16% within one hour after fertilization in Florida pompano (*Trachinotus carolinus*), which is in contrast to the present observation in that the eggs swelled by nearly 5.0%. Swelling of fertilized eggs have also been reported in *Schizothorax curvifrons* (Gupta and Subla 1978), in *Rasbora daniconis* (Sivakami, 1981), and in *Danio rerio* (Gilbert, 2003).

In *Heteropneustes fossilis,* which complete embryonic development within 18-20 hours as in *A. testudineus* in the present case, Thakur *et al.*, (1974) reported the appearance of eye at 8$^{th}$ hour post fertilization, while Mookerjee and Mazumdar (1946) observed the same at 12 hour 45 minutes in *A. testudineus* in Bengal waters. However, the eyes appeared by 14$^{th}$ hour post fertilization in the present study in *A. testudineus*. Further, pigmentation of eye, and the lens that appeared before hatching in the case of *Heteropneustes fossilis,* appeared only by

12th hour and 2 days post hatching respectively in *A. testudineus* in the present study. However, the beginning of heartbeats was observed at comparable periods in both cases. Mookerjee and Mazumdar (1946) found pectoral fin buds and eye lens appearing 9 h post hatching in *A. testudineus* in Bengal, whereas in the present study, pectoral fin buds were visible by 6 h post hatching while the lens appeared only on 2nd day of hatching. Further, body pigmentation was observed by 9th h post fertilization during the present study, which is in sharp contrast to the observation of Mookerjee and Mazumdar (1946) who reported the same to occur at 13th hour. According to Gupta and Sharma, (1996), appearance of pigments do not follow any set pattern in different fish species, but may follow a pattern of appearance within the same species, and can be of some value in the aging of the embryo. Apart from that, it appears that the first appearance of body pigmentation, which is characteristic of a species, could be of considerable value in identification of the embryo or larva. Pigmentation of the eye as observed at 12 h post hatching in the present study, matched well with the observations of Mookerjee and Mazumdar (1946) in *A. testudineus* of Bengal waters. In *Xenentodon cancila,* a freshwater belone inhabiting lotic streams of Jammu, most of life history stages (including pigmentation) are passed within the egg and the larva is born as an adult with well developed mouth and jaws, otherwise formed later in the larval stages of other fish species (Gupta and Sharma, 1996).

Various workers have reported different ways of hatching, in different fish species. Mookerjee and Mazumdar (1946) in *Anabas testudineus,* Thakur *et al.*, (1974) in *Heteropneustes fossilis*, Gupta and Subla (1978) in *Schizothorax curvifrons* and Prasad and Prasad (1985) in *Colisa fasciatus,* found the tail coming out first during hatching, while

Venkitaramanujam and Ramanathan (1994) found the head coming out first in *Ambassis commersoni*. However, in the present study, apart from these modes of hatching a different mode of hatching was observed in which the yolk sac came out first [Plate: 6. 4. (A)]. In *Ambassis commersoni,* Venkitaramanujam and Ramanathan (1994) found an aperture of hatching, which was never observed in *A. testudineus* in the present study. Hora and Pillay (1962) and Chaudhuri (1969) reported the hatching in *A. testudineus* to occur in about 24 hours whereas Mookerjee and Mazumdar (1946) found that hatching occurred between 18 ½ and 20 h at ambient temperatures. In the present study, hatching was observed in about 19 hours post fertilization at a temperature range of 26-28$^0$ C.

The gills appear for the first time in a distinct branchial chamber at different times in the life history of fishes (Gupta and Sharma, 1996). They appear at 4 days post hatching in the belone *Xenentodon cancila* (Gupta and Sharma, 1996), within 3 days in *Tor khudree* (Kulkarni, 1970) in 48 h in *Cirrhina mrigala* and in 24 h in *Labeo rohita* (Chakrabarty & Murty, 1972). Available literature makes no mention about the onset of hemoglobin synthesis in *A. testudineus*. In the present study, it was found that along with the appearance of gill filaments [Pl: 6. 5 (A)] on 2$^{nd}$ day, blood got coloured [Pl: 6. 4 (H)] indicating the onset of hemoglobin synthesis marking the switch over from the cutaneous mode of respiration to that through the gills. Gill lamellae appeared on third day post hatching, and blood became distinctly red coloured [Pl: 6. 5 (F)] so that gills became fully functional.

Sridhar *et al.*, (1998) reported that in *Ompok bimaculatus*, 3 days after hatching, the mouth was completely formed and the larvae began to

ingest exogenous feed consisting of cooked egg yolk from day 4 post hatching, besides utilizing their endogenous yolk. Mookerjee and Mazumdar (1946) found, in *A. testudineus* in Bengal waters, that feeding started on 2nd day post hatching when mouth opened. The present study also endorsed these findings. Surprisingly, Banerji and Prasad, (1974) reported that the fish larvae of this species in Bihar region started feeding within 24 hours of hatching.

One obvious feature of newly hatched fish larva is the yolk sac, which keeps them independent from an external food source during the first few days of larval life. Duration of the yolk sac period differs widely among species and is related to temperature (Bagenal and Braum, 1968), the quantity of yolk present in the larvae and the exogenous mode of feeding (Gupta and Sharma, 1996). In a freshwater belone *Xenentodon cancila,* the yolk sac gets absorbed only on 11th day (Gupta and Sharma, 1996). Chakrabarty and Murty (1972) found yolk sac to resorb on 4th day in the larvae of the carps *Cirrhina mrigala, Catla catla* and *Labeo rohita.* Kharbuli *et al.*, (2004) observed complete yolk absorption only by 12th day in *Danio aequipinnatus* larva.

Dhanya *et al.*, (2005) stated that the embryonic period begins with activation and is characterized by exclusively endogenous nutrition from the yolk. The larval period commences with the transition to exogenous feeding and lasts until metamorphosis. Morphological development continues during the yolk sac period and the larva becomes fit to shift from internal to external food sources. The yolk is absorbed by phagocytic activity of the inner part of the syncytial layer (the vitellolysis zone), and is degraded into substances of low molecular weight and then transported into blood (Kamler, 1992).

There is little evidence of a critical period of the completion of yolk resorption (Bone *et al.*, 1996). In the present study, though in most of the unfed larvae, yolk got absorbed on the third day, in many of the fed larvae it persisted even up to 8$^{th}$ day [Pl: 6. 6 (G)]. Since larvae over a wide range of length and development have been observed with yolk reserves, it appears that yolk resorption may be related to early feeding success and food availability.

The hatchlings of *Monopterus cuchia* and *Monopterus albus* are quite different from those of other air-breathing fishes (Singh, 1993). In these fishes while the yolk is being absorbed, the yolk sac is transformed into a ribbon-like yellow structure extending up to the anal pore in *M. cuchia* and up to the neck region in *M. albus*. Further, it took nearly 22-24 days for the hatchlings to be completely free from the yolk. aforsaid structures were not noticed in *A. testudineus* larvae during the present study. The different characters, occurring in fishes at various intervals of their life history, vary very much, which might be an independent phenomenon.

Some abnormal larvae were observed in one of the induced breeding attempts during the course of present study. These larvae exhibited various types of deformities like bent tail, malformed lower jaw etc. Sahoo *et al.*, (2004) categorised deformed larvae into the following four categories: 1) indeterminate- body of the embryo is not differentiated into head, trunk and tail; 2) Acephala- no dstinct head; 3) Tunicate (Pigmy larvae)- small larvae with body differentiated into head, trunk and tail and 4) Vertebral imparity-axial imparity showing an arc in any part of the vertebral column. The deformed larvae observed during

the present study showed characteristics matching the last of the, above mentioned, two categories. The causes of deformities may be metabolic (Muramoto, 1981), physiological (Bengston and Larson, 1986), nutritional (Oda and Kayano, 1988), toxicological (Reash and Berra, 1989) or genetic (van Eden *et al.*, 1996). Because of hormonal induction, ovulation of premature or overripe eggs can not be ruled out. Such eggs were viable and able to fertilize, but led to unusual development (Sahoo *et al.*, 2004). Jordan, (1982); Oozeki and Hirano, (1985) reported that environmental factors like temperature or depletion of oxygen is responsible for impaired egg development leading to malformations. In the present study, the temperature (26-28$^0$ C) and dissolved oxygen (7 - 8 mg $L^{-1}$) were in the normal range and there was only a single instance among the score of successful induced breeding trials that abnormal larvae were observed. So it appears that impaired embryogenesis might have lead to the hatching of deformed larvae. Defects in development can often be traced to changes in a particular group of cells (Driever *et al.*, 1994; Haffter *et al.*, 1996). Occurrence of abnormal larvae leads to growth depression and mortality. Therefore, the assessment of these malformations could be used as a tool to estimate the larval quality of a species.

Development of suitable methods for rearing the larvae is very important to ensure reliable and regular supply of fry, and successful controlled method of larval culture is dependent on a sound knowledge of the nutritional requirements of the larvae. Meske, (1984) tested a varying mixture of finest trout feed and larvae of *Artemia salina* on the fry of *Sarotherodon galileus* and *Clarias lazera* and demonstrated that the more and longer the larvae were fed with Artemia, the highest is the survival rate and the less when dry feed was used. Pal *et al.*, (1977)

experimented with 6 different types of feeds on 3 day old spawn of *A. testudineus* and found that the young larvae could be reared up to fry stage (15 day old) on cooked diet with over 70 % survival. They however, noted that feeding with zooplankton gave relatively better rate of growth but a lower survival of 48 to 58 %. Growth rate and survival of *A. testudineus* larvae in the present study were found to be considerably influenced by the type of diet offered. Live food Artemia was found superior to the conventional diet of cooked egg yolk, yielding a high rate of growth (76.5 ± 9.1 μ g per day). However, when both were offered, greater survival (55.5 ± 0.92 %) with medium growth (57.5 ± 7.3 μ g per day) was observed. Several earlier workers (Lubzens *et al.*, 1984; Prinsloo and Schoonbee, 1986; Jana and Chakrabarty, 1990) have observed enhanced growth rate in carp larvae fed live food than those fed conventional diet. Though the best growth performance of larvae in the present study was found with diet II (Artemia only), best survival and medium growth rate was found with Diet III. Therefore, diet III comprising Artemia and Egg yolk may be recommended the best diet for growth and survival under the given conditions for rearing *A. testudineus* larvae upto a period of 15 days post hatching.

The great natural mortality during the egg and larval phase is a fundamental aspect for the assessment of fish production (Bagenal and Braum, 1968). Because of the great differences in modes of reproduction among fishes, widely different methods are needed to obtain data about mortality and survival during early stages of life. According to Yakupitiyage *et al.*, (1998) Anabas fry feed on zooplankton and phytoplankton 3 days after hatching and the best initial feed is *Moina*. The survival rate of *A. testudineus* fry was highest if post larvae were fed rotifer (47%), compared to *Moina* (4%), boiled egg (3%) and chlorella

(0.4%) (Doolgindachabaporn, (1988). During the larval period, 3-17 days from the inception of feeding, they can be fed only live food organism (Doolgindachabaporn, 1994). But, during the present study, egg yolk was also found to be well accepted by the larva, though it yielded lower survival and growth.

In rearing experiments, there has sometimes been a catastrophic mortality when larvae change to the post larval stage. The phenomenon is called the 'critical period' for the larvae and has been associated with a lack of suitable food when external feeding begins (Bagenal and Braum, 1968). However there was little evidence for a period of catastrophic mortality in the given conditions of the present experiment. Cannibalism [Pl: 6. 6 (H)] during rearing observed by Banerji and Prasad (1974) in *A. testudineus* in Bihar waters was observed in the present study also beginning on day 15 post hatching. Larval deaths due to overfeeding on Artemia nauplii as reported by Bryan and Madraisau, (1977) in *Siganus lineatus* was not observed during the present investigation.

To conclude, the various stages in initial development observed in *A. testudineus* in the present study, matched well with those described in other teleosts. However, the time of hatching and the beginning of feeding, time of appearance of pectoral fin bud, eye and lens, and body pigmentation, differed with the earlier reports of Mookerjee and Mazumdar (1946) and Banerjee and Prasad (1974) in the species from North India. An aperture of hatching, as reported by Venkitaramanujam and Ramanathan (1994) in *Ambassis commersoni* was not observable in *Anabas testudineus* in the present study. The different modes of hatching like 'head coming first' and 'tail coming first' reported by some earlier workers in different fish species do not match well with the current

observations. Rearing of *A. testudineus* larvae up to a period of 15 days post hatching was found successful with a diet composed of *Artemia franciscana* nauplii and chicken egg yolk. Beginning of hemoglobin synthesis on the second day of hatching, as reported here, is the first such report in the species. Further, the gills became fully functional, with the appearance of gill lamellae on the third day post hatching. The present study on the life history of *A. testudineus* is expected to enable the larval fish ecologists or fish biologists to identify the larvae of the species and to roughly back calculate the time of spawning and predict the time of hatching in similar conditions.

# Chapter 7

# *Summary*

# SUMMARY


1. In the present work, basic aspects of reproduction, maturation and larval development of an air-breathing fish of anabantid family, *A. testudineus* (Bloch) have been investigated.

2. Following a review of relevant works done in Anabantids and on *Anabas species*, the basic aspects of reproduction, histology of the ovary, effects of photothermal treatment on the ovaries, induced breeding, development of larvae and their rearing have been detailed.

3. *A. testudineus* is bisexual. Sexual dimorphism is not marked. Juveniles of *A. testudineus* did not exhibit internal differentiation of sex. At about a size of 6.5-7.0 cm (total length) the females could be distinguished from males by the presence of pinkish, translucent jelly like soft gonadal tissue, occupying one-fourth of the posterior part of the body cavity. Ovaries consisted of two slightly unequal sized cylindrical lobes resting on the ventral wall of body cavity, ventral to the kidneys. Though the left ovary appeared to be slightly longer, Student's t-test revealed no significant difference.

4. An arbitrary five-stage maturity scheme was adopted, based on gross morphology of ovary such as size, colour and texture, and

microscopic observations on the oocytes. The characteristic features of each stage are discussed in detail.

5. A close relation was found between surface water temperature and GSI values in the present study. Consequent to an increase in temperature beginning with February, the mean GSI value also rose and increased steadily to reach a peak in May.

6. A Wide range in GSI values, a single seasonal peak in mean GSI value in the month of May and a similar peak in ovary weight in maturity stage IV, and from the occurrence of only one distinct of batch of ripe eggs as may be clear from the oocyte size frequency profiles, it may be concluded that *A. testudineus* has a "group synchronous" ovary. The single and short spawning season of May to June, in *A. testudineus* in the present study, coincided with the onset of southwest monsoon in Kerala.

7. Minor fluctuations only were seen in the monthly values of condition factor 'K'. The same trend was observed with the values of relative condition 'Kn'. These observations indicated that the fish remained in good condition throughout the spawning period. Low variations in the 'K' values show that the condition factor 'K' is least influenced by the breeding cycle.

While GSI values accurately reflected the stage of maturity, HSI and 'K' values were poor indicators.

8. From the maturity curve, the size at first maturity was found to be 9.8 cm in total length. This is in agreement with the results of studies on changes of relative condition 'Kn' with respect to length, which indicated that first sexual maturity began from 9.1 cm onwards. The length at maturity would be a useful index for determining the size of the exploitable stock.

9. Fecundity ranged from 1000 to 59,022 in fish size range of 8.9-18.7cm/12.74-125.4g. The relationship between fecundity and total length was found to be curvilinear. The exponential value 4.3283 indicated that fecundity increased at a rate greater than the cube of the length. The statistically significant coefficients of correlations derived between fecundity and various body parameters such as body length and body weight, may be of immense help in enumerating the fecundity of fish, without sacrificing them.

10. When all the samples were pooled, a ratio of 1.0 male to 0.94 female was obtained and the low chi-square ( $\chi^2$) value indicated that the ratio did not differ significantly from the hypothetical 1:1 ratio.

11. In the present work, oogenesis has been described in eight stages, with the descriptions based on easily recognizable anatomical and size differences. The classification schemes developed, based on histology, is expected to help identify the discrete stages of oocyte development and the different ovarian maturity stages with precision.

12. Histological observations of ovaries of various maturity stages in different seasons, confirmed that the fish is a "group synchronous spawner", with a single short spawning season.

13. The yolk nucleus, was first observed in the early perinucleolus stage oocyte closely apposed to the wall of the germinal vesicle, which later migrated to the periphery. The yolk nucleus disappeared far before the onset of vitellogenesis and hence apparently it had no role in vitellogenesis.

14. Histological examinations revealed that previtellogenesis in A. *testudineus* occurred in oocytes ranging from 120.0 to 240.0 µm; while, Vitellogenesis started when the oocytes reached a diameter of 240.0 µm.

15. The development of oogonia from follicular cells of corpus luteum as reported by Dutt & Govindan (1969) in *Anabas scandens* was not observed in the present study. Similarly, the

present study also disagrees with the same authors regarding the direction of migration of the yolk vesicles; the yolk vesicles in the present study have been found to follow the centripetal sequence of movement as reported in most of the teleosts.

16. Preliminary experiments on induction of ovarian recrudescence gave encouraging results. Photothermal treatment, involving a temperature of 26-27$^0$C and long photoperiod of 14 hours, for a period of three months during the post spawning season, led to the retention of viable yolk vesicle stage oocytes, beyond the natural spawning season, bringing in ovarian recrudescence. Further, a high temperature of 30-31$^0$C was found detrimental to oogenesis as evidenced by massive atresia of the oocytes, including those of the perinucleolar oocytes.

17. The present study showed that *A. testudineus* neither bred naturally under captivity nor at altered temperatures. The attempt on induced breeding however was found effective when undiluted ovaprim was injected as a single dose (1-3 ml Kg$^{-1}$ body weight), which is slightly higher than that recommended by the manufacturer. The fishes spawned an average number of 387 eggs g$^{-1}$ body weight.

18. Ovaprim administration produced high rates of ovulation, fertilization and hatching of normal larvae in *Anabas testudineus* in the present study. The positive response of both male and female to a single dose of ovaprim is significant from the point of commercial seed production, as it helps save a considerable amount of time and the need for excessive handling of brood fish.

19. The various stages in initial development observed in *A. testudineus* in the present study, matched well with those described in other teleosts. However, the time of hatching and the beginning of feeding, time of appearance of pectoral fin bud, eye and lens, and body pigmentation, differed with the earlier reports in the species from North India.

20. An aperture of hatching, as reported by some authors, was not observed in the present study. The different modes of hatching like 'head coming first' and 'tail coming first' reported by some earlier workers in different fish species did not match well with the current observations.

21. Onset of hemoglobin synthesis on the second day of hatching, as reported here, is the first such report in the species. The appearance of gill lamellae on the third day post hatching

indicated that the gills became fully functional by the third day. The present study on the life history of *A. testudineus* is expected to enable the fish biologists to identify the larvae of the species and to roughly back calculate the time of spawning and predict the time of hatching in similar conditions.

22. Rearing of *A. testudineus* larvae up to a period of 15 days post hatching was found successful with a diet composed of *Artemia franciscana* nauplii and chicken egg yolk.

# *References*

## REFERENCES


Abraham, M., Blanc, N. and Yashouv, A. 1966. Oogenesis in five species of grey mullets (Teleostei, Mugilidae) from natural and landlocked habitats. *Israel J. Zool.*, **15** (3-4): 155-172.

Acharya, P. 1990. Studies on maturity, spawning and fecundity of *Nemipterus japonicus* (Bloch) off Bombay coast. *J. Indian. Fish. Association,* **20**: 51-57.

Agarwal, S. K., T. K. Banerjee and Mittal, A.K. 1980. A histochemical study of the epidermis of the climbing perch, *A. testudineus* (Anabantidae, Pisces). *Z. Mikrosk. Anat. Forsch,* **94** (1): 143-159.

Ahyaudin, B.A. 1992. Rice farming development in Malaysia: past, present and future. p 69-76 *In*: C. R. dela Cruz., C. Lightfoot., B. A. Costa-Pierce., V. R. Carangal and M.A.P. Bimbao (eds.). Rice-Fish Research and development in Asia. *Iclarm Conf. Proc,* **24**: Manila, Philippines, 457 pp.

Ako H., C. S. Tamaru and C. S. Lee. 1994. Chemical and physical differences in milkfish *(Chanos chanos)* eggs from natural and hormonally induced spawns. *Aquaculture,* **127**: 157-167.

AL- Daham, N. K. and Bhatti, M. N. 1979. Annual changes in the ovarian activity of the fresh water teleost *Barbus luteus* (Heckel) from Southern Iraq. *J. Fish. Biol.,* **14**: 381-387.

Alikunhi, K. H., Vijayalaksman, M. A. and Ibrahim, K. H. 1960. Preliminary observations on the spawning of Indian carps, induced by injection of pituitary hormones. *Ind. J. Fish.,* **7**: 1-7.

Alikunhi, K. H. 1953. Notes on the bionomics, breeding and growth of the murrel, *Ophiocephalus striatus* (Bloch). *Proc. Indian Natl. Sci. Acad.,* **38**: 10-12.

Alikunhi, K. H. 1957. Fish culture in India. *Farm. Bull. Indian Council. Agri. Res.,* New Delhi, **20**: 114 p.

Alok, D., Krishnan, T., Salunke, D., Gupta, H., Talwar, G, P. and Garg, L. C. 1994. Precocious ovarian recrudescence and induced off-season spawning of *Heteropneustes fossilis* by D-Lys$^6$ salmon gonadotropin releasing hormone analog (snRH-A). *J. Fish. Biol*, 45: 909-915.

Anand, J. N. 1973. Experiments on induced breeding of Indian major carps by pituitary hormone injections in Uttar Pradesh. *J. Inland. Fish. Soc. India,* **5**: 37-45.

Anna Mercy, T.V., K. G. Padmanabhan and Pillay, N. K. 1982. Morphological studies of the oocytes of Blind Cat fish *Horaglanis krishnai Menon. Zool. Anz., Jena,* **209** ¾, S. 211-223.

Aravindan, C. M. and K. G. Padmanabhan. 1972. Source of new crop of oocytes in *Tilapia mossambica. Helgolander Wiss. Meeresnuters*, 184-192.

Arocha, F. 2002. Oocyte development and maturity classification of swordfish from the North-Western Atlantic. *J. Fish. Biol.*, **60**: 13-27.

Babiker, M. M. and H. Ibrahim. 1979. Studies on the biology of reproduction in the cichlid *Tilapia nilotica* (L): Gonadal maturation and fecundity. *J. Fish. Biol.*, **14**: 437-448.

Bagarinao, T., Chua, T. E. and Dizon, L. B. 1986. Egg size and larval size among teleosts: Implications to survival potential. Maclean, J. L; Hosillos, L.V. (eds.), *In*: Proceedings of the first Asian fisheries forum, Manila, philippines, 26-31 May 1986. pp. 651-656.

Bagenal, T. B. and Braum, E. 1968. Eggs and early lifehistory. *In:* Methods for assessment of fish production in freshwater. (W. E. Ricker, ed.). Blackwell Scientific, Oxford, IBP Handbook No. 3, pp. 159-181.

Baglin, R. E. Jr. 1982. Reproductive biology of Western Atlantic blue fin tuna. *Fish. Bull.*, 80 p.

Baker, J. R. 1938. The evolution of breeding seasons. *In*: "Evolution," de Beer (Ed.). Plenum Press, New York. pp. 533-567.

Baksi, S. and J. Pal. 1993. Influence of physico-chemical properties of water of North Bengal on the survivability of the hatchlings of the fish *Anabas testudineus* . *Environ. Ecol.,* **11** (1): 96-100

Bakthavalsalam, R. 1986. Effect of lindane and carbofuran on the survival times and total evaporative water loss of the fish *Anabas testudineus* at submerged condition and on exposure to air. *Environ. Ecol.,* **4** (4): 533-535

Balakhnin, I. A. and Savchenko, A. L. 1996. Influence of selection, domestication, and rearing conditions on the anti-B lectin level in the oocytes of cultured fish. *Journal of Ichthyology*, **36** (3): 277-287.

Bandyopadhyay, A. and S. Battacharya. 1994. Purification of putative thyroid hormone receptor from the ovarian nuclei of fresh water perch, *Anabas testudineus*. *Fish. physiol .biochem.,* **13** (5): 387-398.

Banerji, S. R. and D. Prasad. 1974a. Observations on reproduction and survival of *Anabas testudineus* (Bloch) in Bihar region. *J. Inland. Fish. Soc. India,* **6**: 6-17.

Banerji, S. R. and D. Prasad. 1974b. Observations on the spawning behaviour of *Anabas testudineus* (Bloch), *Indian j. Anim Sci.,* **51**(6): 651-54.

Bara, G. 1960. Histological and cytological changes in the ovaries of the mackerel *Scomber scomber* L. during the annual cycle. *Rev. Fac. Sci. Uni.*, **25**: 50-91.

Battacharya, S. and Sen, S. 1981. Role of Thyroxine and Gonadotropins on the Mobilization of Ovarian Cholesterol in a teleost *Anabas testudineus* (Bloch). *Indian. j. exp. biol.,* **19** (5): 408-412

Beams, H. W. and R. G. Kessel. 1973. Oocyte structure and early vitellogenesis in the trout, *Salmo gairdneri. Am. J. Anat.*, **136**: 105-122.

Bengston, B. E. and Larson, A. 1986. Vertebral deformities and physiological effects in fourhorn sculpin (*Myxocephalus quadricornis*) after longterm exposure to a simulated heavy metal containing effluent. *Aquatic toxicol.*, **9**: 215-229.

Besra, S. 1997. Growth and Bioenergetics of *A. testudineus* (Bloch). Narendra Publishing House, New Delhi-06, pp.139.

Bhatt, V. S. 1968. Studies on the biology of some fresh water fishes. Part III *Heteropneustes fossilis* (Bloch). *Indian J. Fish.*, **15**: 99-115.

Bhatt, V. S. 1970. Studies on the biology of some fresh water fishes. Part IV *Mystus seenghala* (Sykes) *J. Bombay. Nat. Hist. Soc.* **67**, 194-211.

Bhatt, V. S. 1971. Studies on the biology of some fresh water fishes. Part VI *Mystus cavassius* (Ham). *Hydrobiologia.* **38**, 289-302.

Bhattacharya, S. and Banerjee, P. P. 1988. Impact of environment on animals and aquaculture. *In:* Natl. Symp. on environmental impacts on animals and aquaculture, Manna, G.K., Jana, B. B (Eds.). pp. 85-90.

Bhatti, M. N. and M. Y. Javaid. 1973. Annual cyclical changes in the ovarian activity of a freshwater teleost, *Colisa fasciatus. Punjab Fish. Bull., Directorate of fisheries,* Punjab, Pakistan, pp. 33.

Bhowmick, R. M., Kotwal, G. V., Jana, R. K. and Gupta, S. K. 1979. Observations on the use of various hormones and clomiphene citrate in hypophysation of Indian major carps. *In:* Symposium on Inland Aquaculture (Abs.), February 12-14, 1979: 10. CIFRI, Barrackpore.

Billard, R., Breton, B., Fostier, A., Jalabert, B. and Weil, C. 1978. Endocrine control of the teleost reproductive cycle and its relation to external factors: Salmonid and cyprinid models. *In*: *Comparative*

*Endocrinology,* P. J. Gaillard and H. H Boer, (Eds.). Elsevier/North Holland Biomedical press, Amsterdam. pp. 37-48.

Binoy, V. V. and Thomas, K. J. 2004. The climbing perch, *Anabas testudineus* (Bloch), a fresh water fish, prefers larger unfamiliar shoals to smaller familiar shoals. *Curr. Sci.*, 86 (1): 207-211.

Bisht, J. S. and M. L. Joshi. 1975. Seasonal histological changes in the ovaries of a mountain stream teleost, *Schizothorax richardsonii* (Gray/Hard); *Acta anat.*, **93**: 512-525.

Blanco-Racedo, J. A. 1983. The condition factor of *Mugil incilis* (Hancock)(Pisces: Mugillidae) and its seasonal changes in the Cienaga Grande of Santa Marta (Colombia). *An. Inst. Invest*, Mar.Punta de Betin, **13**: 133-142.

Blay Jr, J. and Eyeson, K. N. 1982. Observations on the reproductive biology of the shad *Ethmalosa fimbriata* (Browdich), in the coastal waters of Cape coast Ghana. *J. Fish. Biol.*, **21**: 485-496.

Boheman, C. G., Lambert, J. G. D. and Peute, J. 1981. Annual changes in plasma and liver in relation to vitellogenesis in the female rainbow trout. *Salmo gairdneri. Gen. and Comp. Endocrinol.*, **44**: 94-107.

Bone, A. N., B. Marshall and J. H. S. Blaxter, (Eds). 1996. Biology of fishes, Chapman and Hall, London, pp. 171-194.

Booth, A. J. and Weyl, O. L. F. 2000. Histological validation of gonadal macroscopic staging criteria for *Labeo cylindricus* (Pisces: Cyprinidae). *African Zoology*, **35**: 223-231.

Booth, D and R. Alquezar. 2002. Food supplementation increases larval growth, condition and survival of *Acanthochromis polyacanthus. J. Fish Biol.*, **60** (5): 1126-1133.

Breton, B., Jalabert, B. and Fostier, A. 1975. Induction de decharges gonadotropes hypophysaires chez la carp (*Cyprinus carpio* L.) á 1:

aide due citrate de cis clomiphene. *Gen. and Comp. Endocrinology,* **25**: 400-404.

Brusle' J. 1981 a. Sexuality and biology of reproduction in grey mullets, IBP No. 6, Cambridge University. Press, U.K., pp. 99-154.

Bryan, P. G. and Madraisau, B. B. 1977. Larval rearing and development of *Siganus lineatus* (Pisces: Siganidae). *Aquaculture*, **10** (3): 243-252.

Brzuska, E and R. Grzywaczewski. 1999. Artificial spawning of carp *Cyprinus carpio* (L.): differences between the effects on reproduction in females of Israeli strain Dor-70 and its cross-breed treated with carp pituitary and Ovopel. *Aquaculture Research,* **30**: 559-570.

Brzuska, E. and Adamek, J. 1999. Artificial spawning of European Catfish, Silurus glanis L, stimulation of ovulation using LHRH-a, Ovaprim and carp pituitary extract. *Aquaculture Research*, **30**: 59-64.

Brzuska, E. 1999. Artificial spawning of carp (*Cyprinus carpio* L.). Differences between propagation effects in females of Polish and Hungarian provenance treated with ovopel. Lecture on conference of the Commission of Agricultural Sciences. Polish Academy of Sciences. Sept. 29, 1998. University of Opol Komunikaty, Rybackie, I, 14-17.

Bye, V. J. 1990. Temperate marine teleosts. *In:* Reproductive seasonality, in teleosts. Environmental influences. A. D. Munro. A. P. Scott and T. J. Lam (eds.) CRC. Press, Inc., Boca Raton, Florida. pp. 125-143.

Campbell, C. M. and D. R. Idler. 1980. Characterization of an estradiol-induced protein from rainbow trout serum as vitellogenin by the composition and radioimmunological cross reactivity to ovarian yolk fractions. *Biol. Reprod.*, **22**: 605-619.

Chacko, P.I. 1955. Observations on the biology and ecology of the inland water fishes of Madras with special reference to their suitability for culture. Fish. Station Reports & Year book, Dept. of fisheries, Madras.

Chakrabarty, R. and D. S. Murty. 1972. Life history of Indian major carps *Cirrhinus mrigala* (Ham.), *Catla catla* (Ham.) and *Labeo rohita* (Ham.), *J. Inland. Fish. Soc. India*, **4** : 132-161.

Chakraborti, P. and Battacharya, S. 1982. Influence of gonadotropins and gonadal hormones on climbing perch thyroid nucleic acids. E*ndokrinologie*, **80** (2): 213-219.

Chakraborti, P. and Battacharya, S. 1984. Plasma thyroxine levels in freshwater perch: Influence of season, gonadotropins, and gonadal hormones. *Gen. Comp. Endocrinol.*, **53** (2): 179-186.

Chakraborti, P., Maitra, G. and Bhattacharya, S. 1986. Binding of thyroid hormone to isolated ovarian nuclei from a freshwater perch, *Anabas testudineus*. *Gen. comp. endocrinol.*, **62** (2): 239-246

Chakraborti, P., Rakshit, D. K. and Bhattacharya, S. 1983. Influence of season, gonadotropins, and gonadal hormones on the thyroid activity of freshwater perch, *Anabas testudineus* (Bloch*). Can. j. zool.*, **61** (2): 359-364

Chan, E. H. and Chua, T. E. 1980. Reproduction in the green-back grey mullet *Liza subviridis* (valenciennes 1836). *J. Fish. Biol.,* 16 (5): 505-519.

Chanchal, A. K., Pandey, B.N., Singh, S. B. 1978. Studies on some aspects of the biology of *Anabas testudineus*- (Teleostei: Anabantidae). *Matsya,* **4** : 15-19

Chanchal, A.K., Pandey, B.N. 1979. Effect of gonadal steroids on respiratory metabolism of *Anabas testudineus* (Bloch). *Endokrinologie,* **74** (3): 297-303

Chang, J. P. and Peter, R. E. 1983. Effect of pimozide and des-Gly$^{10}$ [D-Ala$^{6}$] luteinizing hormone-releasing hormone ethylamide on serum gonadotropin concentrations, germinal vesicle migration, and

ovulation in female goldfish *Carassius auratus, Gen. Comp. Endocrinol.*, **52**: 30-37.

Chaturvedi, S. K. 1976. Spawning biology of tor mahseer, *Tor tor* (Ham.). *J. Bombay Nat. Hist. Soc.*, **73** (1): 63- 73.

Chaudhury, H. 1960. Experiments on induced spawning of Indian carps with pituitary injections. *Indian J. Fish.*, **7** (1): 20-48.

Chaudhury, H. 1969. Techniques of hypophysation of Indian carps. FAO/UNDP Regional Seminar on induced breeding of cultivated fishes. *Cal. Bom.* Delhi. 15 July-18 Aug.

Chopra, H. C. 1958a. A Morphological and histochemical study of the oocytes of the fish, *Ophiocephalus punctatus*, with particular reference to lipids. *Quart. J. micr. Science,* **99**: 149-158.

Chopra, H. C. 1958 b. Morphological and histochemical study of oocytes in the fish, *Barbus licto* (Puntius), with particular reference to lipids. *Res. Bull. Punjab. Uni.,* **152**: 211-221.

Chopra, H. C. 1960. Cytological and cytochemical study of lipids in the oocytes of the fish, *Boleophthalmus dussumerii. La cellule,* **60**: 303-318.

Choudhury, C., Ray, A. K., Bhattacharya, S. 1993. Non lethal concentrations of pesticide impair ovarian function in the freshwater perch, *Anabas testudineus*. *Environ. Biol. Fish,* **36** (3): 319-324

Choudhury, M., Chandra, R. and Kolekar, V. 1990. Observations on some biological and fishery aspects of *Hilisa ilisha*(Ham) of river Brahmaputra. *J. Inland Fish. Soc. India*, **22**: 66-74.

Chouinard, L.A. 1963. Sites of formation of the extra nucleoli during early oocyte growth in the freshwater teleost, *Salvelinus fontinalis. Canad. J. Zool.*, **41**: 997-1010.

Chubb, C. F. and Potter, I. C. 1984. The reproductive biology and estuarine movements of the gizzard shad *Nematalosa vlaminghi* (Munro). *J. Fish. Biol.*, **25**: 527-543.

Clark, F. N. 1934. Maturity of the California sardine *Sardina caerulea* determined by ova diameter measurements. *Calif. Dep. Fish game Fish. Bull.*, **42**: 1-49.

Clemens, H. P. 1967. A review of selection and breeding in the culture of warm water food fishes in North America. *FAO Fish Rep.*, **4** (44): 67-80.

Conceicao, L., Verreth, J., Scheltema, T. and Machiels, M. 1993. A simulation model for the metabolism of yolk sac larvae of the African catfish, *Clarias gariepinus* (Burchell). *Aquacult. Fish. Manage.*, **24** (3): 431-433.

Cone, R. S. 1989. The need to reconsider the use of condition indices in fishery science. *Trans. Amer. Fish. Soc.*, **118**: 510-514.

Copeman, L. A., Parrish, C. C., Brown, J. A. and Harel, M. 1999. Effect of dietary ratios of DHA, EPA and AA on early growth, survival and pigmentation of yellowtail flounder *Pleuronectes ferrugineus*. *In:* Bull. Aquacult. Assoc., Can Waddy, S. (ed.).

Coward, K. and N. R. Bromage. 1998. Histological classification of oocyte growth and the dynamics of ovarian recrudescence in *Tilapia zilli*. *J. Fish. Biol.*, **53**: 285-302.

Crim, L. W. 1991. Hormonal manipulation of fish reproductive cycles. *In:* Proc. 4$^{th}$ Int. Symp. Reproductive Physiology, of Fish. A. P. Scott., J. P. Sumpter., D. E. Kime, and M.S. Rolfe (Eds). Noewich, U.K., pp. 43-47.

Crim, L. W. and Bettles, S. 1997. Use of GnRH analogues in fish culture. *In:* Recent advances in Department of marine biotechnology. Fingerman,

M., Nagabhushanam, R. and T. Mary-Frances (Eds.). Oxford & IBH Publishing Co. Pvt. Ltd. New Delhi.

Crim, L. W., R. E. Peter and G. Van Der Kraak. 1987. The use of LHRH analogues in aquaculture, *In*: LHRH and its analogues. Contraceptive and therapeutic applications. B.H. Vickery and J. J. Nestor (eds.) MTP Press, Lancaster, England, pp. 489-498.

Crim, L. W. 1985. *In:* Reproduction and culture of Milkfish, C. S. Lee and I. C. Liao (Eds.). Oceanic Institute and Tungkang Marine Laboratory, 22-24: 1-13.

Crupkin, M., Montecchia, C. L. and Trucco, R. E. 1988. Seasonal variation in the gonadosomatic index, liversomatic index and myosin/actin ratio in actomysin of mature hake (*Merluccius hubbsi*). Comp. Biochem. Physiol., **89 A** (1): 7-10.

Cyrus, D. P. and Blaber, S. J. M. 1984. The reproductive biology of Gerres in Natal estuaries, *J. Fish. Biol.*, **24**: 491-503.

Dabrowski, K., Ciereszko, A., Ramseyer, L., Culver, D. and Kestemont, P. 1994. Effects of hormonal treatment on induced spermiation and ovulation in Yellow Perch (*Perca flavenscens) Aquaculture,* **120**: 171-180.

Das, S. M. and H. A. Khan, 1962. The pituitary and pisciculture in India with an account of techniques and literature on the subject. *Ichthyologica,* **I** (1): 43-58.

Das, S. M. 1964. A study on the fecundity of some fresh water fishes of India, with a note on a new concept of comparative fecundity, *Ichthyologica.* (1-2): 33-36

Dasgupta, D. and Sircar, A.K. 1986, Glycogen level of a freshwater fish *Anabas testudineus* in relation to breeding cycle. *Environ. Ecol.*, **4** (1): 79-81

Davis, T. L. and West, G. J. 1993. Maturation, reproductive seasonality, fecundity, and spawning frequency in *Lutjanus vittus* (Quoy and Gaimard) from the north West shelf of Australia. *Fishery Bull.*, **91**: 489-506.

Day, 1889. The fauna of British India, including Ceylon and Burma, Fishes. 1: 548., 2: 509. London, Taylor and Francis.

Day, F. 1865. The fishes of Malabar, London, Quritch; 293 p.

Day, F. 1875-78. The fishes of India, being a nat. hist. of the fishes known to inhabit the seas and fresh waters of India and Ceylon. Text and Atlas in four parts, London XX+778, 195 pls.

De Graf, G., J. Galemoni and Huisman, E. A. 1999. Reproductive biology of pond reared Nile Tilapia, *Oreochromis niloticus* L. *Aquaculture research*, **30**: 25-33.

De Jong, J. K. 1939. A preliminary investigation of the spawning habits of some fishes of Java sea. *Treubia*, **8**: 199-218.

de Leeuw, R., Goos. H. J. Th., Richtyer, C. J. and Eding, E. J. 1985. Pimozide- LHRH induced breeding of the cat fish, *Clarias gariepinus* (Burchell). *Aquaculture*, **44**: 295-302.

De Vlaming, V. L. 1972. Reproductive cycling in the estuarine gobiid fish, *Gillichthys mirabilis. Copea,* **2**: 278-291.

de Vlaming, V. L. 1975. Effects of pinealectomy on gonadal activity in the cyprinid teleost, *Notemigonus crysoleucas. Gen. Comp. Endocrinol.*, **26**: 36-49.

De Vlaming. V. L., Grossman. G. and Chapman, F. 1982. On the use of gonadosomatic index. *Comp. Biochem. Physiol.*, **73** (A): 31-39.

De, P. K. 1980. Maturity, fecundity and spawning of post-monsoon run of *Hilsa ilisha* (Hamilton) in the upper stretches of the Hooghli estuarine system. *J. Inland Fish. Soc. India.*, **12**: 54-63.

Deb, S. and Bhattacharya, S. 1988. Mechanism involved in gonadotropin stimulation of ovarian steroidogenesis. *Indian j. biochem. biophys.*, **25** (4): 344-349

Degani, G. and R. Boker. 1992 a. Sensitivity to maturation-inducing steroids and gonadotropin in the oocytes of blue gourami *Trichogaster trichopterus*, (Anabantidae, Pallas, 1770). *Gen. Comp. Endocrinol.*, **85**: 430-439.

Degani, G., R. Boker., Gal, E. and K. Jackson. 1997. Oogenesis and steroid profiles during the reproductive cycle of female angelfish *Pterophyllum scalare* (Cichlidae).

Dehadrai, P.V., Thakur, N.K. and Das, S. K. 1973b. Sexual dimorphism in certain air-breathing teleosts. *J. Biol.*, **5**: 71-77.

Delahunty, G. and de Vlaming, V. L. 1980a. Seasonal relationship of ovary weight, liver weight and fat stores with body weight in the gold fish, *Carassius auratus* (L.). *J. Fish. Biol.*, 16 (1): 5-13.

Delahunty, G. and De Vlaming, V. L. 1980b. Photoperiod, temperature interaction on liver and plasma metabolites in the gold fish, *Carassius auratus*. *Comp. Biochem. Physiol.*, 66A; 507-512.

Devaraj, M. 1973. Biology of the large snake-head *Ophiocephalus marulius* (Ham.) in Bhavanisagar waters. *Indian J. Fish.*, **20**: 280-303.

Dhanya, S., S. Rajagopal., Khan. A. and T. Balasubramanian. 2005. Embryonic development in alligator pipefish, *Syngnathoides biaculeatus* (Bloch, 1785). *Current. Sci.*, **88** (1): 178-181.

Dodd, J. M. and P. J. Sumpter, 1984. Fishes . *In:* "Marshall's Physiology of reproduction" (G.E. Lamming, ed.), 4th ed. Volume I, Churchill-Living-stone, Edinburgh and London. pp. 1-126.

Dodd, J. M., 1977. The structure of the ovary of non-mammalian vertebrates. *In*: 'The ovary' (L. Zuckermanand B. J. Weir. Eds.), 2nd Volume. Academic press, New York, pp. 219-263.

Donaldson, E. M., Hunter, G. A. and Dye, H. M. 1981. Induced ovulation in cobo salmon (*Oncorhyncus kitsutch*). Preliminary study on the use of LH-RH and two high potency LH-RH analogues. *Aquaculture*, **26**: 129-141.

Donaldson, E. M. and Hunter, G. A., Dye, H. M. and Van der Kraak, G. 1982. Induced ovulation in pacific salmon using LH-RH analogues and salmon gonadotropin. *In*: Proceedings of the ninth International symposium on comparative endocrinology, B Loffs (Ed.). Univ. of Hong Kong.

Donaldson, E. M. and Hunter, G. A. 1983. Induced final maturation, ovulation and spermiation in cultured fish. *In:* Fish Physiology, Reproduction. Volume. IX B (Hoar, W. S., Randall, D. J. and Donaldson, E. M., eds.), pp 351-403. Academic Press. New York.

Doolgindachabaporn, S. 1994. Breeding of climbing perch, *A. testudineus* (Bloch). Master Degree Thesis. Kasetsart University, Bangkok, Thailand. pp. 64.

Driever, W., D. Stemple, Schier, A. and A. Solnica-Krezel. 1994. Zebrafish, genetic tool for studying vertebrate development. *J. Clin. Invest.*, **97**: 1788-1794.

Driever, W. and 11 others. 1996. A genetic screen for mutations affecting development in Zebrafish. *Development*, **123**: 37-46

Drori. S., M. Ofir., Levavi Sivan, and Z. Yaron. 1994. Spawning induction in common carp (Cyrinus carpio) using pituitary extract on GnRH superactive analogue combined with metaclopramide: Analysis of hormone profile, progress of oocyte maturation and dependence on temperature. *Aquaculture*, **119**, 393-407.

Dutt, N. H. G. and P. Govindan. 1967. The localization of lipids during oogenesis in *Anabas scandens* (Cuvier). *Z. mikr. Anat. Forsch..***76**: 390-403.

Dutt, N. H. G. and P. Govindan. 1969. The localization of polysaccharides, -SH and S-S groups in the oocytes of *Anabas scandens*. *Z. Mikr. Anat. Forsch.*, **80**: 399-418.

Dutt, N. H. G. and P. Govindan. 1975. Histology of the ovary of *Anabas scandens*. *J. Anim. Morphol. Physiol.*, **22**: 159-173.

Dutt, S., Ramaseshaiah, M. 1980. Chromosome number in *Anabas testudineus* (Bloch, 1795) and *A. oligolepis* (Bleeker, 1855) (Osteichthyes: Anabantidae). *Matsya,* **6:** 71-74

El Maghraby, A. M., Hashem, M. T. and El- Sedfy, H. M. 1974. Sexual maturity, spawning migration and fecundity of *Mugil capito* in lake Borullus. *Bull. Inst. Ocanogr. Fish.*, **4**: 35-36.

Elorduy, J. F. and S. Ramirez-Luna. 1994. Gonadal development and spawning of female ocean whitefish, *Caulolatilus princeps* (Pisces: Branchiostegidae) in the Bay of La Paz, B. C. S., *Mexico. J. Fish. Biol.*, **44**, 567-590.

Erickson, D. L., Hightower, J. E. and Grossman, G. D. 1985. The relative gonadal index: An alternative index for quantification of reproductive condition. *Comp. Biochem. Physiol.,* **81** (1): 117-120.

Fermin, J. D.T., Marte, C. L., Ueda, H., Adachi, S. and Yamauchi, K. 1999. Effect of season on oocyte development and steroid hormones in LHRHa and Pimozide- injected catfish *Clarias macrocephalus* (Gűnther). *Fisheries Science*, **65** (6): 865-870.

Fishelson, L. 1977. Ultrastructure of the epithelium from the ovarian wall of *Dendrochirus brachypterus. Cell. Tissue. Res.*, **177**: 375-381

Fontaine, Y.A., Salmon, C., Fontaine-Bertrand, E., Burzawa-Gerard, E. and Donaldson, E. M. 1972. Comparison of the activities of two purified fish gonadotropins on adenyl cyclase activity in the goldfish ovary. *Canad. J. Zool.* **50**: 1673-1676.

Forberg, K. G. 1982. A histological study of development of oocyte in capelin *Mallotus villosus villosus* (Müller). *J. Fish. Biol.*, **20**: 143-154.

Foucher, R. P. and R. J. Beamish. 1980. Production of nonviable oocytes by pacific hake (*Merluccius productus*). *Can. J. Fish. Aqua*tic. Sci., **37**: 41-48.

Fowler, A. J., McLeay, L. and Short, D. A. 1999. Reproductive mode and spawning information based on gonad analysis for the king George whiting (Percoidei: Sillaginidae) from South Australia. *Marine and fresh water research*, **50**: 1-14.

Fugita, T., W. Hamaura., Takemura, A. and K. Takano. 1997. Histological observations of annual reproductive cycle and tidal spawning rhythm in the female Porcupine fish *Diodon holocanthus, Fisheries Science*, **63**: (5) 715-720.

Geevarghese, C. and John, P. A. 1982. Maturation and spawning of a gobiid fish, *Oligolepis acutipennis* (cuv.and Val.), from the southwest coast of India. *J. Fish. Biol.*, **23**: 611-624.

Gilbert, S. F. 2003. Developmental biology. VII Edn, Sinauer Associates, Inc. Sunderland, Massachussettes.

Giridhar, S. L. and Rao, H. K. 1976. The effect of copper sulphate on the breeding of *Labeo fimbriatus. Matsya,* **2**: 83-84

Goetz, F. W. 1983. Hormonal control of oocyte final maturation and ovulation in fishes. *In:* W. S. Hoar, D. J. Randall and E. M. Donaldson (Eds.), Fish physiology: Reproduction. Academic Press, New York, IX B: 117-170.

Goldberg, S. R. and H. Herring Dayal. 19981. Histological gonad analysis of late summer-early winter collections of big eye tuna, *Thunnus besus*, and yellow tuna, *Thunnus albacores*, from the northwest Atlantic and the Gulf of Mexico. *Noa Tech. Memo. La-Jolla-Ca*-USA-Noaa-Nmfs. 11p.

Goos, H. J. Th., Joy, K. P., de Leeuw, R., van Ordt, P.G., W. J. van., Delft, A. M. L. and Gielen, J. Th. 1987. The effect of luteinizing hormone-releasing hormone analogue (LHRHa) in combination with different drugs with anti dopamine and anti-serotonin properties on gonadotropin release and ovulation in the African catfish, *Clarias gariepinus, Aquaculture,* **63**: 143-156.

Gopalakrishnan, A. 1991. Studies on reproductive physiology of female grey mullet, *Mugil cephalus*. Ph. D. thesis, CUSAT , Kochi.

Gopi, K. C. 2000. Fresh water fishes of Kerala state. 56-76, *In:* Ponniah, A. G, and Gopalakrishnan, A. (Eds),. Endemic fish diversity of Western Ghats,. NBFGR-NATP Publication-1, 347p. National Bureau of Fish genetic resources, Lucknow, U.P., India.

Greenblatt, R.V., Barfield, W. E., Jungk, E. C. and Ray, A. W. 1961. Induced ovulation with MR/L/41 (Clomiphene citrate). *J. Am. Med. Assn.,* **178**: 101-105.

Gregor, H. C. 1972. Nucleolus and its genes in oogenesis. *Biol. Rev.,* **47**: 177-219.

Grimes, B. and Huntsman. 1980. Reproductive biology of the vermillon snapper, *Rhomboplites aurorubens* from North Carolina and South Carolina. *Fish. Bull.*, **79**: 137-146.

Guin, S., Bandyopadhyay, A., Jana, N.R., Bhattacharya, S. 1993. Thyroid hormone stimulates progesterone release from the ovary of a fish, *Anabas testudineus* . *Curr. sci.*, **64** (5): 327-329.

Gupta, S. S. and B. A. Subla. 1978. Embryonic and larval developments of *Schizothorax curvifrons* (Heckel) as observed in the laboratory. *Ind. J. Fish.*, 101-111.

Gupta, S. C. and Sharma, K. K. 1991. Biology of Indian Belone, Xenentodon cancila (Ham.). *In*: Advances in fish and wild life ecology, Bansilal Kaul (Ed.), pp. 159-186.

Gupta, S. 1975. The development of carp gonads in warm water aquaria. *J. Fish. Biol.*, 7: 775-782.

Guraya, S. S. and Kaur, S. 1979. Morphology of the post ovulatory follicle (or corpus luteum) of teleost (*Cyprinus carpio* L.) ovary. *Zool. Beitr.*, **25** (1): 403-410.

Guraya, S. S. 1979. Recent advances in the morphology, cytochemistry and function of Balbiani's vitelline body in animal oocytes. *Inter. Rev. Cytol.*, **59**: 249-321.

Guraya, S. S. 1986. The cell and molecular biology of fish oogenesis, S. Karger Publi., Basel and New York. pp. 223.

Haffter, P. and 16 others. 1996. The identification of genes with unique and essential functions in the development of the Zebra fish, *Danio rerio. Development*, **123**: 1-36.

Hails, A.J. and Abdullah, Z. 1982. Reproductive biology of the tropical fish *Trichogaster pectoralis* (Regan). *J. Fish. Biol.*, **21**: 157-170.

Halder, S., Sen, S., Bhattacharya, S., Ray, A.K., Ghosh, A. and V. G. Jhingran. 1991. Induced spawning of Indian major carps and maturation of a perch and a catfish by murrel gonadotropin releasing hormone, pimozide and calcium. *Aquaculture*, **97** (4): 373-382

Haniffa, M. A., Raj, A. J. A. and Sridhar, S. 1999. Weaning diet for striped murrel *Channa striatus*. *Fish. Technol. Soc. Fish. Technol.*, (India). **36** (2): 116-119.

Hartridge, H. 1936. Essentials of Physiology (Ed. H. Hartridge), London.

Henderson-Arzapalo, A. and Colura, R. L. 1987. *Prog. Fish. Cult.*, **49**:60-63.

Hickling, C. F. and Rutenberg, E. 1936. The ovary as an indicator of spawning period of fishes *J. Mar. biol. Ass.*, U. K. **21**: 311-317.

Hile, R. 1936. Age and growth of the Circo, *Leucichthys artedi* (Le Sueur) in the lakes of northeastern highlands, Wisconsin. *Bull. U. S. Bur. Fish.*, **48**: 211-317.

Hjort, J. 1910. Report on herring investigation until January 1910. *Publ. Circ. Cons. Explor. Mer.*, **53**: 1-36.

Hoar, W.S. 1969. Reproduction. *In*: " Fish physiology'' (W.S Hoar and D. J. Randall, eds.). **3**: 1-72, Academic Press, New York.

Hora S.L. and Pillay, T .V. R. 1962. Handbook on fish culture in the Indo-Pacific region. FAO *Fish. Biol. Tech. Pap.*, **14**: pp. 240.

Hora, S. L. 1938. A preliminary note on the spawning grounds and bionomics of the so-called Indian shad, *Hilisa ilisha* (Ham.) in the Indian River Ganges. *Rec. Ind. Mus.*, **40**: 147p.

Horváth, L., Szabó, T. and Burke, J. 1997. Hatchery testing of GnRH analogue-containing pellets on ovulation in four cyprinid species. *Polskie Archivum Hydrobiologii*, **44**: 221-226.

Htun-Han, M. 1977. ' The effects of photoperiod on reproduction of fishes-An annotated bibliography". *Mines Agric. Fish Food*, Directorate of fisheries research, Lebr. Inf. Leafl. No. 6, Lowestoft, U. K., pp. 1-30.

Hoff, F. H., Mountain, J., Frakes, T. and Halcott, K. 1978. Spawning, oocyte development and larvae rearing of the Florida pompano *Trachinotus carolinus* . *In*: Proc. Annu. Meet. World Maricult. Soc. Georgia, Avault, J.W., Jr. (ed.). January 3-6, 1978., pp. 279-297

Hogendoorn, H. 1979. Controlled Propagation of the African catfish, Clarias lazera (C. and V.). Reproductive biology and field experiments. *Aquaculture*, **17**: 323-333.

Holt, G. J. 1998. Larval fish feeding and nutrition: Status and concerns. Aquaculture '98 Book of Abstracts. pp. 248-249.

Htun-Han, M. 1978a. The reproductive biology of the dab *Limanda limanda* (L.) in the North Sea: Seasonal changes in the ovary. *J.Fish. Biol.*, **11**: 293-308.

Htun-Han, M. 1978b. The reproductive biology of the dab *Limanda limanda* (L.) in the North sea: gonosomatic index, hepatosomatic index and condition factor. *J. Fish. Biol.*, **13**, 369-378.

Hubbard, J. W. 1894. The yolk nucleus in *Cymatogaster aggregatus. Proc. Am. Phil. Soc.*, 33: 74-83.

Hughes, G. M. and J. S. Datta Munshi. 1973. Fine structure of the respiratory organs of the climbing perch *Anabas testudineus* (Pisces: Anabantidae). *J. Zool., London*, **170**: 201-205.

Hughes, M., J. S. Datta-Munshi. and Ojha, J. 1986. Post-embryonic development of water and air-breathing organs of *Anabas testudineus* (Bloch), *J. fish. biol.*, **29** (4): 443-450

Hunter, J. R. and B. J. Macevicz. 1985. Rates of atresia in the ovary of captive and wild northern anchovy, *Engraulis mordax. Fishery Bulletin,* **83**: 119-136.

Hunter, J. R., B. J. Macevicz and Sibert, J. R. 1986. The spawning frequency of skipjack tuna *Katsuwonus pelamis*, from the south Pacific. *Fishery Bull.*, **84**: 895-903.

Idler, D. R. and I. Bitners. 1960. Biochemical studies on sockeye salmon during spawning migration. IX Fat, protein and water in the major internal organs and cholesterol in the liver and gonads of the standard fish. *J. Fish. Res. Bd. Canada*, **17**: 113-122.

Iwamatsu, T., Ohta, T., Shima, E. and Sakai, N. 1988. oogenesis in the medaka *Orizias latipes*. Stages in oocyte development. *Zool. Sci.*, **5**: 353-373.

Jaba, M., S. Bhattacharya and Panchanan Nath. 1989. Annual changes in ovary and vitellogenin content of liver, serum and ovary of murrel *Channa punctatus* (Bloch). *Indian J. Exp. Biol.*, **27**: 764-769.

Jacobson, A., Marshall, J. R., Ross, G. T. and Gargille, C. M. 1968. Plasma gonadotropins during clomiphene induced ovulatory cycles. *Am. J. Obstet. Gynec.*, **102** (2): 284-290.

Jalabert, B. 1976. *In vitro* oocyte maturation and ovulation in rainbow trout (*Salmo gairdneri*), northern pike (*Esox lucius*) and gold fish (*Carassius auratus*), *J. Fish Res. Board. Can.*, **33**: 974-988.

James, P. S. B. R. and V. M. Baragi. 1980. Ovary as an indicator of frequency of spawning in fishes. Summer school on Fishery Biology (H. Y. Mohan Ram, Ed.). *Indian natn. Sci. Acad.*, pp. 149-159.

Jana, N.R. and Bhattacharya, S. 1993. Binding of thyroid hormone to freshwater perch Leydig cell nuclei-rich preparation and its functional relevance *zool. sci.*, **10** (3): 489-496.

Jana, B. B. and Chakrabarti, R. 1990. Exogenous introduction of live plankton-a better approach to carp growth than the direct manured system. *Prog. Fish Cult.*, **52**: 252-260.

Janssen, M. E. 1993. Induction of ovulation in Arctic char held under unchanging temperature and photoperiod. *Prog. Fish Cult.*, **55**, 32-34.

Jayaprakash, V. and B. S. Sindhu. 1996. Effect of hormones on the growth and food utilization of the Indian major carp, *Cirrhinus mrigala. Fishery technology*, **33**(1): 21-29.

Jayaram, K. C. 1999. The fresh water fishes of the Indian region, *Narendra publishing house,* Delhi. **27** : p. 551,18pls.

Jayasankar, P. and K. Alagar swamy. 1994. Histological and Histochemical observations on the oocytes in the sand whiting *Sillago sihama* (Forsskal). *Proc. Indian. Natn. Sci. Acad.*, B **60** (2): 173-182.

Jha, K. K. 1993. Effect of water from different sources on the survival of hatchlings of certain Indian teleosts. *J. freshwat. biol.*, **5** (2): 153-157.

Jhingran, V. G., Natarajan, A V., Banerjee, S. M. and David. A. 1969. *Bull. Cent. Inland Fish. Res. Institute*, Barrackpore, 12.

Jhingran, V. G., 1991. Fish and fisheries of India, Hindustan Publishing Corporation (India), Delhi. pp. 728.

Jones, R. E., 1978. 'The vertebrate ovary: Comparative Biology and Evolution'. Plenum press, New York.

Jordan, D. S. 1982. Relations of temperature to vertebrae among fishes. *In:* Proceedings of United States National Museum, **40**: 154-155.

Jose, T.M. and Sathyanesan, A.G. 1980. Pituitary cytology of the freshwater teleost, *Anabas testudineus* (Bloch). *Z. Mikrosk. Anat. Forsch. Leipzig*, **94**: 123-132.

Joshi, S. N. and Khanna, S. S., 1980. Relative fecundity of *Labeo gonius* (Ham.) from Nanak Sagar Reservoir, *Proc. Indian Acad. Sci. (Anim. Sci.)*, **89** (5): 493-503.

June, F. C. 1953. Spawning of yellowfin tuna in Hawaiian waters. United States Fish and Wild life service, *Fishery Bull.*, **54**: 47-64.

Jyothi, M. K. 1973. Studies on feeding and gonadal cycles in some fishes of J&K state. Ph.D. thesis. University of Jammu, Jammu (J&K).

Kagawa, H., Tanaka, H., H. Ohta and K. Hirose. 1995. Changes in fertilization and hatching rates with time after ovulation induced by 17, 20 β-dihydroxy-4-pregnen-3-one in the Japanese eel, *Anguilla japonica. Aquaculture*, **139**: 291-301.

Kaippallil, J. D. and Oommen, O.V. 1994. Influence of estrogen on intermediary metabolism in a teleost, *Anabas testudineus* and the lizard *Calotes versicolor j. exp. zool.*, **270** (5): 467-473

Kamisaka, Y., Tagava, M. and Tanaka, M. 1999. Semi-annual Reproductive cycle of a small Flounder *Tarphops oligolepis* in Wakasa Bay. *Fisheries Science*, **65**: 98-103.

Kamler, E. 1992. Early life history of fish- An energetics approach, Chapman & Hall, London, New York, Tokyo.

Kapoor, K. and Toor, H. S. 1978. The effect of clomiphene citrate on the steroidogenesis in the ovary and testis of gonadotropin-deprived freshwater teleost, *Cyprinus carpio*. 5th Int. cong. Horm. Steroids, N. Delhi.

Karekar, P. S. and Bal, D. V. 1960. A study of the maturity and spawning of *Polydactylus indicus* (Shaw). *Indian J. Fish.*, **7**: 147-165.

Kase, N. 1973. Induction of ovulation with clomiphene citrate. *Clin. Obst. Gyn.*, **16**: 192-200.

Kaur, D. and M. D. Srivastava. 1965. The structure and behaviour of chromosomes in fresh water teleosts. *Caryologica*, **18**: 181-191.

Kenawy, S. M. 1993. Performance of natural food organisms and tested diets in fish nursing. *J. Inland. Fish. Soc. India*, **25** (1): 39-43.

Kerrigan, B. A. 1997. Variability in larval development of the tropical reef fish *Pomacentrus amboinensis* (Pomacentridae): The parental legacy, *J. Mar. Biol.*, **127** (3): 395-402.

Khan, H. 1938. Ovulation in fish. (Effect of administration of anterior lobe of pituitary gland). *Curr. Sci.*, **7** (5): 233-234.

Khan, H. A. 1972a. Induced breeding of air-breathing fishes, *Indian Fmg.*, **22** (4): 44-45

Khan, H. A. and S.K. Mukhopadhyay. 1972b. On the fecundity of climbing perch, *A. testudineus* (Bloch), *J. Inland. Fish. Soc. India*, **4**: 212-13

Khan, H. A., B. B. Ghosh and Mukhopadhyay, S.K. 1976. Observations on the salinity tolerance of *Anabas testudineus* (Bloch). (Central. Inl. Fish. Res. Inst., Barrackpore, West-Bengal, India. *J. Inland. Fish. Soc. India,* **8** : 111-112

Khanna, D. V. 1958. Observation on the spawning of major carps at a fish farm in the Punjab. *Indian. J. Fish.*, **5** : 282-290.

Khanna, S. S. and R. Sanwal. 1971. Cyclic changes in the ovary of a freshwater teleost *Channa gachua. Zool. Beit.*, **17**: 311-326.

Khanna, S. S. and Sanwal, R. 1972. Influence of long and short photoperiod on the maturation of ovary in a fresh water teleost *Channa gachua. Zool., Beit.*, **83**: 71-88.

Kharbuli, S., S. Dey., Nongkynrih, J. and U. C. Goswami. 2004. Ovaprim induced spawning and subsequent development in an Aquarium fish, *Danio aequipinnatus. J. Inland. Fish. Soc. India,* **36** (1): 58-62.

Khoo, K. H. 1975. The corpus luteum of gold fish (*Carassius auratus* L.) and its functions, *Can. J. Zool.*, **53**: 1306-1323. N'da and Deneil, 1993.

Kime, D. 1993. Classical and non-classical reproductive steroids in fish. *Rev. Fish. Biol. Fish.*, **3**:160-180.

Klodzinńska, H. and Okoniewski, Z. 1998. Ovopel- new preparation for stimulation of fish reproduction, Hatchery, 1997-98 Eddition Institute of Inland Fisheries, Olsztyn, pp. 45-49.

Kobayashi, M., K. Aida and R. van der Hurk. 1988. Hormone change during the ovulation cycle in gold fish . *Gen. Comp. Endocrinol.*, **69**: 301-307.

Koya, Y., T. Hamatsu. and Matsubara, T. 1995. Annual reproductive cycle and spawning characteristics of female Kichiji rock fish *Sebastolobus macrochir. Fisheries science*, **61**: 203-208.

Kucharczyk, D., A. Mamcarz., Kujawa, R and A. Skrzypczak. 1998. Development of cannibalism in larval northern pike, *Esox lucius* (Esocidae). *Ital. J. Zool.*, **65**: 261-263.

Kulkarni, C. V. 1970. Spawning habits, eggs and early development of the Deccan mahaseer, *Tor khudree* (Sykes). *J. Bombay Nat. Hist. Soc.*, **67**: 510-521.

Kurup, B. M., 1994. Maturation and spawning of an indigenous carp *Labeo dussumieri* (Val.) in the river Pamba. *J. Aqua. Trop.*, **9**: 119-132.

Kurup, B. M. 2002. Rivers and streams of Kerala part of Western Ghats- hot spots of exceptional fish biodiversity and endemism: *In*: riverine and

reservoir fisheries of India. (Eds. Bhoopendranath, M. R., Meenakumari, B., Joseph, J., Sankar, T.V., Pravin, P. and Edwin, L.). *Society of Fishery Technologists* (India), Cochin, pp. 204-217.

Lal, B., T. P. Singh., H. N. Singh and S. Harikrishnan, 1995. Regulation of hydromineral balance and spermiation by mGtH and prolactin in freshwater catfish, *Heteropneustes fossilis. In:*, *The Proceedings of the Fifth International Symposium on the Reproductive Physiology of Fish.* (F.W.Goetz and P.Thomas., Eds.) The University of Texas, Austin, U.S.A., pp. 368.

Lam, T. J., Nagahama, Y., Chan, K. and Hoar, W. S. 1978. Over-ripe eggs and post-ovulatory corpora lutea in the three-spined stickleback *Gasterosteus aculeatus* L., *Canadian J. Zool.*, **56**: 2029-2036.

Lam, T. J., Lavens, P., Sorgeloos, P., Jaspers, E. and Olevier, F. 1991. Hormones and egg/larval quality in fish. LARVI,91., Spl. Pub., Europ. Aquacult. Soc., 15: p. 197.

Lambert, J. G. D. 1970a. The ovary of the Guppy, *Poecilia reticulata.* The atretic follicles, a corpus atreticum, or a corpus luteum preovultionis. *Z. Zell. Forsch. Mikrosk. Anat.*, **107**: 54-67.

Lambert, J. G. D. 1970b. The ovary of the Guppy, *Poecilia reticulata.* The granulosa cells as sites of steroid biosynthesis. *Gen. and Comp. Endocrinol.*, 15: 464-476.

Langer, S., S. Khauria and T. Kour. 2002. Growth responses in Rohu *Labeo rohita* larvae, a function of prey type. *J. Inland. Fish. Soc. India*, **34** (1): 35-38.

Larson, G. L. 1974. Liver weight of brook trout in a high- mountain lake in Washington state. *Progve. Fish. Cult.*, **35**, 234-236.

Le Cren, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*), *J. Anim. Ecol.*, **20** (1): 201-219.

Le Menn, F., B. Davail., C. Pelissero., P. Ndiaye., E. Bon., L. Perazzolo and Nunez Rodriguez, J. 2000. A new approach to fish oocyte vitellogenesis. *In:* Proceedings of the 6th International symposium on the reproductive physiology of fish. (Norberg, B., Kjesbu, O. S., Taranger, G. L., Anderson, E. and Stefanson S. O. (Eds). Inst.Mar. Res. & Univ. Bergen, Norway, pp. 499.

Lehri, G. K. 1968. Cyclical changes in the ovary of the cat fish *Clarias batrachus* (Linn.). *Acta. anat.*, **69**, 105-124.

Lin, H., Peng. C., Lu. L., Zhou, X., Van Der Kraak, G. and Peter, R. E. 1985. *Aquaculture*, **46**: 333-340.

Lin, H. R. and R. E. Peter. 1996. Hormones and spawning in fish. *Asian fish. sci.*, **9** (1): 21-33

Liu, C. K. 1963. Induced spawning of Chinese carps by injection of fish pituitary hormone combined with synahorin. *China Fish. Mon.*, **231:** 1-3.

Livni, N. 1971. Ovarian histochemistry of the fishes *Cyprinus carpio and Tilapia aurea* (Teleostei). *Histochem. J.*, **3**: 405-414.

Lubzens, E. S., Minkoff, G., Meragelman, G. and Schneller, A. 1984. Rotifers (*Brachionus plicatilis*) improve growth of carp (*Cyprinus carpio*) larvae, *Bamidgeh,* **36**: 41-46.

Luna, L. G. 1968. Manual of histologic staining methods of the Armed Forces Institute of Pathology. 3rd edn. New York: McGraw-Hill.

Luther, G. 1963. Some observations on the biology of *Liza macrolepis* (Smith) and *Mugil cephalus* L. with notes on the fishery of grey mullets near Mandapam. *Indian J. Fish.*, **10** (2A): 642-666.

Maddock, D. M. and M. P. M. Burton. 1999. Gross and histological observations of ovarian development and related condition changes in American Plaice. *J. Fish. Biol.*, **53**: 928-944.

Malhotra, Y. R. 1965. Studies on the ovarian cycle of Kashmir fishes, *Schizothorax niger* (Heckel) and *Botia birdi* (Chaudhury) with observations of corpus luteum in fishes. Ph.D thesis. Univ. of Kashmir.

Malhotra, Y. R. 1966. Breeding of some fishes of Kashmir valley. *Ichthyologica*, **5**: 53-58.

Manickam, P. and K.P. Joy. 1989. Induction of maturation and ovulation by pimozide-LHRH analogue treatment, and resulting high quality egg production in the Asian catfish, *Clarias batrachus*, (L.). *Aquaculture*, **83**: 193-199.

Marcus, O. and Kusemiju, K. 1984. Some aspects of the reproductive biology of the clupeid *Ilisha africana* (Bloch) off the Lagos coast, Nigeria. *J. Fish. Biol.*, **25**: 679-689.

Martini, E. E. and Lau, B. B. 1999. Morphometric criteria for estimating sexual maturity in two snappers, *Etellis carbunculus* and *Pristipomoides sieboldii*. *Fish. Bull.*, **97**: 449-458.

Marx, K. K. and S. S. Kumar, 2004. Induced breeding of striped murrel, *Channa striatus* (Bloch). *J. Inland. Fish. Soc. India*, **36** (1): 75-77.

May, R. C. 1974. Larval mortality in marine fishes and the critical period concept. The early life history of fish, *Springer verlag*, Berlin.

Mayer, I., Shackley, S. E. and Ryland, J. S. 1988. Aspects of the reproductive biology of the bass, *Dicentrarchus labrax* L. I. A histological and histochemical study of oocyte development. *J. Fish. Biol.*, **33**, 609-622.

Medford, B. A. and W. C. Mackay, 1978. Protein and lipid content of the gonads, liver and muscle of northern pike *Esox lucius* in relation to gonad growth. *J. Fish. Res. Bd. Canada*, **35**: 213-219.

Meenakaran, S. 1986. Induced spawning of *Pangasius pangasius* (Ham.) carried out in South Sumatra, Indonesia. Manual of small-scale

fisheries development project of the directorate General of fisheries, Indonesia, pp. 1-13.

Mein, V. A. 1927. Observations on the yearly variations in the ovaries of the perch (*Perca fluviatilis* L.), *Russk. Zool. Zh.* 7: p. 4.

Menon, A. G. K. 1974. A stocklist of fishes of the Himalayan and Indo-Gangetic plains. Special publication No. I, *Inland Fisheries Society of India,* CIFRI, Barrackpore, 138p.

Merson, R. R., C. S. Casey., C. Martinez., B. Soffientino., M. Chandlee. and J. L. Specker. 2000. Oocyte development in summer flounder: Seasonal changes and steroid correlates. *J. Fish. Biol.*, **57**: 182-196.

Merve, J. H. J., Vuren, V. and Vermaak, J. F. 1988. Cyclic histomorphological changes in the ovary of mudfish, *Labeo capensis, Aquaculture*, **72**: 349-358.

Meske, C. 1984. Initial feeding of tropical fish. *Inf. fischwirtsch.*, **31**(2): 102-104.

Micale, V., G. Maricchiolo and L. Genovese. 1999. The reproductive biology of the amberjack, *Seriola dumerilii* (Risso 1810). Oocyte development in captivity. *Aquaculture Research,* **30**: 349-355.

Mishra, A.P. and Singh, B.R. 1979. Oxygen uptake through water during early life of *Anabas testudineus* (Bloch). *Hydrobiologia*, **66** (2): 129-133

Misra, A. B. and J. S. Datta Munshi. 1958. On the accessory respiratory organs of *Anabas testudineus*. 15th International congress of Zoology, London (Abstr.): No. 32.

Misra, K. S. 1959. An aid to the identification of the common commercial fishes of India and Pakistan. *Rec. Indian. Mus.*, **57** (1-4): 1-320

Moitra, A. Pandey, A. Ghosh, T. K. and Munshi, J. S. D. 1979. Spawning behaviour, post embryonic development and culture of *Anabas*

*testudineus* (Bloch). *In:* symposium on Inland aquaculture held at CIFRI, Barrackpore, West Bengal, Abstract No. 3: 2-3.

Moitra, S. K. and S. K. Sarkar. 1975. A new method of induced breeding by hypophysation of some major carps in dry bundhs of Bankura, West Bengal. *Proc. Zool. Soc.* Calcutta, **28**: 41-50.

Moitra, S. K. and S. K. Sarkar. 1976a. The morphohistology of the pituitary glands in two fresh water major carps of India, *Labeo rohita* (Ham.) and *Cirrhinus mrigala* (Ham.). *Anat. Anz.*, **139**: 424-441.

Moitra, S. K. and S. K. Sarkar. 1976b. Seasonal variations on the histology of the pituitary gland of *Cirrhinus mrigala* (Ham.), an Indian fresh water major carp, in relation to gonadal activity. *Z. mikrosk. anat. Forsch. Leipzig*, **90**: 154-174.

Moitra, S. K. and S. K. Sarkar. 1977. Seasonal changes in the histology of the pituitary gland of *Labeo rohita* (Ham.) an Indian fresh water major carp, in relation to its spawning behaviour. Gageubaurs. *Morph. Jahrb. Leipzig,* **123**: 84-102.

Moitra, S. K. and S. K. Sarkar. 1978. On the potency of the pituitary gland extract in relation to spawning in an Indian fresh water major carp *Cirrhinus mrigala* (Ham.). *Zool. Anz. Jena,* **200** (3-4): 275-282.

Mollah, M. F. A. 1986. Cyclic changes in the ovary of fresh water cat fish *Clarias macrocephalus* (Gunther). *Indian J. Fish.*, **33** (1): 54-65.

Mookerjee, H. K., and S. R. Mazumdar. 1946. On the life history, breeding and rearing of *Anabas testudineus* (Bloch). *J. Dep. Sc. Cal. Univ.*, **2**: 101-140.

Moteki, M., Yoshida, K., Sahin, T., Ustundag, C. and Kohno, H. 2001. Transition from endogenous to exogenous nutritional sources in larval Black Sea turbot *Psetta maxima. Fish. Sci.*, **67**: 571-578.

Munkittrick, K. R. and Leatherland, J. F. 1984. Seasonal changes in the pituitary- gonad axis of feral gold fish, *Carassius auratus* L., from Ontario, Canada. *J. Fish. Biol.*, **24**: 75-90.

Munshi, J. S. D. 1968. The accessory respiratory organs of *Anabas testudineus* (Bloch) (Anem study). *Ind. Biol.*, **1**: 43-45

Muramoto, S. 1981. Variations of some elements in cadmium-induced malformed fish. *Bull. Environ. Contam. Toxicol.*, **27**: 193-200.

Murugaboopathy, N., Srinivasa-Reddy, Y., Bakthavathsalam, R. 1985. Adaptive changes for survival during starvation in the fish *Anabas testudineus* . *Environ. ecol.*, **3** (3): 366-372

Muthukaruappan, S. 1987. Biochemical aspects of ovarian maturation in *Liza parsia* (Hamilton- Buchanan). Msc. Dissertation, P.G.P. in Mariculture, CMFRI, Cochin- 683 031, 76 p.

Myers, G. S. 1937. Fresh water fishes and West- Indian Zoogeography. Smithsonian Report, 339-364.

Mylonas C. C., J.M. Hinashaw. and C.V. Sullivan. 1992. GnRHa-induced ovulation of brown trout (*Salmo trutta*) and its effects on egg quality. *Aquaculture*, **106**: 379-392.

N'da, K. and C. Deneil. 1993. Sexual cycle and seasonal changes in the ovary of the red mullet, *Mullus surmuletus*, from the southern coast of Brittany. *J. Fish. Biol.*, **43**: 229-244.

Nagahama, Y. 1983. The functional morphology of teleost gonads. *In:* Hoar et al., (eds.), *Fish Physiology*, Vol. 9, Reproduction, Part A, pp. 223-276. (Academic Press, Newyork).

Nagahama, Y., I. Yoshikuni., M. Yamashita. and M. Tanaka. 1994. Regulation of oocyte maturation in fish. *In:* N. M. Sherwood and C. L. Flew (Eds.), Fish Physiology. *Academic Press, New York*, XII: 393-439.

Najim, K., Al-Dahamand Nazir Bhatti, M. 1979. Annual changes in the ovarian activity of the freshwater teleost, *Barbus luteus* (Heckel) from Southern Iraq. *J. Fish. Biol.*, **14**: 381-387.

Nandeesha, M. C., Rao, K. G., Jayanna, R. N., Parker, N. C., Varghese, T. G., Keshavanath, P. and Shetty, H.P.C. 1990. Induced spawning of Indian major carps through single application of ovaprim-C. Proc. of the second Asian fisheries forum Tokyo, Japan. 581-585.

Nargis, A., Hossain, M.A. 1987. Food and feeding habit of koi fish (*Anabas testudineus* (Bloch), Anabantidae: Perciformes). *Bangladesh j. agric.*, **12** (2): 121-127

Natarajan, R. and Subramanyam, K. 1974. A Karyotype study of some teleosts from Portonova waters. *Proc.India. Acad. Sci.*, **79** (5): 173-196.

Nath, P. and B. I. Sundararaj. 1981b. Induction of vitellogenesis in the hyposectomised catfish, *Heteropneustes fosilis* (Bloch): Effects of piscine and mammalian hormones. *General. Comp. Endocr.*, **43**: 191-200.

Nayak, P. K., Singh, B. N. and Ayyapan, S. 2000. *J. Inland. Fish. Soc. India*, **32** (2): 49-56.

Nelson, J. S. 1994. Fishes of the world,. John Wiley and sons, New York; XVII+599

Norman, J. R. 1975. A history of fishes, 3 rd ed., P.H. Greenwood, Ernest Benn Ltd. London, 467 p.

Nuradha, C. H. and T. N. Raju. 1996. Effect of selenium toxicity on dehydrogenases in the fish *Anabas scandens j. ecotoxicol. environ. monit.* **6** (2): 149-151

Oda, T. and T. Kayano. 1988. Effect of several commercial diets on growth, occurrence of colour anomaly and vertebral malformation in hatchery

reared flounder, *Paralichthys olivaceus*. Experimental station, Okayama perfect, *Bull. Fish.*, **3**: 41-46.

Odum, E. P. 1971. Fundamentals of ecology. Saunders & Co., London.

Ojanguren, A. F., Reyes, F. G. and Munoz, R. R. 1999. Effects of temperature on growth and efficiency of yolk utilisation in eggs and pre-feeding larval stages of Atlantic salmon. *Aquacult. Int.*, **7** (2): 81-87.

Ojha, J., N. P. Tiwary and Dandotia, O. P. 1975. Effect of starvation on fat content in the air-breathing organs of amphibious fishes, *Anabas testudineus* (Bloch) and *Clarias batrachus* (Linn.),. *J. Biol. Sci. India*, **18** (2), 81-84

Oozeki, Y. and Hirano, R. 1985. Effects of temperature change on the development of egg of the Japanese whiting *Sillago japonica. Bull. Jpn. Soc. Sci. Fish.*, **51**: 557-572.

Pal, J. 1988. Dominance hierarchy in the Asian climbing perch *Anabas testudineus* in the laboratory. *Environ. ecol.*, **6** (2): 251-256

Pal, J., B. C. Pal., and Banerjee, R. J. 1978. Epithelial carcinoma in *Anabas testudineus. J. Fish. Biol.*, **13** (6): 693-694.

Pal, J. and B. C. Pal. 1985. Reproductive behavior of the Asian climbing perch *Anabas testudineus . Environ. ecol.*, **3** (2): 222-226

Pal, J. and K. Pradhan. 1990. Bacterial involvement in ulcerative condition of air-breathing fish from India. *J. fish. biol.*, **36** (6): 833-839.

Pal, R. N., S. C. Pathak., Singh, D. N. and Dehadrai, P. V. 1977. Efficacy of different feeds in survival of spawn of *Anabas testudineus. J. Inland. Fish. Soc. India*, **9**: 165-167.

Palekar, V. C. and D. V. Bal. 1961. Studies on the maturation and spawning of the Indian whiting *Sllago sihama* (Forskal) from Karwar waters. *Proc. Indian Acad. Sci.*, **54** B (1): 76-93.

Palmer, E. E., P. W. Sorensen, and I. R. Adelman. 1995. A histological study of seasonal ovarian development in freshwater drum in the Red Lakes, Minnesota. *J. Fish. Biol.*, **47**: 199-210.

Pandey, A., P. K. Srivastava., Adhikari, S. and Singh, D.K. 1992. pH profile of gut as an index of food and feeding habits of fishes. *J. freshwat. biol.*, **4** (2): 75-79

Pandey, K., J. P. Shukla. 1982. Behavioural studies of freshwater fishes during a solar eclipse. *Environ. biol. fish.*, **7** (1): 63-64

Pandey, S. and Hoar, W. S. 1972. Induction of ovulation in Goldfish by clomiphene citrate. *Can J. Zool.*, **50**: 1679-1680.

Pandey, A. K., R. S. Patiyal., Upadhyay, J. C., M. Tyagi and P. C. Mahanta, 1998. Induced spawning of the endangered mahseer *Tor putitora* at the state fish farm near Dehradun, *Ind. J. Fish.*, **45** (4) : 457-459.

Pandey, B.N., Chanchal, A.K., Singh, S. B. 1978. Effect of hormones and pharmacological drugs on the blood of *Anabas testudineus* (Bloch). *Folia-Haematol.*, **105** (5): 665-671

Panigrahi, A.K. and B. N. Misra. 1978. Toxicological effects of mercury on a freshwater fish, *Anabas scandens*, Cuv. and Val., and their ecological implications. *Environ. Pollut.*, **16** (1): 31-39

Pankhurst, N. W., Mc Millan, P. J. and Tracy, D. M. 1987. Seasonal reproductive cycles in three commercially exploited fishes from the slope waters, off New Zealand. *J. Fish. Biol.*, **30**: 193-211.

Pathak, S. C., Y. S. Yadav., Singh, D.N. and Dehadrai, P.V. 1980. Observations on the mixed culture experiment on air breathing fishes conducted in derelict and fresh water ponds in Gauahti (Assam). *J. inland. fish. Soc. India*, **12** (1): 112-115

Peter, R. E. and K. L. Yu. 1997. Neuroendocrine regulation of ovulation in fishes: Basic and applied aspects. *Rev. F. Biol. Fish.*, **7**: 173-197.

Peter, R. E. 1983. The brain and neurohormones in teleost reproduction. "Fish physiology" Volume. IX A. (Eds. W. S. Hoar, D. J. Randall and E. M. Donaldson), pp. 97-127. Academic Press, New York.

Peter, R. E., Chang, J.P., Nahorniak, C. S., Omeljaniuk, R. J., Sokolowska, M., Shih S. H., and Billard, R. 1986. Interactions of catecholamines and GnRH in regulation of gonadotropin secretion in teleost fish. Recent Progress in Hormone Research, **42**: 513-548.

Peter, R. E., H. R. Lin., G. Van Der Kraak and M. Little. 1993. Releasing hormones, dopamine antagonists and induced spawning. pp. 25-30. *In*: Recent advances in Aquaculture, vol. IV (J. F. Muir and R. J. Roberts., eds.). Blackwell, Oxford.

Peter, R. E., Trudeau, V. L. and Sloley, B. D. 1991. Brain regulation of reproduction in teleosts. Bulletin of Institute of Zoology, *Academia sinica*, Monograph, **16:** 89-118.

Peute, J., Van der Gaag and Lambert, J. H. D. 1978. Ultrastructure and lipid content of the liver of the zebra fish, *Brachydanio rerio*, related to vitellogenin synthesis. *Cell Tissue Res.*, **186**:297-308.

Pickford, G. E. and J. W. Atz. 1957. The physiology of the pituitary gland of fishes. New York Zoological society, New York: 613p.

Popma, T. J. and Lovshin, L. L. 1996. Worldwide prospects for commercial production of Tilapia. Research and development series No. 41. International center for environment, Auburn univ., Alabama, USA.

Potongkam, 1972. Experiment on feeding climbing perch, *Anabas testudineus* (Bloch), with ground trash fish and pellets. Department of fisheries Annual report, Bangkok.

Prabhu, M. S. 1955. Some aspects of biology of ribbonfish, *Trichiurus haumela* (Forskal). *Indian J. Fish.*, **2** (1): 132-163.

Prabhu, M. S. 1956. Maturation of intra-ovarian eggs and spawning periodicities in some fishes. *Indian J. Fish.*, **3**:59-90.

Pradhan, L. B. and C. V. Reddy. 1964. Fluctuations in mackerel landings at Calicut in relation to hydro-geographical factors. *Indian J. Fish.*, **9** (1): 100-109.

Prakasam, V. R. and P. Johnson. 1988. Scale morphology of three teleosts (*Etroplus suratensis, Anabas testudineus, Sardinella longiceps*) with reference to function. *Indian. j. fish.*, **35** (3): 221-225.

Prasad, M. S. and P. Prasad. 1985. Larval behaviour during growth and development of air-breathing habit in *Colisa fasciatus* (Bl. and Schn.). *J. Inland. Fish. Soc. India*, **32** (2): 185-191.

Prasad, M. S., Kiran, Usha and Singh, B. R. 1993. Oxygen uptake in relation to body size in an air breathing fish, *Osphronemus noblilis* (Mcclelland). *Pol. Arch. Hydrobiol.*, **40**: 217-222.

Prinsloo, J. F. and Schoonbee, H. J. 1986. Comparison of the early larval growth rates of the Chinese grass carp (*Ctenopharyngodon idella*) and the Chinese silver carp (*Hypophthalmichthys molitrix*) using the live and conventional feed. *Water*, **12**: 229-234.

Qadri, M. Y., Mir, S. and Yousf, A. R., 1983. Breeding biology of *Schizothorax richardsoni.* Grap and Hard. *J. Indian Inst. Sci.*, **64** (6): 73-81.

Qasim, S. M. and A. Qayyum. 1961. Spawning frequencies and breeding season of some fresh water fishes with special reference to those occurring in the plains of Northern India. *Indian J. Fish.*, **3** (1): 27-43.

Qasim, S. Z. 1973. An appraisal of the studies on maturation and spawning in marine teleosts from Indian waters. *Indian J. Fish.*, **20** (1): 166-181.

Radhakrishnan, N. 1957. A contribution to the biology of the Indian sandwhiting *Sillago sihama* (Forskal). *Indian J. Fish.*, **4** (2): 254-283.

Rahman, M. K., Akther, J. N., Mazid, M . A. and Halder, G. C. 1993. First record of induced breeding of Thai *Pangas Pangasius* sutchi (Fowler) in Bangladesh, *J. Inland. Fish. Soc. India*, **25** (2), 26-30.

Rahmani, A. R. and S. M. Khan. 1981. Development of the secondary lamellae in the olfactory epithelium of *Anabas testudineus*. *Jap. j. ichthyol*, **28** (2): 177-180.

Rai, B. P. 1967. Cyclic changes in the ovary of *Tor tor*. *Acta. Zool.*, **48**: 289-307.

Rai, S. C. and B. P. Rai. 1981. Structure and histological changes in the ovary of the freshwater tropical teleost *Colisa fasciatus* (Bl. & Sch.). *Environment India*, **4**: 37-42.

Ram, R. N., D. M. Singh, Singh, I. J. and Singh, U. P. 2001. Induction of maturational events in gonads of *Labeo rohita* in response to pituitary extract and Ovaprim treatment. *Ind. J. Fish*, **48** (3): 259-267.

Ramaseshaiah, M. 1985. The fecundity of anabantid fishes *Anabas testudineus* (Bloch) and *A. oligolepis* (Bleeker) (Osteichthyes: Anabantidae) from Lake Kolleru. *Matsya*, **11**: 78-81

Ramaseshaiah, M. and S. Dutt. 1984. Comparative electrophoretic studies of *Anabas testudineus and A. oligolepis* (Osteichthyes: Anabantidae) (climbing perch) from Lake Kolleru, Andhra Pradesh, India. *Hydrobiologia*, **119** (1): 57-64

Ramaswamy, L. S. and B. I. Sundararaj. 1956. Inducing spawning in Indian catfish. *Science*, **123** (3207): 1080.

Ramaswamy, L. S. and B. I. Sundararaj. 1957. Induced spawning in the catfish *Clarias batrachus*. *Naturwissenschaften*, **44**: 384p.

Ramaswamy, L. S. 1962. Endocrinology of reproduction in fish and frog. *Gen. Comp. Endocrinol.*, **1**: 286-289.

Rangaswamy, C. P. 1972 a. Observations on the growth of *Mugil cephalus* (L.) in the Ennur and Adayar estuaries. *Curr. Sci.*, **41** (15): 567-568.

Rao, B. and V. Seshagiri. 1968. Systematic studies on *Anabas testudineus* (Bloch, 1792) and *A. oligolepis* (Bleeker, 1855) *Proc. Indian Acad. Sci.*, **67**(5): 207-214.

Rao, B. and V. Seshagiri. 1971. The Indian Climbing perch. *Science Reporter*, **8** (2): 63-65.

Rao, J. B. and Karamchandani, S. J. 1986. On the spawning biology of *Ompok bimaculatus* (Bloch) from Kulgarhi reservoir of Madhya Pradesh. *J. Inland Fish. Soc. India*, **18** (2): 40-47.

Rao, P.V.S., V.P. Sarada. and Rao, M.V.S. 1994. Observations on decline of climbing perch, Anabas in Krishna District, A.P. Threatened fishes of India. Proceedings of the national seminar on endangered fishes of India held at national bureau of fish genetic resources, Allahabad on 25 and 26 April, 1992. Dehadrai, P.V., Das, P. and Verma, S.R. (eds.) Muzaffarnagar-India, **4**:73-76.

Raven, C. P. 1961. Oogenesis: The storage of developmental information. Pergamon, Oxford, 164p.

Ray, A.K. and B. C. Patra. 1989. Growth response, feed conversion and metabolism of the air-breathing fish, *Anabas testudineus* (Bloch) to different dietary protein sources. Fish nutrition research in Asia. Proceedings of the third Asian fish nutrition network meeting. Silva, S. S. (ed.), **4** :123-129.

Reash, R. J. and Berra, T. M. 1989. Incidence of fin erosion and anomalous fishes in a polluted stream and a nearby clean stream. *Water Air Soil Pollut.*, **47**: 47-63.

Redding, M. J. and R. Patino. 1993. Reproductive physiology. *In:* The physiology of fishes (Evans., D. H., ed.) pp. 503-534. Boca Raton. FL: CRC Press.

Reddy, T. G. and G. M. Natarajan. 1970a. Studies on the respiration of *Anabas scandens* (Cuv.), *J. Annamalai. Univ. Sci.*, **28**: 155-162.

Reddy, T. G., and G. M. Natarajan. 1970b. On the function of labyrinthine organ of *Anabas scandens* (Cuv.). *J. Annamalai. Univ. Sci.*, **29**: 149-157.

Reihl, R. 1976. The yolk nucleus in young oocytes of *Noemachilus barbatulus*. *Z. Natur.Forsch. Biosc.*, **31**: 761-762.

Richter, C. J. J., Eding, E. H., Goos, H. J. T., De Leeuw, R., Scott, A.P. and Van Oordt, P. G. W. J. 1987. *Aquaculture*, **63**: 157-168.

Rideout, R. M., D. M. Maddock and M. P. M. Burton. 1999. Oogenesis and the spawning pattern in Greenland halibut from the North-West Atlantic. *J. Fish. Biol.*, **54**: 196-207.

Rita Kumari, S. D. and K. C. Padmanabhan. 1976. Oogenesis in the pearl-spot Etrolus suratensis. *Zoological. Anz.*, **196**: 133-143

Robb, A.P. 1982. Histological observations on the reproductive biology of the haddock, *Melanogrammus aeglefinus*(L.). *J. Fish. Biol.*, **20**: 397-408.

Rosas, J., Millan, J. and Cabrera, T. 1998. Proximate analysis of *Brachionus plicatilis*, *Oithona ovalis, Methys sp.*, *Metamisydosis insularis* and nauplii of Artemia. Creswell, R.L. (ed), Proc. Gulf Caribb. Fish. Inst., **50**: 640-647.

Rothbard, S. and Yaron, Z. 1995. Carps (Cyprinidae) (Chapter13). *In:* Bromage, N. R. and Roberts, R. J. (eds.), Broodstock Management and egg and larval quality. Blackwell Scientific Publications, Oxford, pp. 321-352.

Saat, T. V. and A. E. Veersalu. 1990. Changes in the oocyte structure in the carp, *Cyprinus carpio*, during *in vitro* maturation . *J. of Ichthyol.*, **30** (1): 17-26.

Sadlier, R. M. F. S. 1973. Reproduction of vertebrates. Academic press, New York.

Sahoo, T. K., S. S. Giri, T. Swain. and A. K. Sahoo. 2004. Observations on some common abnormalities in induced bred *Clarias batrachus* hatchlings. *J. Inland. Fish. Soc. India,* **36** (1): 67-71.

Sahu, K. C. 1983. Histochemical studies of cyclical changes in the ovary of certain fishes. Ph. D. Thesis, Berhampur University, Berhampur.

Saidapur, S. K. 1989. Reproductive cycles of Indian vertebrates, Allied publishers Limited, New Delhi. pp. 465.

Saksena, D.N., Raizada, A.K. 1984. Cellular and dynamic aspects of oocyte growth in teleosts. *Int. j. acad. ichthyol.,* Modinagar. **5** (1-2): 11-22

Sangrattanakhul, C. 1989. Effect of pelletized feed diets containing various levels of protein on growth and survival of climbing perch, *Anabas testudineus* (Bloch). Master degree thesis. Kasestat University. Bangkok, Thailand, pp. 74.

Santos, R. S. 1995. Anatomy and histology of secondary sexual characters, gonads and liver of the rock-pool blenny, *Parablennis sanguinolentus parvicornis*, (Pisces; Blennidae) of the Azores Archipelago, *Cienc. Biol. Mar. Sci.*, **13**: 21-28.

Sanwal, R. and Khanna, S. S. 1972. Seasonal changes in the testes of a freshwater fish *Channa gachua. Acta. Anat..*, **83**: 139-1748.

Sarma, D., S. Dey., A. Dutta and N. Bhuyan. 2003. Changes in surface structure and histological features of *Labeo rohita* (Ham.) gonads with reference to mid-altitude and low temperature conditions. *J. Inland. Fish. Soc. India*, **35** (2): 32-40.

Sathyanesan, A. G. 1960. seasonal cycle in the gonads of *Cirrhina reba* (Ham.). *Indian J. Vet. Sci.*, **29**: 27-32.

Sathyanesan, A.G. 1961. Spawning periodicities as revealed by seasonal histological changes in the gonads of *Barbus stigma* (Cuv. & Val.). *Proc. Zool. Soc. India*, **14**: 15-26.

Saxena, K. C. 1964. Studies on the physiology of respiration in fishes. Functional anatomy of the respiratory organs of the air-breathing fish *Anabas testudineus. Ichthyologica*, **3**(1&2): 77-85.

Schaefer, K. 1987. Reproductive biology of black skipjack, *Euthimas lineatus*, an eastern Pacific tuna. *Inter-American Tropical Tuna Commission Bulletin,* **19**: 169-260.

Selman, K. and R. A. Wallace. 1982. Oocyte growth in the sheepshead minnow; uptake of exogenous proteins by vitellogenic oocytes. *Tissue Cell*, **4**: 555-571.

Sen, S., D. Mukherjee., Chakraborty, P., and Bhattacharya, S. 1979. 17~Hydroxysteroid dehydrogenase activity in the ovary and head kidney of two teleosts, *Anabas testudineus and Ophiocephalus punctatus. Proc. Indian Natl. Sci. Acad.*, Part-B, **45** (5): 534-538.

Sen, S. and S. Bhattacharya. 1982. Hormonal influence on perch ovarian 17 beta -hydroxysteroid dehydrogenase activity in *in vitro* system. *Indian. j. exp. biol.*, **20** (9): 664-667.

Sen, S., S. Deb. and Bhattacharya, S. 1985. Equilibrium of free and ester cholesterol in a fresh water perch, *Anabas testudineus*, in different seasons and in response to gonadotropin and thyroid hormone. *comp. physiol. ecol.*, **10** (2): 66-70.

Shah, M. S., Rahman, M. A., Deppert, D. and Rab, M. A. 1990. Seduced breeding of Indian major carps (*Labeo rohita, Chirrhinus mrigala* and *Labeo calbasu*). *Asian Fish. Sci.*, **3** (2):*257-261.*

Shaji, C. P., P. S Easa and Gopalakrishnan, A. 2000. Fresh water fish diversity of western ghats, *In*: Ponniah. A. G. and Gopalakrishnan, A. Eds). Endemic fish diversity of Western Ghats. NBFGR-NATP Publication-1, 347pp. National Bureau of Fish genetic resources, Lucknow, U.P., India.

Shashi, S. B. and B. P. Akela. 1996. Determination of maturity and spawning period by gonadosomatic index and measurement of mean ova diameter in certain teleosts. *Environ. Ecol.*, **14** (2): 399-403.

Shoesmith, E. 1990. A comparison of methods for estimating mean fecundity. *J. Fish Biol.*, **36** (1): 73-84.

Simpson, A. C. 1951. The fecundity of the plaice. Ministry of Agriculture (U.K). *Fish. Invest. Ser.*, II. **17**: 1-27. Cited in Bagenal & Braum (1968).

Singh, A. and Singh, T. P. 1976. Effect of Clomid, Sexovid and prostaglandins on induction of ovulation and gonadotropin secretion of a freshwater catfish, *Heteropneustes fossilis. Endocrinologie,* **68**: 129-136.

Singh, B. R. 1993.Advances in fish research. Narendra Publishing House, Delhi, India. pp. 400.

Sinha, G.M., S. K Mondal and Ghosal, S. 1982. Autoradiographic studies on the chronology of meiosis and spermiogenesis in a freshwater teleost fish, *Anabas testudineus* (Bloch). *cytologia,* **47** (2): 309-315.

Sinha, V. R. P., Jhingran, V. T and Ganapati, S. V. 1974. A view on the spawning of the Indian major carps. *Arch. Hydrobiol.*, **73**, 518-536.

Sivakami, S. 1961. Study on the induced breeding and larval development of *Rasbora daniconius* (Hamilton- Buchanan). *J. Inland. Fish. Soc. India,* **13** (2): 6-15.

Smith, B. B. and K. F. Walker. 2004. Spawning dynamics of common carp in the river Murray, South Australia, shown by macroscopic and histological staging of gonads. *J. Fish. Biol.*, **64**: 336-354.

Smith, C. J. and S. Haley. 1987. Evidence of steroidogenesis in post-ovulatory follicle of the tilapia, *Oreochromis mossambicus. Cell and Tissue Culture Research,* **247**: 675-687.

Smith, S. H. 1956. Life history of lake herring of Green Bay, Lake Michigan. *U. S. Fish. Bull.,* **57**: 87-138.

Sobhana, B. and Nair, N. B. 1974. Observations on the maturation and spawning of *Puntius sarana subnastus* (Val.), *Indian J. Fish.*, **21** (2): 357-369.

Somavanshi, V. S. 1985. Indices of fecundity in the Hillstream fish *Garra mullya. Indian J. Fish.*, **32** (1): 33-43.

Soto, C. G., J. F. Leatherland and Noakes, D. L. G. 1992. Gonadal histology in the self-fertilizing hermaphroditic fish *Rivalus marmoratus* (Pisces: Cyprinidontidae). *Can. J. Zool.*, **70** (12): 2338-2347.

Spencer, H. 1871. The principles of biology. *Vide* Jhingran, V. G., 1952. General length weight relationship of three major carps of India, *proc., Nat, Inst. Sci, India,* **18** (5): 449-460.

Sridhar, S. C., Vijayakumar and M. H. Haniffa. 1998. Induced spawning and establishment of a captive population for an endangered fish, *Ompok bimaculatus* in India. *Current Sci.*, **75** (10): 1066-1088.

Srivastava, P.K., A. Sen. and Moitra, A. 1990 Mucous-secreting cells in the intestine of *Anabas testudineus* in relation to different dietary conditions: Histochemical observation. *J. freshwat. biol.*, **2** (3): 203-207

Srivastava, S. and K. Swarup. 1979. Cyclic changes in the ovary of a freshwater large murrel, *Channa striatus. Arch. Biol. Brusselles*, **90**: 245-260.

Srivastava, S. 1980. Seasonal changes in the ovary of a freshwater large murrel *Channa marulius. Zool. J. Anat.*, **104**: 492-499.

Stacey, N. E. and Goetz, F. W. 1982. Role of prostaglandins in fish production. *Can. J. Fish. Aquat. Sci.*, **39**: 92-98.

Stenger, A. H. 1959. A study of the structure and development of certain reproductive tissues of *Mugil cephalus. Zoologica* (N. Y.), 44 (2): 53-78.

Sultana, N., M. A. Hossain., Furuichi, M. and Y. Yone. 1989. Rearing of 'Ayu' larvae (*Plecoglossus altivelios*) by artificial diets. II. Effects of Taurine, Urso-20, Glutathione and Tachinase supplements on growth and survival. *Ind. J. Fish.*, **36** (1): 34-40.

Sumpter, J. P. 1990. General concepts of seasonal reproduction. *In*: Reproductive seasonality in Teleosts: Environmental influences (Munro, A. D., A. P. Scott. and T. J. Lam eds.) CRC Press, Boca Raton, F. L.

Sundararaj, B. I. and Vasal, S. 1976. Photoperiod and temperature control in the regulation of reproduction in the female Catfish *Heteropneustes fossilis. J. Fish. Biol.*, **33**: 959-973.

Sundararaj, B. I. And Sehgal , A. 1970. Effects of a long or increasing photoperiod on the initiation of ovarian recrudescence during the preparatory period in the catfish, *Heteropneustes fossilis* (Bloch). *Biol. Reprod.*, **2**: 413-424.

Sunder, S. 1986. On the breeding biology of a snow trout, *Schizothorax longipinnis* from the River Thelum, Kashmir. *Indian J. Fish.*, **33** (2): 201-210.

Swara, Y. 1974. Reproduction of the mosquito fish (*Gambusia affinis*) a freshwater fish introduced into Japan. *Jpn. J. Ecol.*, **24**: 140-146.

Szabó, T. 2001. Hormonally induced ovulation of Northern Pike *via* sustained- release vehicles. *North Ame. J. Aq.*, **63**:137-143.

Szabó, T., Medgyasszay, C. and Horváth, L. 2002. Ovulation induction in nase (*Chondrostoma nasus*, Cyprinidae) using pituitary extract or GnRH analogue combined with domperidone. *Aquaculture*, **203**: 389-395.

Taranger, G. L., S. O. Stefansson and T. Hansen. 1992. Advancement and synchronization of ovulation of Atlantic salmon (*Salmo salar* L.) following injections of LHRH analogue. *Aquaculture*, **102**:169-175.

Thakur, N. K., R. N. Pal and Khan, H. A. 1974. Embryonic and larval development of *Heteropneustes fossilis* (Bloch) *J. Inland. Fish. Soc. India*, **6**: 33-44.

Thakur, N.K. and P. Das. 1986. Synopsis of biological data on koi *Anabas testudineus* (Bloch, 1792). B*ull. cent. Inland. fish. Res. inst.*, Barrackpore. India. **40**: 47 pp.

Tharakan, B. and K. P. Joy. 1996. Effects of mammalian gonadotropin releasing hormone analogue, pimozide and the combination of plasma gonadotropin levels in different seasons and induction of ovulation in female cat fish. *J. Fish. Biol*, **48**: 623-632.

Thiraphan, P. 1984. The determination of age of *Anabas testudineus* (Bloch) by using formation of fish scale method. *J. Fishery Gazette*, **28** (2): 207-215.

Thorogood, J. 1986. Aspects of the reproductive biology of the Southern blue fin tuna (*Thunnus maccoyii*). *Fisheries Research*, **4**: 297-315.

Tripathy, S. D. and B. S. Bhimachar. 1972. Hypophysation of fishes with particular reference to India: 80 p. Jabalpur: The Directorate of Research Services, Jawaharlal Nehru Krishi Vishva Vidyalaya.

Tulasi, S. J., P. U. M. Reddy. and Ramana-Rao, J.V. 1989. Effects of lead on the spawning potential of the fresh water fish, *Anabas testudineus. Bull. environ. contam. toxicol.*, **43** (6): 858-863.

Tyler, C. R. and J. P. Sumpter. 1996. Oocyte growth and development in teleosts *Rev. fish. biol. Fish.*, **6** (3): 287-318.

Upadhyaya, S. N., Bretton. B. and R. Billard. 1978. Ultra structural studies on experimentally induced vitellogenesis in juvenile rainbow trout (*Salmo gairdneri*). *Annls. Biol. Anim. Biochem. Biophys.*, **18**: 1010-1025.

Van Eden, F. J. M. and 16 others. 1996.Mutations affecting somite formation and [patterning in zebrafish, *Danio rerio. Development*, **123**: 153-164.

Veena, K. B., C. K. Radhakrishnan and Chacko, J. 1995. Sialic acid and cholesterol variations during oogenesis of an estuarine teleost. *Oebalia*, **21**: 177-180.

Venkitaramanujam, K. and N. Ramanathan. 1994. Manual of finfish biology, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, 110 001. pp. 46-61.

Venugopalan, V. K. 1962. Study on the cytochemical and biochemical aspects of the ovarian cycle in *Ophiocephalus striatus* (Bloch). Ph. D. Thesis, Annamalai Univ., Porto Novo, Tamil Nadu, India.

Verghese, P. U. 1967. Prolongation of spawning season in the carp *Cirrhina reba* (Ham.) by artificial light treatment. *Curr. Sci.*, **36**: 465-467.

Verghese, P. U. 1970. Preliminary experiments on the modification of reproductive cycle of an Indian carp *Cirrhina reba* (Ham.) by control of light and temperature. *Proc. Indo-Pac. Fish. Counc.*, **13**, 171-184.

Verghese, P. U. 1975. Internal rhythm of sexual cycle in a carp *Cirrhina reba* (Ham.) under artificial conditions of darkness. *J. Inland Fish. Soc. India*, **7**: 182-188.

Verma, G. P. 1997. Growth of teleostean oocytes. *In:* B. R. Singh (ed.). Advances in fish research. Narendra Publishing House, Delhi. **2**: 129-162.

Verma, G. P., A. K. Panigrahi, Verma.V. P. and C. C. Das. 1981a. Studies on the developing oocyte with special reference to its nucleolar activity in a teleost, *Tilapia mossambica. Environ. India*, **4**: 25-30.

Vijayaraghavan, S. and J. V. R. Rao. 1984. Some observations on the biochemical aspects of nutritional stress and carbohydrate loading in a carnivorous fish *Anabas scandens* (Cuvier). *Int. j. acad. ichthyol.*, Modinagar, **5** (1-2): p.15.

Vijayram, K., P. Loganathan and Janarthanan, S. 1991. Liver dysfunction in fish *Anabas testudineus* exposed to sub lethal levels of pulp and paper mill effluent. E*nviron. ecol.*, **9** (1): 272-275.

Wakiyama, A., K. Hiroshi and T. Yasuhiko. 1997. Genetic relationships of anabantoid fishes, *J. Tokyo. univ. fish.*, **83** (1-2): 93-102.

Wallace, R. A. 1985. Vitellogenesis and oocyte growth in nonmammalian vertebrates. *In*: Browder, L. W. (ed.). Developmental Biology- A comprehensive synthesis, Volume. I Oogenesis. Plenum Press, New York, pp. 127-177.

Wallace, R. A. and Selman, K. 1979. Physiological aspects of oogenesis in two species of stickle backs, *Gaterosteus aculeatus* L. and *Apeltes quadracus* (Mitchill). *J. Fish. Biol.*, **14**: 551-564.

Wallace, R. A., Selman, K., Greely, M. S. Jr., Begovac, P. C., Lin, Y. W. P. and McPherson, R. 1987. Current status of oocyte growth. Proceedings of the Third International Symposium on the Reproductive Physiol of fish (Idler, D. R., Crim, L. W. & Walsh, J. M., eds.), pp 167-177. St. John's Canada Memorial University of Newfoundland.

Wallace, R.A. and Selman, K. 1981. Developmental biology of fishes. American Soc. Of Zoologists, USA. *Div. Devel. Biol. and Vertebr. Morphol.*, **2**: 325-343.

Ward, H. W. C. 1973. Antioestrogen therapy for breast cancer: a trial of tamoxifen at two dose levels. *Br. Med. J.*, **1**: p. 13.

Weber, W., 1974. The influence of hydrographical factors on the spawning time of tropical fish. *Proc. Int. Seminar. Fish. Res. and their Management in S. E. Asia*, pp. 269-281.

Weesner, F. M. 1960. General Zoological microtechniques. Williams & Wilkins Company & Scientific Book Agency, Calcutta. XII, pp. 230.

West, G. 1990. Method of assessing ovarian development in fish: A review . *Aust. J. freshwater Res.*, **41**: 199-222.

Willey, A. 1910. Notes on fresh water fisheries of Ceylon. *Spol. Zey., Colombo*, **7**: 88-105.

Wingfield, J. C. and Grimm, A. S. 1977. Seasonal changes in plasma cortisol, testosterone and oestradiol-17 β in the plaice, *Pleuronectes platessa* L. *Gen. Comp. Endocrinol.*, **31**: 1-11.

Witthames, P. R. and Walker, M. G. 1987. An automated method for counting and sizing fish eggs. *J. Fish. Biol.*, **30**:225-235.

Worthington, A. D. and N. A. A. Mcfarlane. 1983. The induced spawning of roach, *Rutilus rutilus* (L.), with the antioestrogens clomiphene and tamoxifen. *J. Fish. Biol.*, **22**: 253-257.

Xie, Xiangyun and Zang, Qiyong. 1990. Studies on the gonadal development in female mudskipper, *Boleophthalmus pectinirostris, J. Oceanogra. Taiwan Strait. Taiwan Haixia*, **9** (3): 217-221.

Yakupitiyage, A., J. Bundit and H. Guttman. 1998. Culture of climbing perch (*Anabas testudineus*); A review, *In: Ait Aqua Outreach*, Working paper No. T-8, http://www.agri.

Yamamoto, K. and F. Yamazaki. 1961. Rhythm of development in the oocyte of the gold fish, *Carassius auratus. Bull. Fac. Fish. Hokkaido University*, **12**: 93-110

Yamamoto, K. 1956a. Studies on the formation of fish eggs. I Annual cycle in the development of ovarian eggs in the flounder, *Liosetta obscura. J. Sci. Hokkaido Univ. ser.*, VI. **12**: 362-374.

Yamamoto, K., H. Kai and Isheda, R. 1959. A preliminary report on the formation of the egg of the trout, *Oncorhynchus masou. Bull. Hokkaido Reg. Fish. Res. Lab.*, **20**: 109-116.

Yaron, Z. 1995. Endocrine control of gametogenesis and spawning induction in carp. *Aquaculture*, **129**: 49-73.

Yuen, H. S. H. 1955. Maturity and fecundity of big-eye tuna in the Pacific. *Spec. Sci. Rep. Fish.*, **150**: 30 p.

Yuen, H. S. H. and F. C. June. 1957. Yellowfin tuna spawning in the central equatorial Pacific. *U. S. Fish. Wild. Serv. Fish. Bull.*, **112** (57): 250-264.

Yufera, M., Fernandez-Diaz, C., Pascual, E., Sarasquete, M., Moyano, F., Diaz, M., Alarcon, F., Garcia-Gallego, M. and Parra, G. 2000.

Towards an inert diet for first-feeding gilthead *seabream sarus aurata* L. larvae *Aquacult. Nutr.*, **6** (3): 143-152.

Zahnd, J. P. 1959. Modifications hepatiques liees au cycle ovarien chez deux poisons ovovivipares: *Xiphophorus helleri* et *Lebistes reticulates. Arch. Anat. Microsc. Et al. Morphol. Exptl.*, **48**: 231-259.

Zohar, Y. 1989. Fish reproduction: Its physiology and artificial manipulation. *In:* Fish culture in warm water systems. Problems and trends. M. Shilo and S. Sarig, (eds.). CRC Press, Inc., Boca Raton, Florida, pp. 65-119.

Zuckerman, S. 1962. The ovary, Volume. I. Academic Press, London & New York.