# STUDIES ON THE REPRODUCTIVE PHYSIOLOGY OF *Lates calcarifer* (BLOCH)

THESIS SUBMITTED

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By

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**(Indian Council of Agricultural Research)**

**KOCHI - 682 031**

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TO
THOSE
WHOM
I
SACRIFICED
FOR
THIS
PREPARATION

## CERTIFICATE

This is to certify that the thesis entitled, **Studies on the reproductive physiology of Lates calcarifer (Bloch.)** is the bonafide record of the research work carried out by **Mr. Kuldeep Kumar Lal** under my supervision and that no part thereof has been presented for the award of any other degree.

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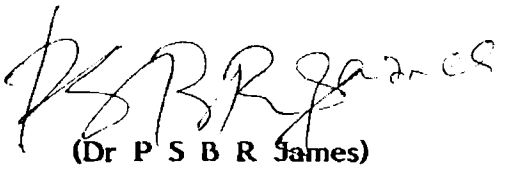

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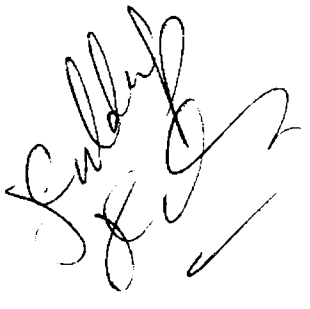
## DELCARATION

I here declare that this thesis entitled, **Studies on the reproductive physiology of Lates calcarifer (Bloch.)**, is a record of original and bonafide research carried out by me under the supervision and guidance of **Dr. P.S.B.R. James, Director, Central Marine Fisheries Research Institute, Kochi** and that no part there of has been presented for the award of any other degree, diploma, associateship, fellowship or other similar recognition.



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## PREFACE

Research on fish reproduction has progressed considerably during the last few years owing to the development of new and varied techniques. However, its application to acquaculture has largely been confined to a few selected species for quite a long time. Recognition of acquaculture as a means to provide easy access to the desired species has led the awareness among culturists, towards the diverisification in terms of variety of fishes being brought under the culture systems.

Lates calcarifer or sea bass, distributed in troprical and semitropical coastal waters of Indo-Pacific is one among the carnivorous fishes, gaining importance, now a days (James and Marichamy, 1987). The demand as well as endearance to consumers as an excellent table fish, coupled with its fast growing and euryhaline nature are considered as valuable attributes by the aquaculturists. Its complex sexuality involving protandric hermaphroditism, makes it an interesting specimen for academic studies.

In India Lates calcarifer, though distributed throughout the coast does not contribute much to the total fish landings except in certain areas in the northeast like Chilka lake, Hooghly-Matlah estuary etc. Though high priced and relished, as a food fish, but capture fisheries resources are limited. Therefore the alternative is to develop culture technology for this fish.

At present, there is paucity of information regarding the breeding biology, especially from southeastern region, which differs in its climatic features from northeastern coastal areas, where some attempts have been made to study the biology of the fish, though not documented in detail (Jhingran and Natarajan, 1969; Patnaik and Jena, 1976; Kowtal, 1977; Roy et al., 1977). The present work, first to undertake detailed investigation into reproductive aspects of the fish from India is not only confined to biology, but extend to the study on histological as well as physiological changes in the processes associated with natural reproductive cycle. The

main objective of the study is to develop a background for the standardisation of subsequent artificial propagation techniques for the sea bass The thesis is presented in three chapters.

The first chapter; general introduction, surveys the literature pertaining to the status of research on Lates calcarifer with specific reference to reproduction and acquaculture.

The second chapter entitled, materials and methods gives description regarding collection of specimens as well as samples in addition to the various analytical methodologies employed during the study.

Results and discussion of the current study, constituting chapter three, is divided further into three sections:

Section one is aimed at the description of sexuality, maturity and reproductive cycle of the fish. Salient features of maturity stages of testis and ovary, identified on the basis of gross morphology and histology of gonads; oocyte diameter frequency profile (in females only); changes in spermatogenic response and quantity of spermatozoa (in males only), changes in gonadal index are presented in detail. The size at first maturity and sex inversion, sex-ratio have been determined. Spawining season has been defined on the basis of seasonal changes in gonadal index, oocyte diameter (in females only),spermatogenic response and quantity of spermatozoa (in males only) and relative abundance of different maturity stages around the year. Fecundity has been estimated and values are correlated with parameters of the body size.

Section two deals with changes occurring at cellular level during the different stages of development of gametes (in both the sexes) studied by light as well as electron microscopy.

Section three draws attention towards the changes in the profile of selected biochemical parameters in gonads as well as certain somatic

tissues with respect to maturation. This preliminary attempt to draw physiological frame work, lay specific emphasis on the parameters associated with vitellogenesis including identification of vitellogenin in the serum.

The major results of the study are summarized followed by listing of the literature cited in the thesis.

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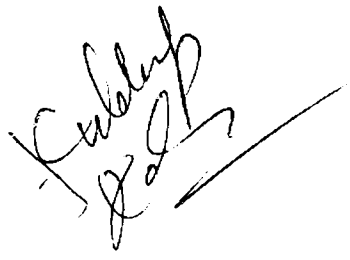


**(Kuldeep Kumar Lal)**

**CONTENTS**

# CHAPTER I
# INTRODUCTION

# CHAPTER I

## INTRODUCTION

Today's world fisheries is facing the problem of stablization of catch and growing demand for fish and fish products giving rise to the necessity of increasing the fishing effort by adopting the newer technologies. But sole dependence on natural resources to meet the growing demand is bound to have adverse effect on fishery stocks. Moreover, technological advancement is not a day's work and had saddled with another problem especially in developing countries like India i.e. clash of interests of traditional fishermen and their mechanised counterparts; may sometimes render the place of the former out of the fishing industry. Keeping in view the future succeeding the present circumstances, contribution of culture fisheries is of vital importance and has wide scope for expansion. Although in some regions of the globe, fish culture has grown into a big enterprise supplying food for human society (China, Philippines, Indonesia, Israel, U.S.A., Japan etc.), on the whole its importance in relation to fisheries is still supplementary besides a fact that the earliest record of aquaculture is found in 2000 B.C., of carp culture in China and bulk of aquatic production is still from hunting and gathering. Man's increasing dependence over the last several decades on cultured as opposed to wild fish, as a source of dietary protein has resulted in an increasing level of scientific effort being directed towards the propagation techniques.

With the advancement of aquaculture, more and more species of aquatic organisms are being studied for their commercial potential. Lates calcarifer, is one of the high priced fish, recognised for the development of aquaculture technology in the recent years. Description of Lates calcarifer or sea bass dates back to 1790 by Bloch, under the name Holocentrus calcarifer. The genus Lates was erected later in 1828 by Covier and Valencinnes. Its placement under the order perciformes and suborder percoidei has been unquestioned but familial position remained controversial for long time, including allocation to Percinae (Day, 1878)

subfamily Centropominae (Boulenger, 1895), Latinae (Fowler, 1928), Serranidae (Fowler and Bean, 1930), Latidae (Munro 1967) and Centropomidae (Katayama, 1956). The latter has been accepted.

L. calcarifer is widely distributed throughout the Indo-West Pacific region including India, Burma, Sri Lanka, Bangladesh, Malay Peninsula, Java, Borneo, Celebes, Philippines, Papua New Guinea, northern Australia, southern China and Taiwan. Its limit of distribution extends to Persian Gulf in the west and southeast tip of Papua New Guinea mainland in the east. It is distributed north to Amoy (Hsia-men) at Latitude 24° 30'N on the Chinese mainland and southern limit being Australian mainland, Noosa river (26° 30'S) on the east coast and the Ashburton river (22° 30'C) on the west coast (Grey, 1987). L. calcarifer has variety of roles to play in the region as it supports extensive commercial as well as recreational fisheries in Australia and Papua New Ghinea, on the other hand provides the basis of an extensive aquaculture industry in Asia apart from being a subject of wild stock exploitation.

L. calcarifer inhabits coastal, brackish, freshwater habitats and being a catadromous migrant as well as protandric hermaphrodite, exhibits one of the most complicated life histories among fishes (Moore, 1979, 1982; Moore and Reynolds, 1982; Reynolds and Moore, 1982; Russell and Garrett, 1983, 1985; Griffin, 1987; Davis, 1982, 1985a, 1987). Several authors have confirmed sea bass to be highly carvinorous (Day, 1878; Mookherjee et. al; 1946,Menon 1948; Pillay, 1954; Chacko, 1956; Dunstan., 1959; 1962; De, 1971; Patnaik and Jena, 1976; Tait, 1981; Russell and garrett, 1983; Davis, 1985b; James and Marichamy, 1987;) and opportunistic predator with ontogenetic progression in diet from microcrustacea to macrocrustacea to fish (Davis, 1985b).

L. calcarifer is dioecious with paired strongly dimorphic gonads (Moore, 1979). Histological events associated with gonadal maturation in male and female sea bass as well as the process of sex inversion has been studied by Davis (1982). The process of maturity was classified into six stages viz. (1) Immature (males) and newly formed (females); (2) developing-

recovering spent (3) maturing (4) mature (5) ripe (6) spent; which differ from Moore's (1980) eight stage classification in that stages 2 and 8 (slight developing and resting) have been combined to form stage '2' and stages 5 and 6 (mature and running ripe), combined to form stage 5.

The studies carried out in northern Australia and Papuna New Guinea (PNG) reveal the occurrence of protandric hermaphroditism (Moore, 1979, 1982; Moore and Reynolds, 1982; Reynolds and Moore, 1982; Davis 1982, 1985a; Russell and Garrett, 1983, 1985; Griffin, 1987). In all the studies, males dominated lower length classes and were absent in the higher ones. Sex inversion has been found to be intimately linked with the spawning season as females are derived from the post-spawning males (Moore, 1979; Davis, 1982, 1987). Oocytes first appear when the testes ripen for the final time. The spent testes directly transform into ovary and the transition is completed shortly after spawning. Morphologically transitional gonads are indistinguishable from testes (Davis, 1982).

The transitional males were detected in tidal waters in northern Australia and Gulf of Carpentaria (Davis, 1982, 1987) in contrast to the situation in Papua New Guinea where sex inversion is reported to occur in inland waters (Moore, 1979). A small proportion of synchronous hermaphrodites has also been observed with well developed ovarian as well as testicular tissue, supposed to be the abnormal developments (Moore, 1979; Davis, 1982). L. calcarifer has been reported to be digynous. Apart from the females derived from males (secondary females), a small proportion of female population develop directly from immature fish (primary females). Gonads of primary and secondary females are indistinguishable from each other; only females too young to have been functional males earlier can be identified as primary females (Moore, 1979; Davis, 1982, 1987). The size at first maturity of sea bass and sex inversion vary between localities (Davis, 1987). Chacko (1956) and Alukunhi (1957) reported that the size of the breeders range from 500 to 600 mm. Jhingran and Natarajan ( 1969) reported the minimum size at first maturity to be 425mm (persumably males; Davis, 1982), when the fish is two years old. Patnaik and Jena

(1976) encountered the smallest mature male of 505 mm TL. Males of more or less same size were used by Wongsomnuk and Maneewongsa (1974) in Thailand for induced spawning. The male sea bass mature in PNG in the length range of 510-700 mm (Moore, 1980). The size at first maturity of males in northern Australia and Gulf of Carpentaria has been reported to be 700-750 mm and 600-650 mm respectively (Davis, 1982, 1987). In Thailand, the females used in induced spawning trials ranged from 640-850 mm (Wongsomnuk and Maneewongsa, 1974). Patnaik and Jena (1976) observed smallest mature female of 700 mm. Moore (1979) reported the length of males detected to be undergoing sex change ranging from 910 to 990 mm with greatest proportion occurring in 950 mm length class in PNG. It was slightly smaller than the point of inflection on the Gompterz curve (calculated to be 896 mm) fitted to the sex ratio data of Reynold (1978) from PNG (Davis, 1982). Davis (1982, 1987) suggested that the predictions based on sex ratio/length relationship in different populations may be a reliable indication of size at which most of the fish are changing sex, particularly useful where direct histological observations are not practicable or feasible. Using this method, Davis (1982) predicted the length of transitional males in northern Australia (Mean length 907 mm TL; range 840-970 mm) and Gulf of Carpentaria (Mean 820 mm TL; range 680-900 mm). Davis (1984a) discovered a sexaually precocious population in the northeast Gulf of Carpentaria, changing sex at half of the size of other fish (mean length 490 mm TL). Any study indicating the occurrence of natural sex inversion in Asian sea bass is conspicuously absent in the literature. For a protandric hermaphrodite species, sex ratio of the population is obvious to favour males. Moore (1979) reported the greater proportion of males (3.8:1) in the sea bass population in PNG.

Most of the authors have described sea bass as annual breeder with single peak spawning (Dunstan,1959; Shetty et al., 1965; De, 1971; Gopalakrishnan, 1972; Patnaik and Jena, 1976; Kowtal, 1977; Moore, 1982; Russell and Garrett, 1983, 1985; Davis, 1985, 1987; Grey, 1987). However, Jhingran and Natarajan (1969) observed two peaks of spawning in Chilka lake, India. Spawning season and duration differ from place to place,however

the breeding is synchronized to the wet season so that larvae can take advantage of the aquatic habitat that results from rains, which provide food rich, predator free environment for the development of the youngones (Moore, 1982; Russel and Garrett, 1983; Davis, 1985, 1987; Grey, 1987; James and Marichamy, 1987).

Sea bass has been observed to be surface spawner (Moore, 1980), so local weather conditions may exert short term effect on the intensity of local spawning activity (Mackinon, 1985). Coastal spawning grounds are virtually close to the supralittoral nursery habitat of larvae and juvenile fish (Rusell and Garrett, 1983). Sea bass has been reported to spawn in the areas of salinity, 28-31 parts per thousand (Moore, 1982; Davis, 1985a). Movement of the fish to spawning areas and maturation of gonads is considered to be triggered by increase in water temperature which occurs at the end of dry season (Grey, 1987).

L. calcarifer is one of the most fecund fishes reported in the literature with maximum number of eggs reported upto $46 \times 10^6$ (Davis, 1984b). Wongsomnuk and Maneewongsa (1974) estimated the facundity of 2.1 to 7.1 million eggs in the weight range of 5.5 to 11.0 kg. Patnaik and Jena (1976) obtained fecundity estimate of $4.5 \times 10^6$ to $6.6 \times 10^6$ eggs in fish of 700 to 900 mm length, approximately equal to 0.76 million eggs per kg body weight. Moore (1982) inferred that the larger specimens being more fecund per kg of body weight. Regression line $F = 1.942\ W \times 10^6 - 13.816 \times 10^6$ was fitted to express the relationship between total weight (W) and fecundity (F) within the weight range of 7.7 to 20.8 kg. Davis (1984b) observed more variability in estimates for larger fish than those for smaller ones. The relationship between fecundity ($F = x\ 10^6$) with length (L,mm) and weight (W,g) was determined seperately as

$$F = 0.3089\ C^{0.035L}$$

$$F = 0.3089 \exp. (0.148\ W^{0.3391})$$

L. calcarifer is becoming commodity of attraction for acquaculture due to its fast growing as well as euryhaline nature and endearance to consumers as an excellent table fish. However, dependence on wild resources for seed had been a bottleneck in the progress of its culture and is still faced by countries including India. Captive seed production has been achieved in Thailand (Maneewong, 1987; Kungvankij, 1987a); Philippines (Fortes, 1987; Nacario, 1987; Garcia, 1988; Garcia et al., 1988; Garcia, 1990) Malaysia (Ali, 1986); Singapore (Lim et al., 1986; Cheong and Yeng, 1987) Australia (Garrett et al., 1987; Garrett and Rasmussen, 1987).

Initial success achieved in 1971 in artificial propagation by stripping the running ripe fish at the spawning ground followed by natural spawning of cultured broodstock in large tanks by hormonal (Wongsomnuk and Maneewongsa, 1974) and environmental manipulations (Kungvankij, 1987a) has been instrumental in the development of aquaculture techniques. Appreciation for the gradual improvements in the technology involved, though need more infrastructure facilities, becomes necessity keeping in view the advantages it promises, to enlist a few:

(i) Use of captive broodstock is not only more predictable in terms of yield and period but provide more scope for manipulation of breeding season and selective breeding (Garcia, et al., 1988; Garcia, 1990). It can avoid potential conflict with wild life stock resources and conservation concerns (Garrett and Rusmussen, 1987).

(ii) Natural spawning in large tanks reduce the chances of mortality due to handling etc. because of lesser stress the fish is subjected to; especially in comparison to hand stripping (Garrett and Rusmussen, 1987).

During the course of development of induced breeding techniques, considerable attention has been paid to the use of different hormones and their mode of administration (Table 1) with one or more aims in view. Apart from widely used hormones viz.,human chorionic gonadotropin (HCG),

**Table 1. Doses and mode of administration of different hormones used by various workers for induced breeding of L. calcarifer.**

| Hormones | Dose | Mode of Administration | Author |
|---|---|---|---|
| Puberogen (63% PSH and 34% LH) | 50-200 IU /Kg* | Injection | Kungvankij (1987a) |
| HCG + Homogenized pituitary glands of Chinese carp | 250-1000 IU/Kg<br>2-3 mg/kg | Injection | Kungvankij (1987a) |
| HCG | 40-250 IU/Kg (for females) 30 IU/Kg (for males) | Injection | Cheong and Yeng (1987) |
| HCG | 20-50 IU/Kg | Injection | Garret and Rasmussen (1987) |
| LH-RH-a | 10 /ug/kg | Injection | Nacario (1987) |
| LH-RH-a | 50-100 /ug/kg | Cholestrol pellet implants. | Nacario (1987) |
| LH-RH-a | 400 /ug. | Osmotic pump | Nacario (1987) |
| LH-RH-a | 50-100 /ug/fish | Cholestrol | Fortes (1987) |
| LH-RH-a | 25 /ug/kg | Injection | Garret and Rasmussen (1987) |
| 17α-Methyl Testosterone | 200 /ug/kg (for females) 100 /ug/Kg (for males) | Injection; cholestrol pellet implants | Garcia et al., (1988); Garcia (1988, 1990) |

* per kilogram body weight.

puberogen and Chinese carp pituitary extracts; luteinizing hormones releasing hormones as well as their analogues (D-Ala$^6$ LH-RH-a or D-Ser$^6$ LH-RH ethylamide) has been found to be effective agents. Slow release of analogues when administered through implanted pellets (cholestrol or cholestrol +cellulose mixture) or osmotic pump has made it possible to induce multiple spawning (Nacario, 1987, Fortes, 1987; Garcia, 1988). Combination of LH-RH analogues and 17 $\alpha$– methyl testosterone has been found to be useful in inducing early onset of sexual maturity as well as spawning (Garcia et al., 1988; Garcia, 1990).

Kunjvankij (1986, 1987a) described the method to achieve successful natural spawning of sea bass in captivity through environmental manipulation; reported to be the most preferred method in Thailand because it has consistently produced the largest quantity of eggs of the highest quality for a prolonged period.

Little work has been done on storage of sea bass sperms. Hogan et al., (1987)reported successful short term storage(20 days) of sperms using egg yolk-citrate diluent at 5°C. Poleung (1987) tried three cryoprotectants viz. dimethyl sulphoxide (DMSO), glycerol and methanol for their ability to cryopreserve viable sperms of sea bass. The dilution ratio of milt to diluent was 1:4 (V/V) and the initial freezing rate was 31°C/min. DMSO at a concentration of 5% with either 15 percent milk powder or 20 percent egg yolk gave the best results (post-thaw motility of spermatozoa was 70-100 percent for 7 min). Glycerol gave acceptable results when used at high concentration (20 percent) with 15 percent milk powder. Methanol provided little protection against freezing damage.

Sea bass culture is finding wide acceptability among aquaculturists in many of the countries in Asia, including India. Though considerable advancement have been achieved yet culture techniques still require improvement. Definite and precise information regarding stocking rate, feeding practices, environmental monitoring and other farm management practices are lacking (James and Marichamy, 1987).

In India, most of the reports on its culture are from extensive or semi-intensive systems accomplished in bhasabadha or bheris, paddy fields, feeder canals and other brackishwater impoundments, (Pillay, 1954; Pillay and Bose, 1957; Ghosh, 1971; Jhingran, 1977; James and Marichamy, 1987). Growth of the fish in these systems has been found to be fast and gross production of 2759.5 kg/ha was recorded in eight months (Jhingran, 1977). James and Marichamy, (1987) suggested better water exchange in the culture site to maintain salinity around 33 parts per thousand, adequate supply of trash fish as favourable conditions for culture. There exist a close relationship between stocking density and production (Genodepa, 1987; Khamis and Hanafi, 1987). Apart from the traditional culture system, fast development has been made in cage/pen culture in many countries like Thailand (Sirikul, 1989); Indonesia (Kungvankij 1987b); Malaysia (Ali, 1987); Singapore (Cheong and Yeng, 1987); India (Prasadam et al 1984).

To improve the efficiency of culture system, certain attempts have been made to grow sea bass in mixed culture which include Mugil tade and Mugil parsia (Anon; 1974); Eleutheronema tetradactylum (Anon, 1977), milkfish and tilapia (Bano and Amar, 1984) and tilapia (Fortes, 1987; Genodepa 1987 and Garcia, 1988). Culture with tilapia seems to be suitable alternative to trash fish as apart from providing forage fish, tilapia contributes to the production and at the same time its prolificity remains in check (Fortes, 1987).

Development and application of formulated diets has also been attempted and found promising to overcome the problems due to cost and non-availability of trash fish at times (Danakusumah and Ismail, 1987; Mackinnon, et. al., 1987). Walford et al (1991) attempted to rear larvae, with microencapsulated diet and observed that the live food facilitate digestion of protein memberane of microencapsules. Chonchuenchob, (1987) gave descriptive account of diseases of sea bass caused by parasitic organisms, bacteria, viruses, malnutrition and environmental stress in culture. Our knowledge pertaining to diseases of sea bass is very much in its infancy and paucity of specific disease data also exist. But the knowledge of

many other fresh water, estuarine and marine fish form the basis of a useful article by Humphray and Langdon (1987) documenting potential diseases with their causes, likely to occur with intensive production of sea bass.

The foregoing text highlights the status of scientific research on L. calcarifer especially in the areas of reproduction and artificial propagation. The technologies so far developed ensuring production of quality seed supply is not only significant to alleviate dependence of culturists on widely fluctuating natural seed resources but has potential to catalyse the future course of research to acquire manipulative skills of far reaching bearing on the advancement of the sea bass culture industry. Modern fish farming is a highly specialized intensive industry, more and more requiring possibilities to improve production, demanding increasing level of scientific effort. Altering growth rates, sexual cycles and sex-ratios, induction of maturation of gametes, ovulation and spemiation, storing over longer periods of time of eggs and sperm and artificial fertilization are techniques, commonly used for several species of commercial fish but still under development for others. The techniques to induce precocious maturity, control of sex differentiation, development and reversal can be of special significance in sea bass culture. In its natural cycle, fish may take around two to four years to mature as males and four to seven years as females as the latter are derived from males after sex reversal (Davis, 1982). Reduction in the length of period associated with sexual maturity can save time and cost besides avoiding loss of broodstock due to mortality etc. Though, such techniques can be achieved by trial and error and some results are impressive. However, more and faster progress demand for the scientific effort oriented towards achieving integration and balance between the knowledge acquired from basic and applied research. The former confines itself to the question "how biological process are controlled" and the latter's interest is in "how it can be governed to obtain a tool in the enhancement of aquaculture. A better understanding of mechanisms regulating biological functions makes it possible to exercise

varying degrees of control on the different stages of life, to adopt the techniques to fit new situations,then to teach and propagate the techniques to fish culturists.

The present work is an attempt towards this direction in addition to exploring the reproductive biology of the fish, which is the need of the time and prerequisite for the artificial propagation of sea bass in India.

# CHAPTER II
# MATERIALS AND METHODS

# CHAPTER - 1

# MATERIALS AND METHODS

## COLLECTION OF SPECIMENS

L. calcarifer does not contribute much to the regular coastal fishery of the Tuticorin area and is categorised among other perches in capture fisheries statistics. Specimens were collected from commercial catches, caught in gill net, shore seine operations. Dead specimens brought at landing centres were purchased through auction and were useful for supplementing to the data on biological parameters. Live specimens necessary for biochemical and ultrastructural studies were procured directly from fishermen.

## COLLECTION OF SAMPLES

Blood (from live specimens only) was taken out through cardiac puncture. After recording total length and body weight, the fish was dissected to remove liver and ovary. The various tissue samples were processed according to the requirements of the respective techniques to be discussed in the following text.

## ANALYTICAL METHODOLOGIES

Various methodologies adopted during the present work are described under the following heads.

### 1. Reproductive biology.

### (a) Morphology of gonad.

Macroscopic characteristics of the fresh gonad like colour, size and shape were recorded and classified corresponding to different stages of maturity.

**(b) Standard Gonad Weight (GS)**

Gonosomatic index (gonad weight/body weight) was found to vary with body size, hence, following the recommendations of deVlaming et al. (1982) logarithms of gonad weight and body weight were used to calculate the index to compare the monthly samples.To eliminate the effect of fish size from monthly samples, gonad weights were adjusted to a fish of standard length according to their sex and stage using the regression parameters from Table 2. The following relationship (Davis, 1985) was used to calculate standard gonad weight.

Where GS = GES X (GO/GEO)
GS = Standard gonad weight
GES= Expected gonad weight at standard length
GES= Expectedgonad weight at observed length.
GO = Observed gonad weight.

The standard length was set at 750 mm for males and 900 mm for females, representing approximately the average lengths of mature fish of each sex sampled during the study. Standard gonad weight was used as an index in place of gonosomatic index.

**(c) Oocyte diameter**

Oocyte size is a good indicator of maturation and gonadal cycle (Greelay et al., 1987; Davis, 1985). A representative piece from freshly collected ovary was teased in a drop of solution of 1 percent formalin in 0.8 percent sodium chloride on a glass slide. Oocyte diameter was measured along its horizontal axis using ocular micrometer, precaliberated with stage micrometer. Oocytes were measured at random till the count reached hundred cells. The data obtained was used to work out largest oocyte diameter and comprehensive oocyte size frequency profile.

(i) Average diameter of ten largest oocytes for each specimen was determined to, (a) sort out maturity stages (Davis, 1982); (b) define gonadal cycle by plotting monthly average of larggest oocyte diameter against the months around the year (Davis, 1985).

Table 2. Parameters of the regression equation log G = a + b log L , where G is gonad weight(g) and L is total fish lenth (mm) for different stages of maturity of L. calcarifer

| Gonad stage | a | b | Correlation coefficient |
|---|---|---|---|
| **Males** | | | |
| 2 | 1.9168 | 0.32028 | 0.72 |
| 3 | 2.01365 | 0.42156 | 0.67 |
| 4 | 1.2362 | -0.4728 | 0.52 |
| 5 | 0.9180 | 0.5668 | 0.76 |
| 6 | 1.6852 | 0.3908 | 0.68 |
| **Females** | | | |
| 2 | 4.1432 | 1.0864 | 0.78 |
| 3 | 2.6283 | 0.9472 | 0.82 |
| 4 | 1.9864 | 1.06194 | 0.61 |
| 5 | 3.1927 | 1.3628 | 0.73 |
| 6 | 2.5870 | 0.9861 | 0.76 |

(ii) A comprehensive oocyte size-frequency profile was developed by plotting frequency of oocyte size for every 50 µ class interval for different maturity stages, to trace the development of oocytes (Greeley et al., 1987).

**(d) Size at first maturity**

The fish specimens with testes undergoing active spermatogenesis (stage 3 onwards) were considered as mature. The frequency of mature specimens collected during spawning season forevery 50 mm length class was determined. The length at which 50 percent of the total individuals are mature was calculated as size at first maturity (Beverton and Holt, 1957).

**(e) Spermatogenic response and quantity of spermatozoa**

Spermatogenic response and quantity of spermatozea were rated in arbitrary units (-to +++) (Nayyar and Sundaraj, 1970) and were useful in the description of testicular cycle as well as different stages of maturity.

**(i) Spermatogenic response:**

-, only spermatogonia and no meiotic stages.
+, initiation of spermatogenesis, mostly primary spermatocytes.
++, Active spermatogenesis, stages of spermatogenesis including spermatids present.
+++, Spermatogenesis active, all stages of spermatogenesis.

**(ii) Quantity of spermatozoa:**

-; No spermatozoa
+, Few spermatozoa
++, More spermatids than spermatozoa
+++, Plenty of spermatozoa.

Each (+) sign was given a numerical value of one and (-) sign was equivalent to zero.

**(f) Fecundity**

Fecundity was estimated by subsampling method following that described by Greeley et al. (1986). Three subsamples weighing 200 mg each were collected from anterior, middle and posterior regions of the gravid ovaries (stage 5). The yolky eggs were separated on a glass slide in a drop of 1 percent formalin in 0.8 percent sodium chloride. Number of eggs were counted in each subsample and mean count was calculated. The fecundity was estimated using the formula:

$$F = \frac{n\,G}{g}$$

F = Fecundity
n = Number of eggs in the subsample.
G = Total weight of paired ovaries in grams.
g = Weight of the subsample in grams.

The regression equation log Y = a + b log X was fitted to the data to determine the relationship between fecundity and body weight (grams) as well as total length (mm). Here Y = fecundity; X = bodyweight or total length.

**(g) Sex ratio**

Total number of individuals belonging to each sex were counted and data was objected to chi-square test to verify if the sex ratio differ significantly from 1:1, male : female ratio (Davis, 1977b).

**2. Histology of gonads**

**(a) Light microscopic studies**

Small pieces of the gonad tissue, dissected out from the live fish, were fixed in Zenker's acetic (for 7 hrs) and 10 percent neutral buffered formalin (for 24 hrs). After thorough washing in running tap water for 6-7 hrs, tissues were dehydrated in graded ethanol ascending series following the standard procedure. Dehydrated tissues were cleared in pure chloroform

for three hours with three changes of one hour each. The tissues were infilterated with and embedded in paraffin wax with ceresin (Glaxo, melting point 58- 60°C).

To maintain the original shape and avoid breakage of yolky oocytes, double embedding (with cellodin and paraffin) method of Khoo (1979) modified by Gopalakrishnan (personal communication) was employed. The Smith's bichromate fixed ovarian tissue samples were washed, dehydrated and left in the 2 percent celloidin solution prepared in enquiproportional ethanol-ether mixture, for five hours in refrigerator. After clearing in pure chloroform for three hours, the tissues were finally embedded in paraffin wax. The blocks were trimmed, catalogued and stored till sectioning.

The serial sections of 4 - 6 µm thickness were cut manually using rotary microtome and placed on glass slides coated with Mayer's egg albumin. The sections were spread over a film of distilled water by placing the slides on the slide warmer maintained thermostatically at 40°C. The slides with well spread sections were left overnight on the slide warmer for drying. The dried sections were deparaffinized, hydrated and stained with Delafield haematoxylin/Herris haematoxylin using Eosin 'Y' as counterstain and Mallory's triple stain.

The stained sections were dehydrated, cleared and mounted using DPX as mounting medium. The histological details were studied and photographed using compound microscope (Carlzeiss Jena) with automatic camera and light control attachment. The negative film of 24x 36 mm (ORWO, 125 ASA) was used for taking photographs.

**(b) Electron micrscopic studies**

Techniques for ultrastructural studies, recommended by Hawkes and Stehr (1980) for marine organisms were adopted for processing the tissues for transmission electron microscopic studies. The gonads were dissected out while the fish was live and small pieces (3-4 mm size cube)

of gonad tissue were fixed in 3 percent gluteraldehyde in 0.1 M cacodylate buffer for 24 hours at 4°C, afterwards tissues were rinsed in buffer, trimmed (approx. 1-2 mm size cube) and washed repeatedly remove excess fixative. Secondary fixation was done by keeping the tissues in 1 percent osmium tetroxide at4°C for one and half hours, followed by thirty minutes at room temperature. After rinsing the tissues thoroughly in cacodylate buffer, the samples were dehydrated gradually in graded ethanol ascending series. Dehydrated tissues were impregnated with spurr resin (Spurr, 1969) by passing it through ethanol-spurr medium, mixture in ratios 1:1, 1:2, 2:1 and finally in pure spurrmedium. The tissueswere embedded in spurr medium using plastic moulds and curred for 8 hours at 60°C in BOD incubator. The polymerizedblocks were removed from moulds and stored till sectioning in labelled polyethene bags.

The blocks were trimmed and sectioned with freshly made glass knife fitted on ultramicrotome. Semithin sections of 1 /um size were stained with Methylene blue-Azure II/Basic Fuchsin or toluidene blue and observed under a compound microscope to select the desired areas. The ultrathin sections of 600 - 700°A thickness were taken over the copper grids (3 mm diameter) and dried. The sections were stained with saturated alcoholic uranyl acetate and Reynold's lead citrate. The dried sections were observed under Hitachi 600/Phillips cm 10 electron microscopes and desired areas photographed.

**3. Biochemical analysis.**

**(a) Muscle, liver and gonad tissues**

Muscle (white muscles from the dorsal part of the body), liver and gonads were dissected out from the live fish Pieces of gonad tissue were excised from the central region. Tissue samples were stored at-20°C till analysis.

**(b) Blood serum**

Blood samples collected from the fish at the collection site were transported in glass stoppered centrifuge tubes under ice.Blood was allowed

to clot at room temperature and centrifuged at 3000 rpm for twenty minutes. The supernatant straw coloured serum was decanted in a screw capped glass vial and stored at -2°C till analysis.

Methodologies adopted for various biochemical analysis are described under the following heads.

**i. Changes in certain biochemical constituents in gonad as well as somatic tissues in relation gonadal maturation.**

**(a) Moisture contents**

Samples wre cleaned and adhering water was blotted. A known weight (W) of the tissue was dried in hot air oven at 70°C and weight of dried sample (a) was determined. Moisture content expressed as percentage of wet sample was calculated as $\frac{w - a}{W} \times 100$

**(b) Total protein**

Total proteins were estimated by Folin-Clocalteu phenol method (Lowry et al., 1951).

Twenty five mg wet tissue (muscle, gonad and liver) was homogenized with 1 ml. cold trichloro acetic acid (TCA) using machanical homogenizer. 0.1 ml of serum was mixed with 1 ml of cold TCA. The extracts were centrifuged at 400 rpm for 10 min and supenatant decanted. The precipitate was dissolved in 5 ml of 1 N NaOH. To the 1 ml of this protein solution, added 5 ml of (50 ml of 2 percent $Na_2 Co_3$ in 0.1 N NaoH + 1 ml of 0.5 percent $CuSo_4 . 5H_2o$ in 1 percent sodium tartarate) and after keeping at room temperature for 10 min, add 0.5 ml of 1 N Folin-Ciocalteau's reagent and vortexed rapidly. The blue colour was allowed to develop at room temperature for thirty minutes and read at 600 nm against blank containing 1 ml of 1 N NaOH. The concentration of protein was calculated from standard curve plotted using 25 to 250 $\mu$/ml linear concentration of bovine serum albumin fraction V

**(c) Total cholesterol**

Total cholesterol was determined following the method of Henly (1957) as described by Varley (1975).

Twenty five mg of wet tissue samples (nuscle, gonad and liver) were homogenized and mixed well respectively with 10 ml of cold ferric chloride-acetic acid reagent (0.5 percent $FeCl_3.6H_2o$ in glacial acetic acid). 0.1 ml of serum was mixed with 10 ml of cold ferric chloride-acetic acid reagent. The mixture were left overnight at 4°C; centrifuged at 3000 rpm for 10 min. 5 ml of supernatant transferred to another glass tube, was treated with 3 ml of concentrated sulfuric acid and thoroughly mixed with cyclomixer. A blank containingg 5 ml of ferric chloride-acetic acid reagent was also run simultaneously. The colour was allowed to develop for 30 min and intensity was measured at 560 nm against blank.

Standard stock solution was prepared by dissolving 100 mg cholesterol per 100 ml acetic acid. To 1 ml stock solution 24 ml of ferric chloride-acetic acid mixture was added (final concentration - 0.2 mg/5 ml). Five ml of this mixture was transferred to another test tube and treated along with test samples.

Amount of cholestrol was calculated as following;

mg cholesterol/100 ml serum - $\frac{\text{OD of test sample}}{\text{OD of standard}} \times \frac{100}{0.05} \times 0.2$

mg cholesterol/100 mg muscle - liver/gonad tissue $\frac{\text{OD of test sample}}{\text{OD of standard}} \times \frac{100}{12.5} \times 0.2$

**(d) Total lipids**

Total lipids were estimated by sulphophosphovanillin method of Barnes and Black stock (1973). Ten mg of muscle, gonad and 5 mg of liver tissues were thoroughly homogenized in 1 ml of choloroform: methanol, 2:1 (V/V) each in cold and homogenate allowed to stand overnight at 4°C followed by thorough mixing and centrifuged at 2000 rpm for 10 min in glass stoppered centrifuge tubes. The clear supernatant solution containing

lipids was separated out in clean dry glass tube. For extraction of lipids from serum, 0.1 m of serum was thoroughly mixed with 1 ml of 2:1 chloroform-methonol (V/V) and left overnight.

0.5 ml of lipid extract contained in glass tubes were dried in vacuo over silica gel in a dessicator. 0.5 ml of concentrated sulfuric acid was added to the dried samples and shaken well. The tubes plugged with non-absorbent cotton, were heated at 100°C in a boiling water bath. The tubes were cooled to room temperature rapidly under running tap water. 0.1 ml of this acid digest was transferred to another clean glass tube, added 2.5 ml of phosphovannillin reagent and vortexed well. The colour was allowed to develop at room temperature for 30 minutes. The intensity of pink colour developed was read against blank at 520 nm.

Eighty mg of cholesterol was dissolved in 100 ml chloroform-methanol (2:1 V/V) mixture (equivalent to 100 mg of total lipid in 100 ml chloroform-methanol (2:1 V/V) mixture) and used as standard. This solution was freshly prepared. To prepare the working solution different concentrations of stock solution in the range (50 - 500 mg) were taken and the treated as in test samples. Standard graph was prepared by plotting concentration of standards against optical density and amount of lipids in the test sample was calculated .

**Serum protein bound phosphorous (alkali labile phosphorous**

The method of determination is based on that described by Craik and Harvey (1984). A volume of 0.5 ml serum was mixed with 5 ml 20 percent TCA and allowed to stand overnight at 4°C. The tubes were centrifuged for 10 min at 2000 g and supernatant was discarded. Compact lipophosphoprotein precipitate was washed again with 5 ml TCA, repeating the process. The solid precipitate was broken up with a glass rod and sequentially extracted with the absolute ethanol at 60°C for 10 min; ethanol-diethyl ether-chloroform (2:2:1), acetone, diethyl ether. The tubes were allowed to stand 2-3 hr at room temperature to dry the solids. The samples were treated with 1 ml 2 N sodium hydroxide, heated in a boiling water

bath for 20 min, cooled, treated with 1 ml 2 N hydrochloric acid, 5 ml isobutanol-toluence (1:1), 1.0 ml 0.2 M tungstosilicic acid and 1 ml 40 mm ammonium molybdate in 1.25 M $H_2So_4$. After shaking the tube for 25 sec, the tubes were centrifuged to seperate the phases and 1 ml of the upper (organic solvent) phase was removed and treated with 0.2 ml freshly prepared dilute stannous chloride (1 ml of percent $SnCl_2$ $2H_2o$ in 2.5 ml Conc. HCl diluted to 200 ml with 1 N $H_2So_4$) and 3.8 ml 2 percent ethanolic $H_2S_4$. The optical density of blue colour was read at 650 nm, against a blank (reagents mixture only) within 2 hrs of mixing.

Standard phosphate solution was prepared by dissolving 219.5 mg anhyd. $KH_2PO_4$ per litre of distilled water (1 ml = 0.05 mg/.50 /ugP) 0.5 ml of the standard solution was treated identically to the sample tubes in triplicate. The amount of protein bound phosphorous was calculated.

Protein bound phosphorous ( /ug/ml serum)

$$= \frac{\text{OD of the test sample}}{\text{OD of the standard}} \times 50$$

**Total serum calcium**

A volume of 0.1 ml serum was digested in 2 ml conc. nitric acid and 1 ml perchloric acid at 150°C for 2 hrs. The samples were diluted to 5 ml with 2 ml of 0.1 percent lanthanum chloride solution. The samples were analysed by atmoic absorption spectrophotometer (Perkin - Elmer-2380) using air-acetylene flame, at the wavelength of 422.7' mm against blank (0.1 percent lantharum chloride). Calcium content were calculated from standard curve prepared using different concentrations of calcium corbonate 1.249 g of calcium carbonate (predried at 120°C for 4 hrs and cooled in a desiccator) was dissolved in 50 ml of deionized water and 10 ml of conc. HCl was added dropwise. The solution was diluted to 1 Litre with deionized

water (1 ml = 0.5 mg/500 µg Ca). A series of standard metal solution containing 50 to 500 µg/ml were prepared by diluting with 0.1 percent lanthanum chloride solution.

### (ii) ELECTROPHORETIC IDENTIFICATION OF SERUM VITELLOGENIN

Samples of serum at different maturity stages were analysed electrophoretically to identify the vitellogenin (the female specific protein) in serum. The polyacrylamide gel disc electrophoresis method of Davis (1964) as given by Subhashini and Ravindranath (1981) has been followed with necessary modifications.

### REAGENTS

**A. Running gel:**

a) Acrylamide : 4% in double distilled water

b) N,N' methylene bisacrylamide (bis) : 2.1% in double distilled water

c) Small pore buffer : 1 N HCL - 48 ml
Tris - 36.6 g.

N, N, N', N' - Tetramethylethelene (TEMED) - 0.23 ml.
diamine.

Dissolved in double distilled water and final volume made upto 100 ml with double distilled water; pH adjusted to 8.9

d) Catalyst : 0.149 ammonium per sulphate in double distilled water.

e) Double distilled water.

**B. Spacer gel:**

a) Monomer solution : 10g acrylamide and 2.5 g methylene bis acylamide in 100 ml double distilled water (the concentration of spacer gel monomer is 3%)

b) Riboflavin : 4 mg per 100 ml. double distilled water.

c) Sucrose : 40 g per 100 ml double distilled water.

**C. Tank buffer:**

**Stock solution:**

Tris - 6 g
Glycine - 28.8 g

Dissolved in 100 ml double distilled water and volume made upto one litre; pH adjusted to 8.3.

**Working solution**

Diluted 60 ml of stock solution to 600 ml with double distilled water.

**D. Indicator solution:**

Stock solution: - 0.1% bromophenol blue in double distilled water.

Working solution: - 0.5 ml of stock bromophenol blue diluted to 5 ml with 40% sucrose.

**Procedure:**

**Polymerization of gel:**

**Running gel:** Running gel was prepared by mixing solutions of acrylamide, small pore buffer, double distilled water and catalyst in the ratio 3.5:2:4.5:5.5 so as to get final concentratiopn of acrylamide and bisacrylamide 7% and 2% respectively in the gel. Gel tubes (Total length = 75 mm, internal diameter - 5 mm) were fitted over a stand with one end sealed tightly. Reagent mixture prepared was poured carefully in gels avoiding trapping of air bubbles. A few drops of double distilled water were overlaid so as to avoid miniscus formation. The stand with gel tubes was left undisurbed at room temperature for 10-25 minutes to allow complete polymerization.

**Spacer gel:** Spacer gel was prepared by mixing large pore buffer, monomer-solution, riboflavin and sucrose in the ratio 1:2:1:4.

After the polymerization of running gel, overlying water layer was blotted and 4-5 mm thick layer of spacer gel was poured, a few drops of water were overlaid and polymerized by photoactivation under ultraviolet light.

**Sample preparation:** Serum samples were prepared just prior to use, by mixing 0.2 ml of clear serum in 1 ml ice cold double distilled water.

Water column above the polymerized spacer gel was blotted out. Serum samples (60 µl/tube) were loaded carefully over the gel using a digital finnpipette (Lab systems, Finland). Following this 40 µl of marker dye (bromophenol blue) was added to each tube and mixed with sample gently using finnpipette. The remaining space in the gel tubes was filled upto brim by dropping diluted tank buffer using a syring, along the sides of the tubes.

The gel tubes were carefully removed from the stand and inserted into the grommets of the upper buffer tank. Drops of dilute buffer were suspended from the lower end of the gel tubes so as to avoid trapping of air bubbles during the run. About 300 ml of the working buffer was poured in the lower tank and the upper tank with tubes was placed over this. The remaining tank buffer was carefully added along the sides of the upper tank using a clean glass rod, taking care not to disturb the sample in the tubes. After covering the upper tank with the lid, the whole unit was placed inside the refrigerator to avoid rapid increase of temperature during the run. The tanks were then connected to the powerpack and a steady current of 1 mA/tube was supplied until the marker dye reached the lower end of the spacer gel, after which the current was increased to 3 mA3tube. A steady current was maintained till the marker dye reached the lower edge of the gel tubes.

On the completion of the run, the tubes were removed from the grommets and placed in order in a petridish containing double distilled water. The gels were removed from the gel tubes carefully by forcing

a jet of water between the gel and inner wall of the glass tube using a syringe fitted with a thin needle. The gels were stained with apprropriate stains.

**Staining procedures:**

**a. Detection of protein fraction:** The gels were incubated in 10% trichloro-acetic acid (TCA) solution for 30 minutes till the bromophenol blue mark became yellow, followed by staining in 0.25%. Coomassie brilliant blue staining solution (0.25 g stain in methanol, double distilled water and acetic acid in the ratio 5:5:1) for 20-30 min in dark. The gels were destained in darkness in methanol, water and acetic acid mixture in the ratio 5:5:1 for 30 minutes with two changes. Destained gels were stored in 7% acetic acid in dark.

**a. Localization of lipoprotein moiety:** The gels were incubated in saturated oil red 'O' solution of 50% methanol containing 10% TCA for 3 hrs at 60'C. Development of reddish orange colour indicates the presence of neutral lipids. The gels were stored in staining solution itself.

**c. Localization of polysaccharide moiety:** The gels prefixed in 12.5% TCA were rinsed with distilled water for one hour and placed in 1% periodic acid in 3% acetic acid for another one hour. The gels were washed thoroughly in distilled water for one hour followed by treatment with Schiff's reagent for one hour in dark at 4°C. The gels were destained in 1% sodium metabisulphite and stored in 7% acetic acid in dark.

**d. Localization of calcium containing protein fraction:** Freshly removed gels were treated with Alizarin Red-S (pH 6.5) for 10 min., followed by rinsing in the distilled water and acid ethanol (0.1% HCl in 95% ethanol) until the background is destained completely. The gels were stored in 7% acetic acid in dark. Calcium bound proteins stain deep reddish orange.

**Interpretation of results:**

Relative mobility value ($R_f$ = Distance travelled by the; protein fraction/ marker dye) for each band was calculated and electrophorograms were drawn for each maturity stage.

# CHAPTER III
# RESULTS AND DISCUSSION

## SECTION I

## SEXUALITY, FECUNDITY AND SEASONNAL GONADAL CYCLE.

Most teleost species exhibit reproductive cycling. The reproductive cycles are characterized by pronounced variations in gonad size and structure; distinguished into different stages of maturity. Usually examination of female gonad is preferred for the description of reproductive cycle (West, 1989). However, analysis of male reproductive stages becomes necessary in certain situations when specific information like size of maturity, sexuality etc. are desired or females are scarce in the population (Davis, 1982).

Evaluation of gonad maturation has been used extensively for determining reproductive status as well as the stock that is reproductively active, of several commercially important fishes. Detailed information regarding the breeding requirements is necessary to allow an accurate estimation of potential hatchery production (Mackinon, 1985).

L. calcarifer, a large centropomid teleost, is important as a valuable natural resource for many countries in the Indo-Pacific region. It exhibits divergence in the characteristics of its sexuality, maturity and reproductive cycle in different localities; hence needed to be established throughout the range. It warrants biological investigations to get a better understanding of its behaviour under natural conditions and assist as well as improve traditional culture of the species.

Detailed information on the breeding biology of sea bass from Indian waters remains obscure despite wide spread interest in its culture and exploitation. A few existing reports are, of Patnaik and Jena ( 1976); Kowtal (1977); Roy et al., 1977 ; James and Marichamy (1987) reviewed the status of sea bass culture in India and attributed the lack of adequate information to several factors like non-availability of the large number of specimens at times and high cost.

The ongoing chapter deals with the description of sexuality, maturity fecundity and seasonal reproductive cycle in the Tuticorin area (south-

east coast of India) .

## DESCRIPTION OF STUDY AREA.

As shown in fig. 1, the area under present study has one major river, Tambraparni, besides a few creeks. Tambraparni river originates from the western ghats, after passing through the plains of Thirunelveli and Chidambarnar districts enters into Gulf of Mannar, through deltaic system at three places Kayalpattinam, Punnaikkayal and Palayakayal. (Fig.1 and 2). Across the river, at the Pappanasam area three reservoirs have been built up for conservation of water as well as electricity generation. The water discharge is strictly regulated released just sufficient to cater the agricultural and other human related requirements. Hence, river maintains poor flow of freshwater throughout the year.

The region understudy experiences poor rainfall, the major rains are due to north east monsoon from October to December (Fig.3). Moreover, the rains are mostly discontinuous (Fig.4). The average monthly temperature ranges from 25° to 31°C.

The shore line is partly muddy and sandy; the areas adjoining Tambraparani estuary and Korampallam creek are swampy, infringed with thick mangroove vegetation.

Sea bass though, distributed throughout the region, is more concentrated near Punnaikkayal.

## RESULTS

Gonads of L. calcarifer are paired and strongly dimorphic.

### Testes:

The testes are elongated flat structures positioned between swim-bladder and ventrolateral wall of the abdominal cavity, with their smooth dorsal surface apposed to the former. Ventral surface is characterized

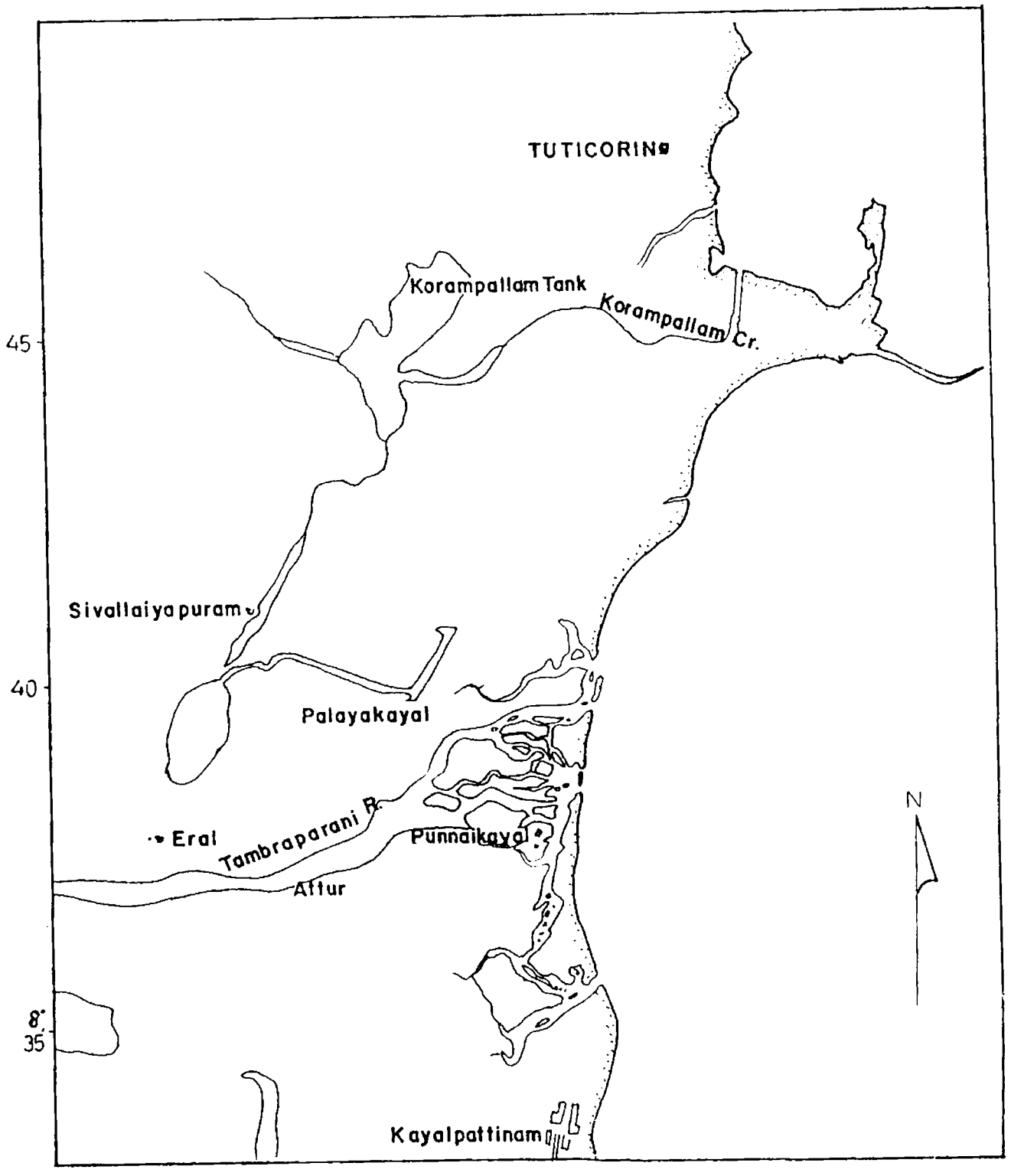



FIG. 1. Map of the area of southeast coast of India studied, showing major river system and creeks.

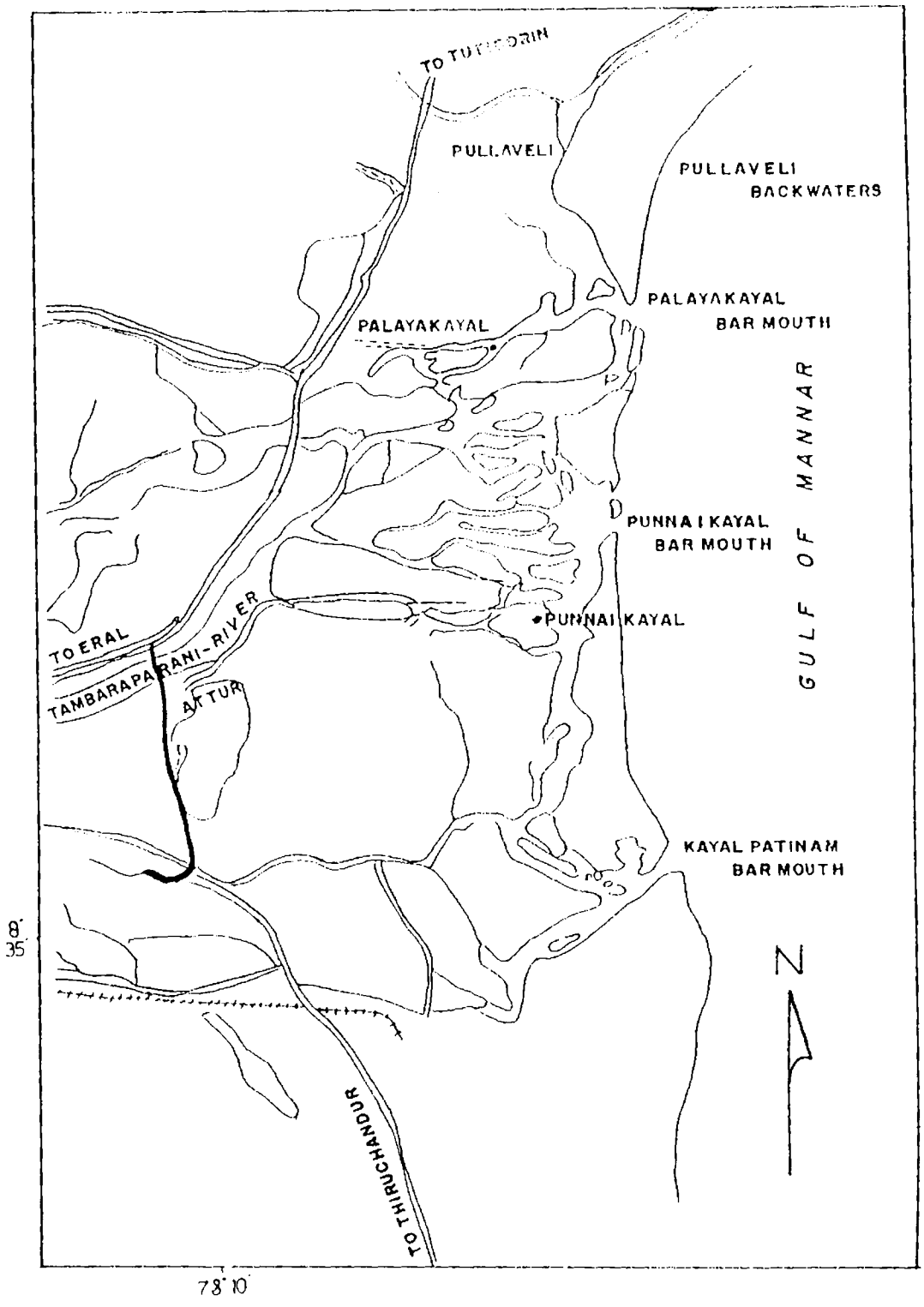



FIG. 2. Map of the estuary of Tambraparani river.

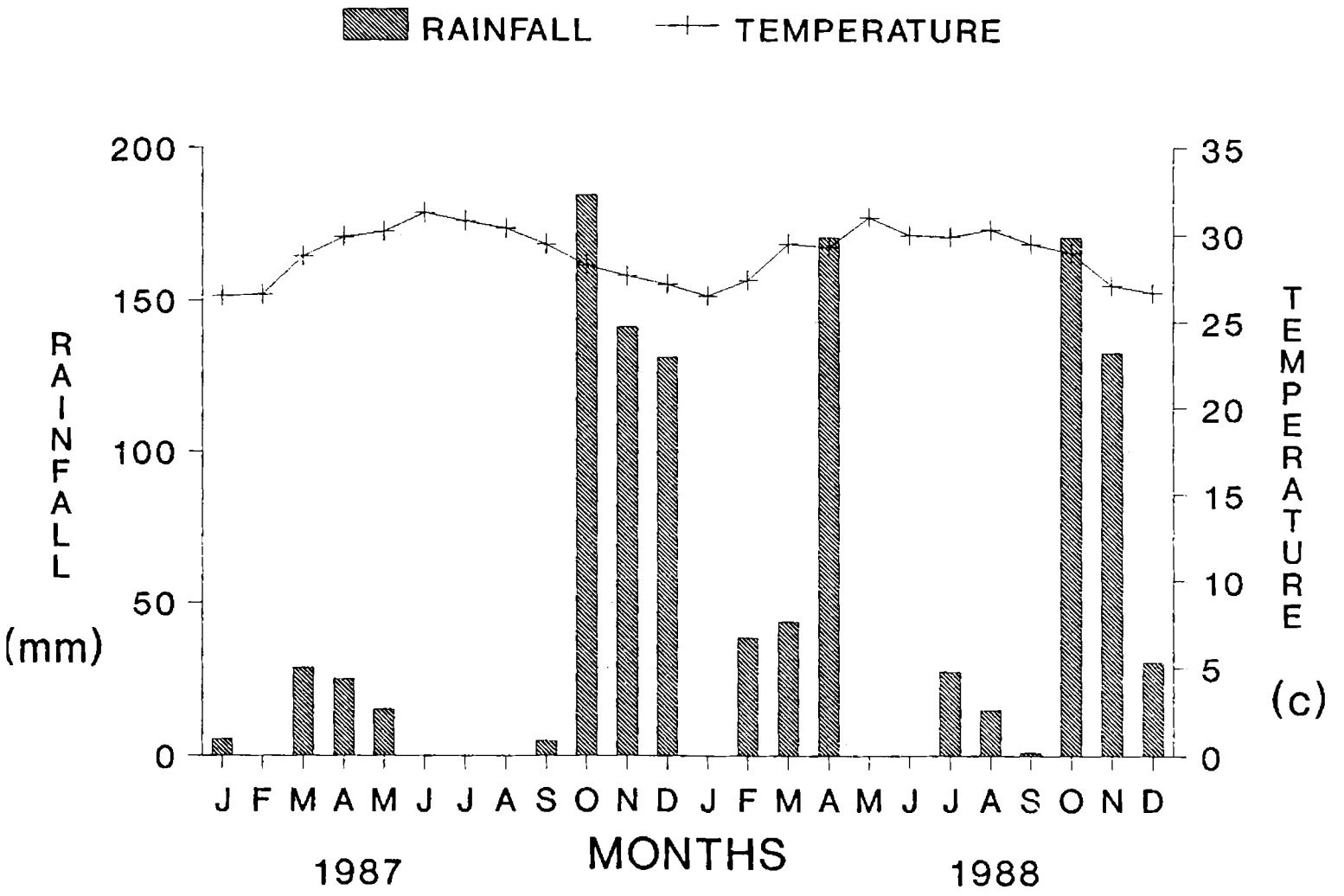



FIG. 3. Monthly rainfall and temperature variations, in the study area for the years 1987 and 1988.

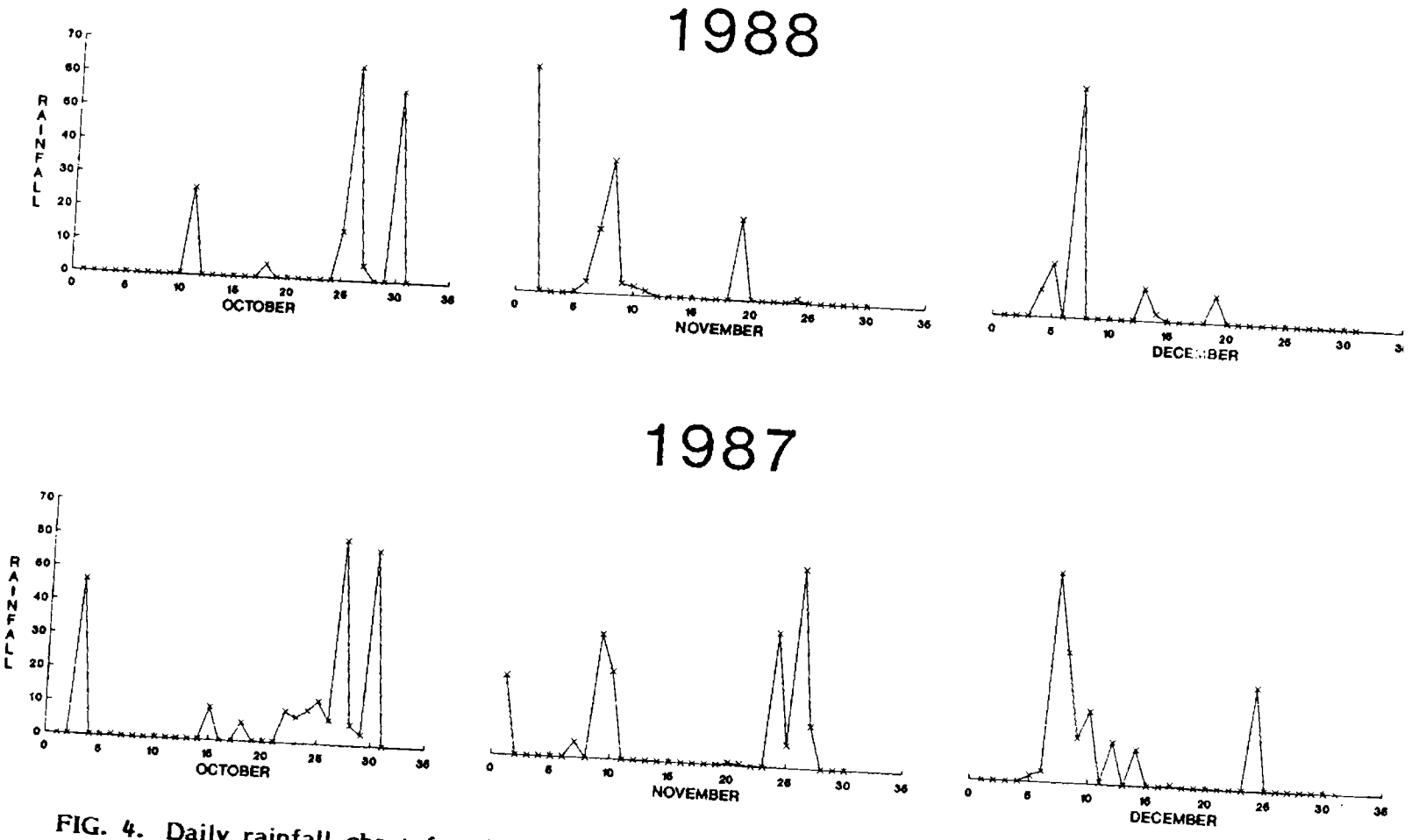



FIG. 4. Daily rainfall chart for the months October to December during the years 1987 and 1988.

by the furrowed appearance due to longitudinal elongation of leaf-like lobes. Two testes join posteriorly to open through a common aperture. There is no seminal vesicle or other specialised storage organ.

**Ovaries**

The ovaries are located ventrolaterally on either side of the abdominal cavity. Internally it consists of numerous lamellae; site for the oocyte development and growth. These lamellae are conspicuously absent in an area in the ventrolateral wall which broadens posteriorly, as a longitudinal channel, leading to common oviduct formed by tapering and joining of the posterior ends of the two ovaries.

Salient features of testes and ovaries corresponding to various stages of maturity are described in the Tables 3 and 4 respectively. Though largest oocyte diameter is useful criteria to trace the stages of ovarian recrudescence; but it failed to distinguish itself, between previtellogenic and postspawn ovaries. Comprehensive oocyte size-frequency profile (Fig.5) illustrate better, the pattern of oocyte development as well as ovarian staging system. It is evident from the oocyte size frequency profile that L.calcarifer has group-synchronous type oocyte development, where a discrete clutch of oocytes distinguishens from rest of the population in stage 3. The oocytes in this clutch enter gonadotropin-dependent phase (cortical alveoli stage, Fig.6). Out of this clutch, second clutch is recruited into vitellogenic phase at stage 4 (Fig . 5,6), forming the single leading clutch of synchronously developing oocytes. The pattern of oocyte development does not provide any evidence of multiple spawning in L. calcarifer.

**SEXUALITY**

Except the two female specimens detected with total length 480 mm and 500 mm, the fish less than 775 mm were exclusively males. Fig. 7 presents relationship between total length (TL) and maturation within the length range of 200 to 800 mm. Out of 210 specimens 14.2 percent were detected to be at stage 0 (TL range 225 to 475 mm), 29.9 percent at stage 1 (TL range 275 to 675 mm and 55.9 percent at stage 2-6 (TL, range 475 to 725 mm).

Table 3. Salient features of testes of L. calcarifer corresponding to various stages of maturity

| Stage | Macroscopic characteristics | Histological characteristics | Spermatogenic response | Quantity of spermato-zoa | Standard gonad weight GS(g) |
|---|---|---|---|---|---|
| State 0 | The testes indistinguishable from the viscera. | | | | |
| Stage I (imma-ture) | The testes are light pale, thin and semitransparent, distinguishable from rest of the viscera by the appearance of characteristic longitudinal furrows on the ventral surface. | The undifferentiated stroma cells with large vacuoles form major part of the lobe primordial germ cells with highly basophilic nuclei present in clusters. | 0.00 | 0.00 | 1.8217 |
| Stage 2 (deve-loping) | The testesare opaque, light pale with deep longitudinal furrows. | Can be classified into developing virgin and recovering spent.<br>Lobules contain mainly spermatogonia 'type A' along the periphery, with a few cysts of spermatogonia 'type B' and primary spermatocytes. | 0.60 | 0.00 | 4.6903 |
| Stage 3 (matur-ing) | The testes thicken and are wedge-shaped in cross section. The spermatozoa squeeze out from the cut testes. | Most of the interstital areas are occupied by the lobules, contain mainly the cysts of spermatocytes and spermatids with spermatozoa in the lumina and spern ducts ducts some spermatogonia are still present. | 1.48 | 0.32 | 6.8814 |
| Stage 4 (mature ) | The testes are thick with rounded lateral margins and prominent ventral lobes. | Lobules are characterized by thin walls and lumina are packed with spermatozoa. Cysts of spermatocytes and spermatids still present but no active spermatogonia. | 2.96 | 2.14 | 10.7391 |
| Stage 5 (ripe) | The ventral lobes are enlarged considerably. Pressure on the belly causes milt to extrude from urogenital aperture. | Lobules and ducts are packed with spermatozoa . | 2.80 | 3.00 | 15.1014 |
| Stage 6 (spent) | The testes appear collapsed and appear like a strap. | The lobule walls are wrinkled and start thickening; lumina still have residual spermatozoa. | 0.12 | 1.11 | 6.8838 |

**Table 4.** Salient features of ovaries of L. calcarifer corresponding to various stages of maturity.

| Stage | Macroscopic characteristics | Histological characteristics | Largest oocyte diameter ( /um) | Standard gonad weight Gs (g) |
|---|---|---|---|---|
| Stage 1 (newly formed) | The ovaries are deep reddish in colour, recently derived from testes after sex inversion. | Ovigerous lamellae are packed with chromatin nucleolus and early perinucleolus stage oocytes with a few at late perinucleolus stage. | 110 | 78.1041 |
| Stage 2 (developing virgin/ recovering spent) | The ovaries are thick walled, translucent, with pinkishred hue. | Chromatin nucleolus stage oocytes present but perinucleolus stage oocytes dominate. | 140 | 82.9406 |
| Stage 3 (maturing) | The ovaries increase in size, translucent and cream in colour | Perinucleolus stage oocytes are present; oocytes entering gonadotropin dependent phase (cortical alveoli stage) predominate the population. | 160 - 270 | 168.1038 |
| Stage 4 (mature) | The ovaries are markedly larger in size, with thinner wall and yellow hue start appearing | Ovigerous lamellae are packed with yolky oocytes visible to the naked eye. Most of the oocytes are at early and late vitellogenic stages, forming the leading clutch Previtellogenic stages also coexist with this clutch. | 260 - 500 | 406.7025 |
| Stage 5 (ripe) | The ovaries are deep yellow in colour, with thin walls, distended and occupying most of the body cavity | The oocytes undergo final maturation, migration of germinal vesicle and break down, coalescence of oil droplets. | 450 - 700 | 628.793 |
| Stage 6 (spent) | The ovaries are flaccid, reduced in size, with purple yellow colour. The wall becomes tough and wrinkled. | Post ovulatory follicles; previtellogenic oocytes dominate along with a few residual yolky oocytes. | 140 | 127.4911 |
| Stage 5A. (involuting) | The ovaries are deep red in colour, progressively decrease in size and have watery consistency. | Vitellogenic oocytes at various stages of atresia, often surrounded by blood vessels. | | |

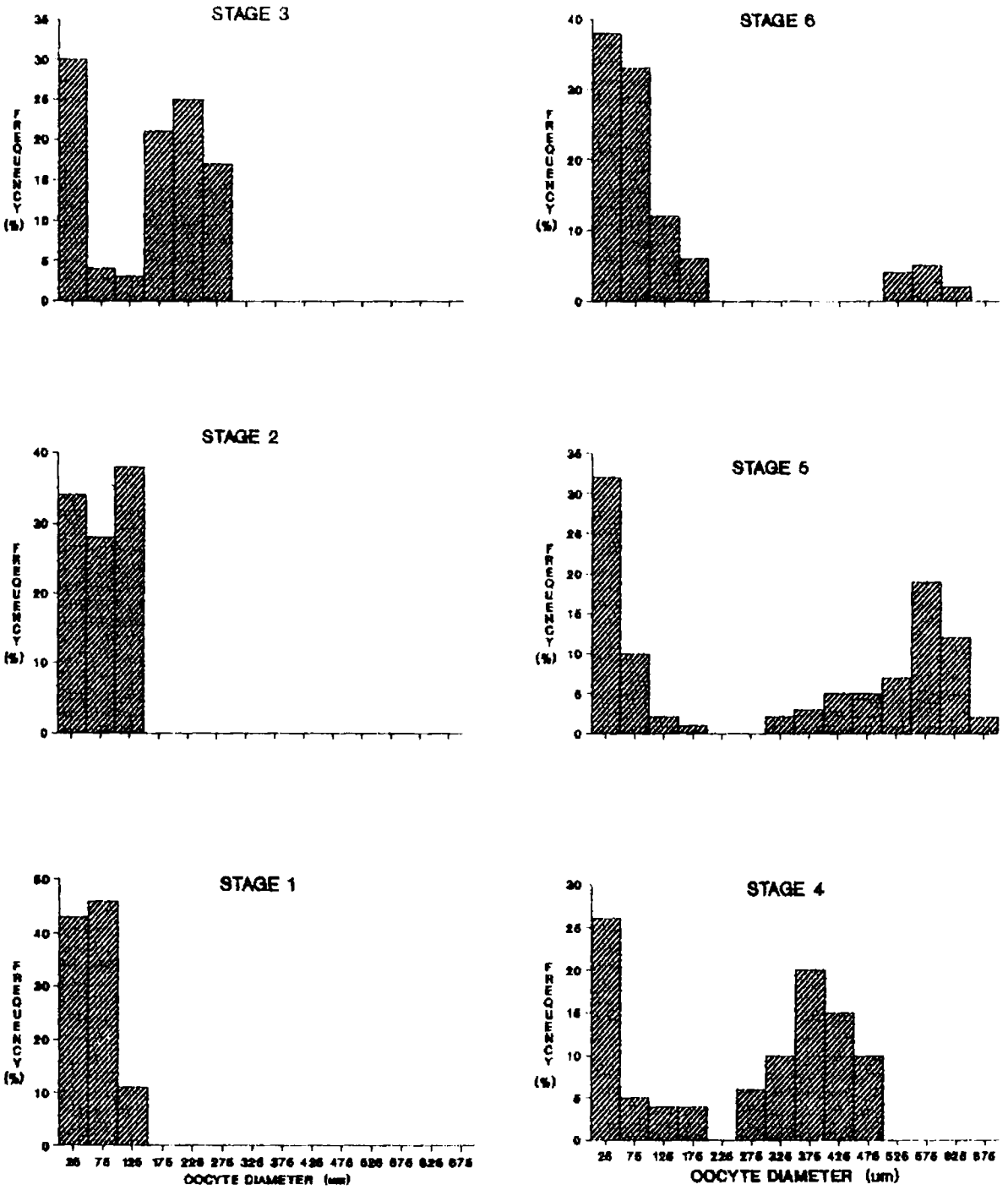



FIG. 5. Representative oocyte size-frequency profiles corrresponding to stages of ovarian recrudescence in L. calcarifer.

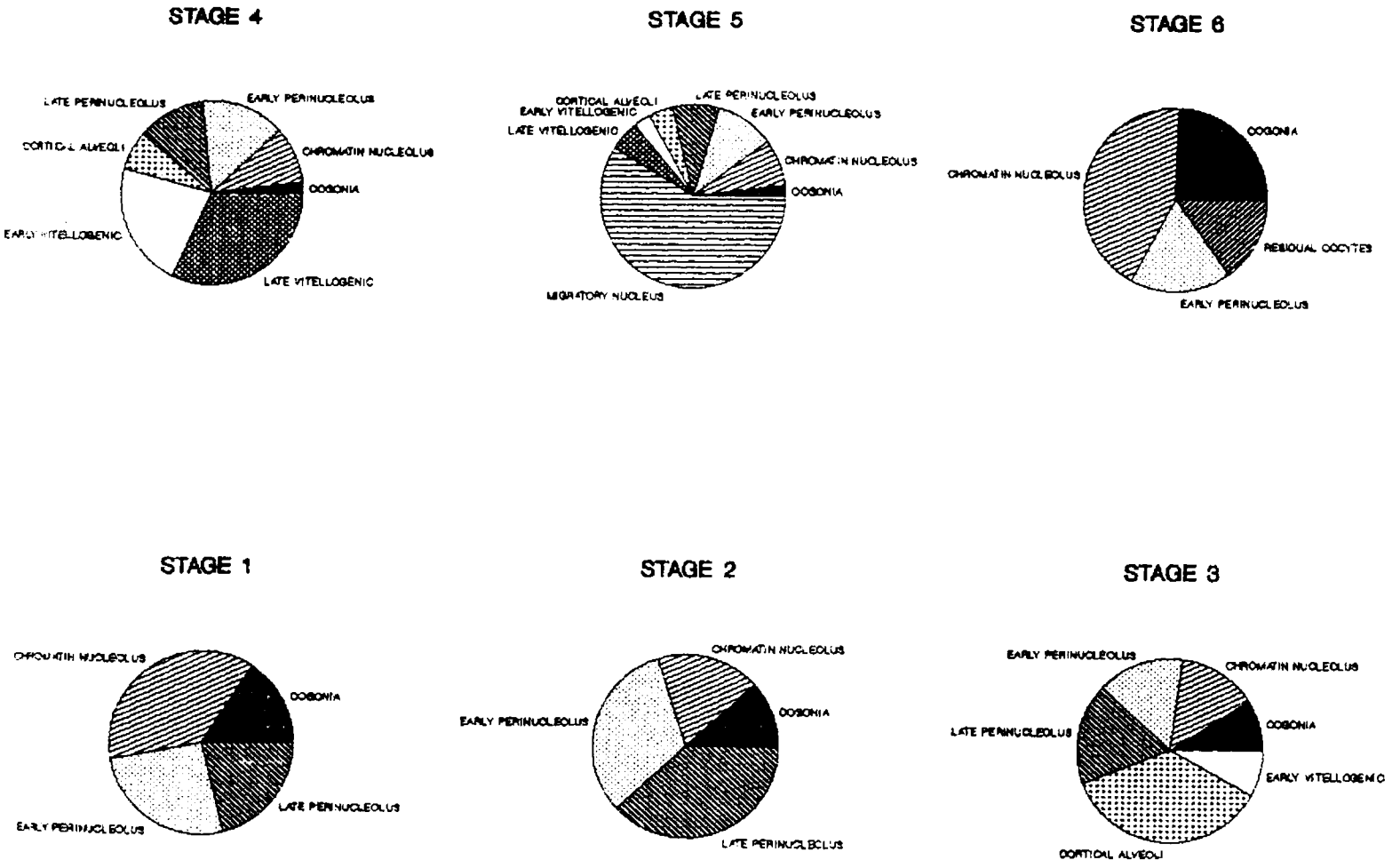



FIG. 6. Representative stages of oocyte development corresponding to stages of ovarian recrudescence in L. calcarifer.

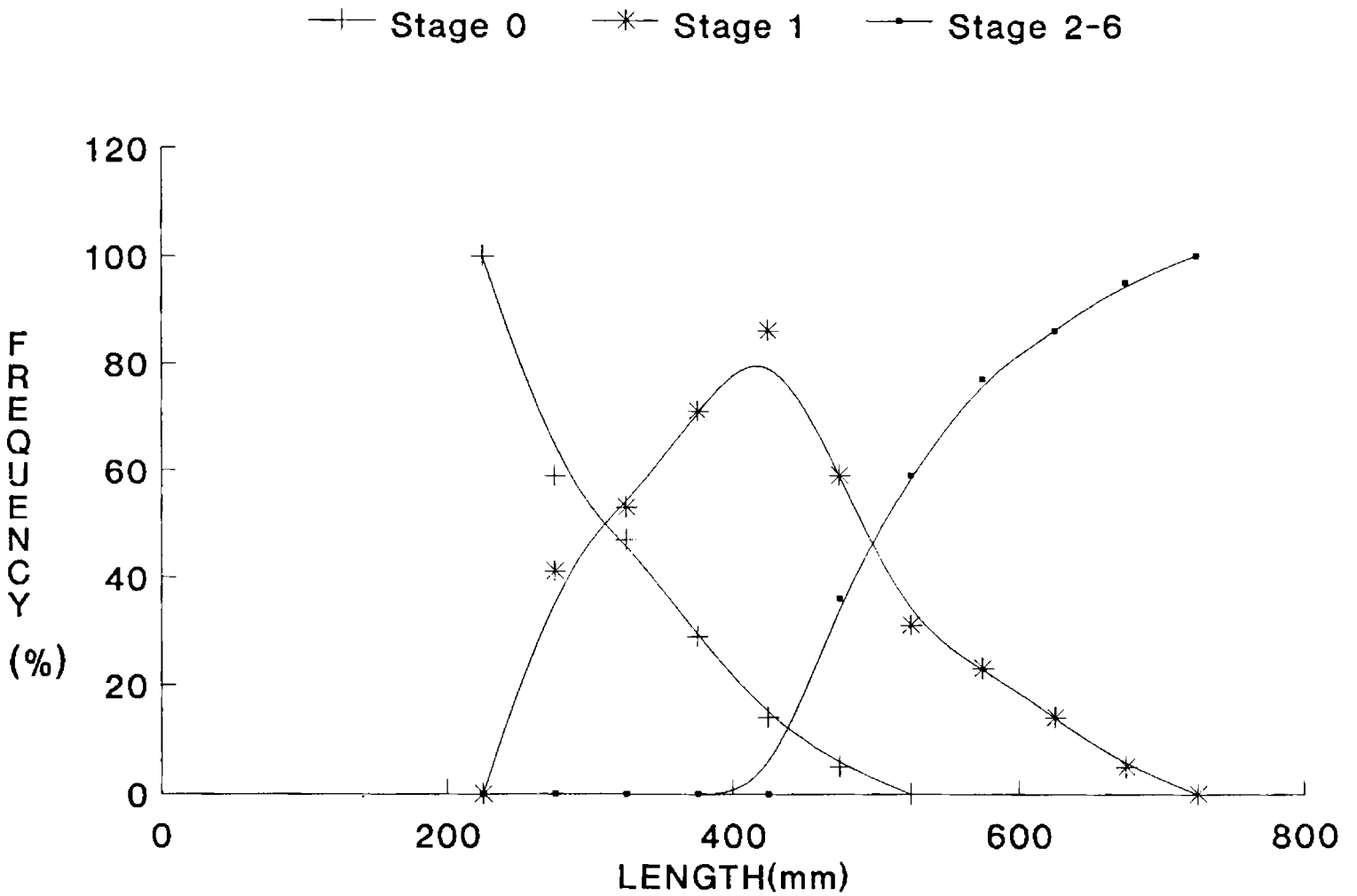



FIG. 7. Relationship between total length and maturation of L. calcarifer in the length range of 200 to 800 mm.

Analysis of length frequencies of 570 adult specimens (Fig.8) reveal a zone of overlap in the length range of 750 to 1000 mm occupied by both the sexes, with progressive decline of male proportion, as length increases. The male peak in length frequencies occurs at 775 mm TL; females constituting only 5.67 percent of the length class. The female peak occurs at 975 mm TL, the males constituting only 3.78 percent of the length class. The length class 1025 mm and above consisted of only females. The shift of female zone towards right with respect to the male zone in the figure, indicates that the L. calcarifer is a protardric hermaphrodites. The majority of the females are derived from the previously functioning males through sex inversion, hence designated as secondary females.

**Primary Females:**

Two female specimens of 480 and 500 mm TL, with gonads morphologically similar to that of secondary females, were collected. They were too young (even smaller than the size at first maturity of males) to have functioned as females previously and might be representing the population, directly derived from the immature fish. Both the specimens were collected during the breeding season in the year 1988, but histological examination reveals the predominence of chromatin-nucleolus stage oocytes along with perinucleolus ones but no sign of vitellogenic growth.

**Large size males:**

One male specimen of 1275 mm TL (19.5 Kg) with stage 4 testes was collected, indicating that all the males not necessarily undergo sex inversion.

## LENGTH AT FIRST MATURITY

The milt is discrenible first at stage 3 and onwards. Till spent stage (stage 6), the testes undergo active spermatogenesis. All the males between stage 3 to 6 are considered to be mature. The smallest mature male encountered was of 570 mm TL. The length at first maturity is


MALE
FEMALE
LENGTH (mm)
575
625
675
725
775
825
875
925
975
1025
1075
1125
1175
1225
ZONE OF OVERLAP
25
20
15
10
5
0
5
10
15
20
FREQUENCY (%)

defined according to Beverton and Holt (1957) as the length at which fifty percent of the males mature. The length frequency analysis of mature males collected during the months of greatest gonadal activity (August to December indicate that this level is reached at 605 mm TL (Fig.9).

## LENGTH AT SEX INVERSION

The percentage distribution of females in each length class is plotted (Fig.10), based on the data from dissection of 303 specimens. The point of greatest slope (the point of inflection) on the curve corresponds to the length at which highest proportion of males are changing sex (Davis, 1982). The length comes out to be 885 mm.

Histological examination detected 14 transitional males. The mean length of transitional males was found to be 835 mm (length range 790 to 910 mm TL .)

The relative distribution of transitional males detected, for different length classes (Fig. 11) reveals that the highest sex change occurs in 825 mm length class.

## TIMING OF SEX INVERSION

The transitional males were detected from November to February with highest proportion occuring in January (Fig. 12).

## SEX RATIO

The sex ratio was found to be
0.83:1,in favour of females ($\chi^2$ = 4.6864)

## FECUNDITY

Fecundity estimates were found in the range of $3.85 \times 10^6$ to $30.18 \times 10^6$ eggs in the fish of length range 855 mm to 1275 mm. The relationship of fecundity to body weight (W; Fig.13, and total length (L; Fig. 14) observed, are given below.

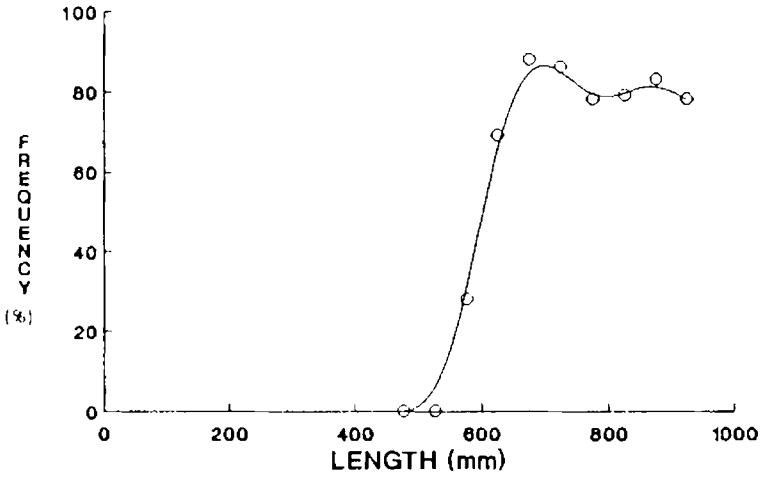



FIG. 9. Variation of the frequencies with length of mature male L. calcarifer

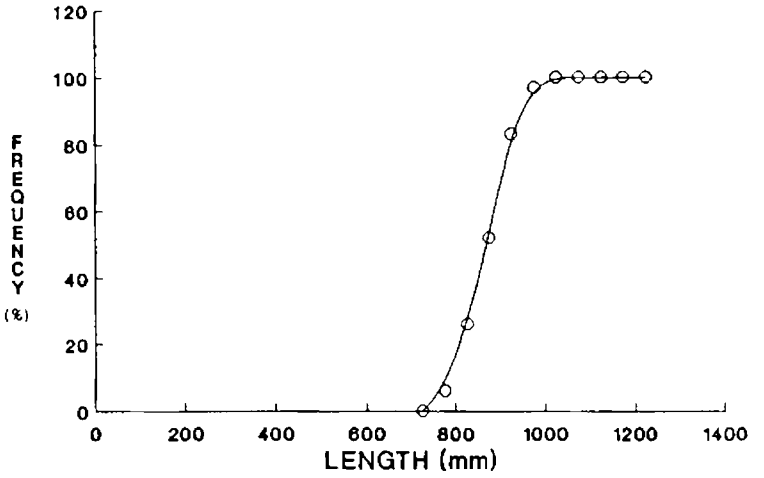



FIG. 10. Variation of the frequencies with length of female L. calcarifer.

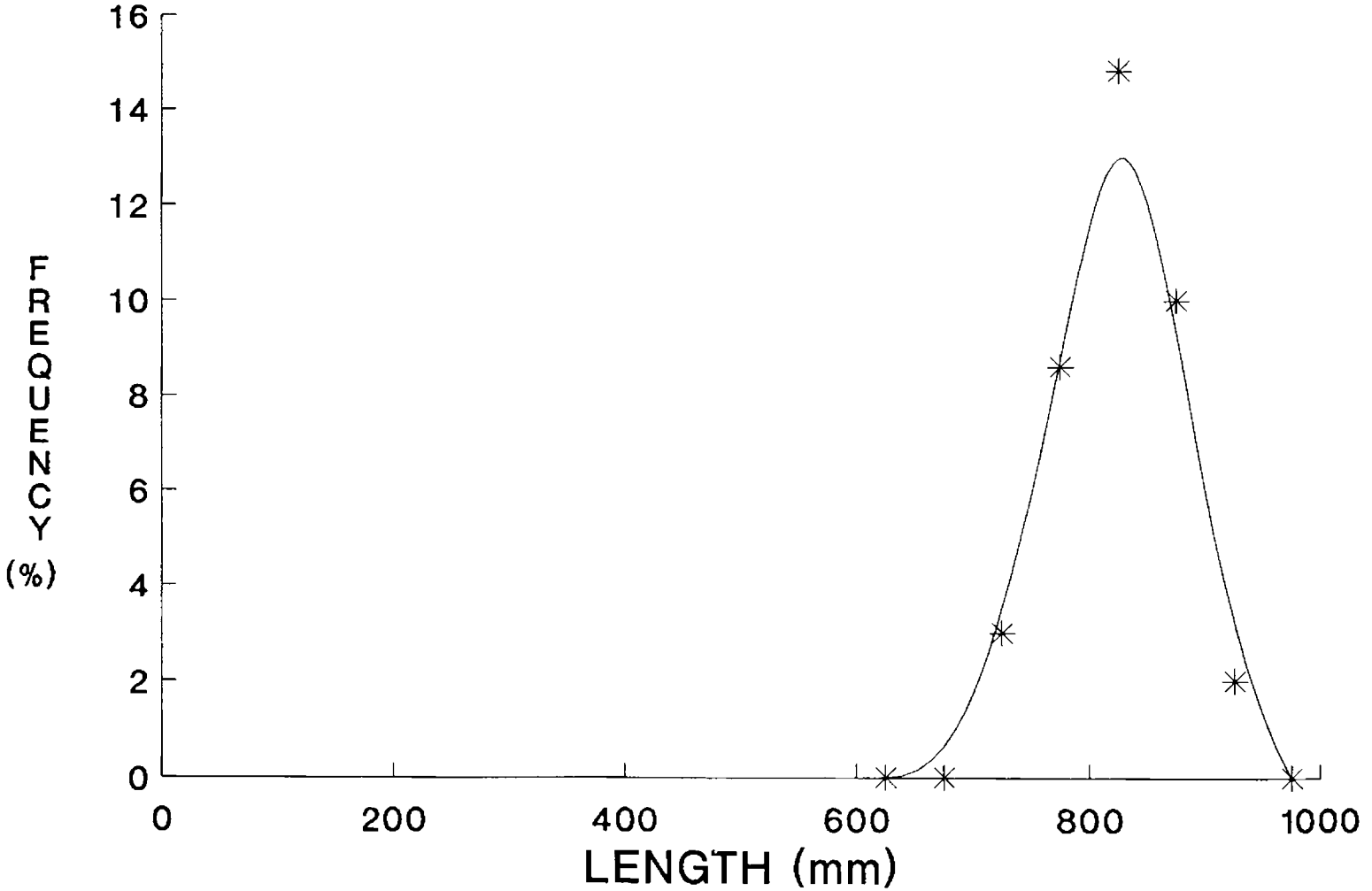



FIG. 11. Percentage of male L. calcarifer examined that were changing sex.

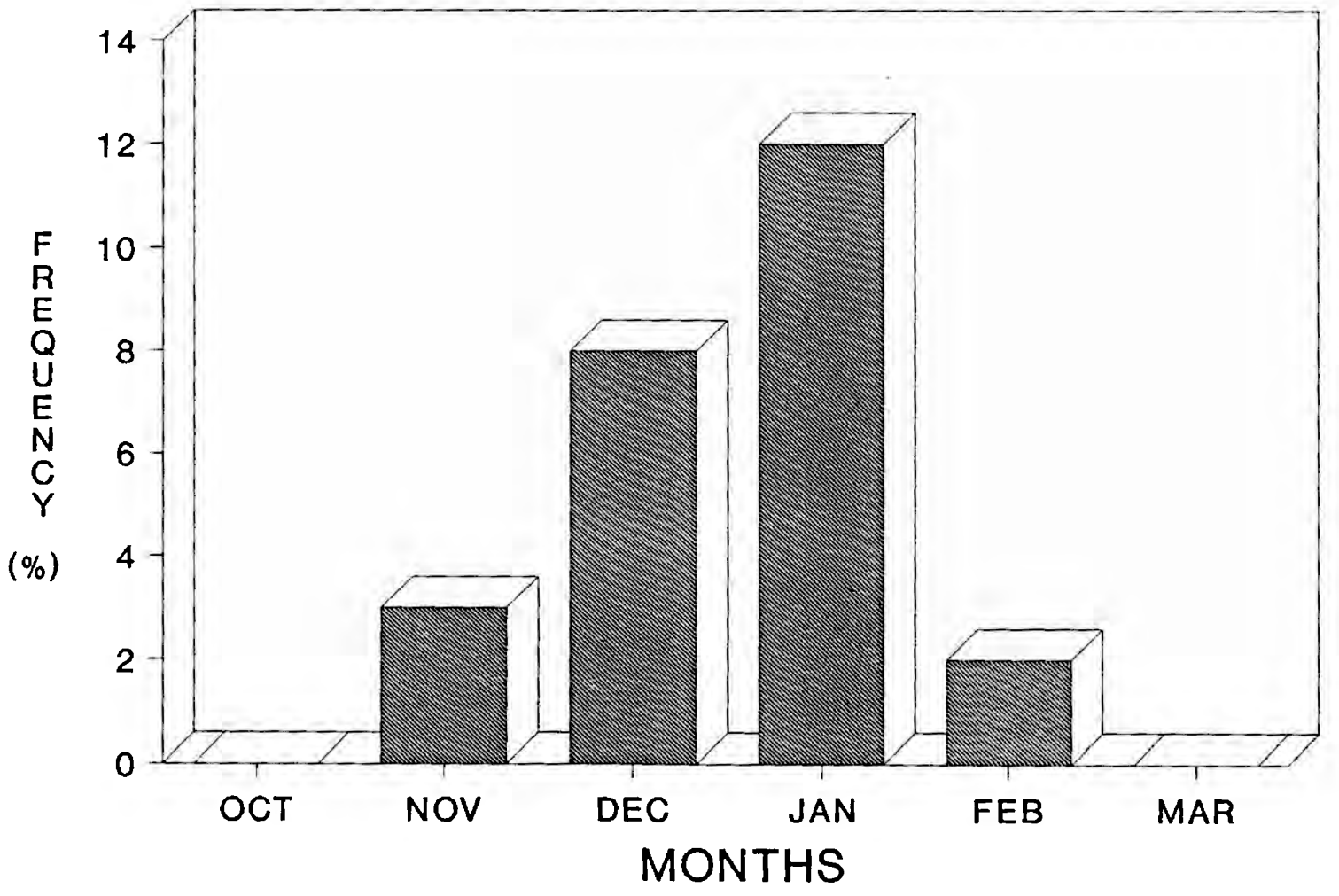



FIG. 12. Percentage of transitional male L. calcarifer detected during the months from October to March.

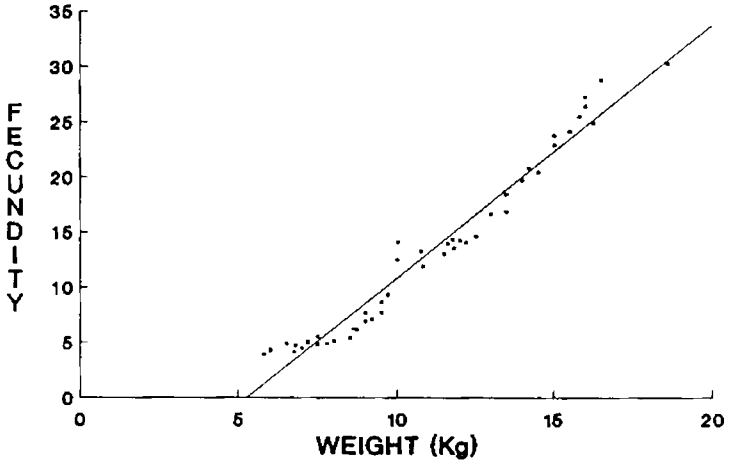



FIG. 13. Relationship between fecundity ( $x10^6$) and body weight (Kg) of L. calcarifer.

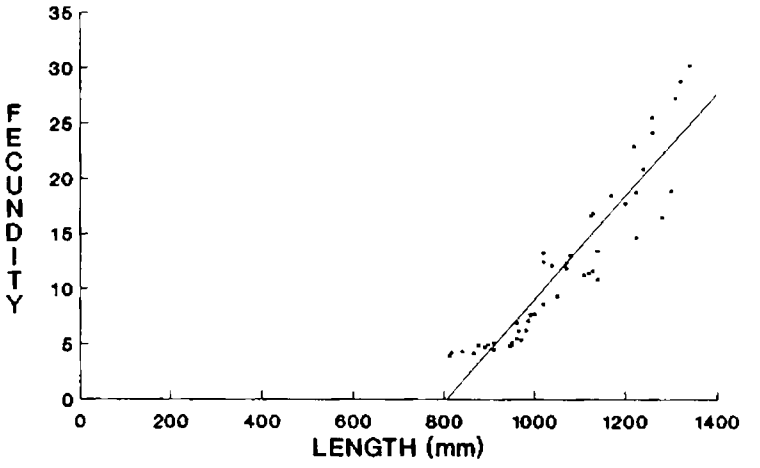



FIG. 14. Relationship between fecundity ( $x10^6$) and total length (mm) of L. calcarifer.

$\log F = 1.4143 \log W + 4.6552$, $r^2 = 0.6808$
$\log F = 2.8903 \log L - 7.6841$, $r^2 = 0.7312$

## SEASONAL GONADAL CYCLE

**Male:**

Standard gonad weight (Gs) rise sharply from July onwards reaching its peak value in November (Fig. 15) followed by drop in December. Gs value declines gradually till resting weight in February. The recovery of testes weight proceed slowly through March to June upto July. The observed monthly variations in spermatogenic response and quantity of spermatozoa represented in fig. 16, is in agreement with that indicated by gonad weight. Spermatogenic response value attain its minimum in February and remain low till April, afterwards rise sharply to its peak value in September and maintain high values in October and November followed by decline to resting weight in January. On the other hand, quantity of spermatozoa attain highest value in October and remain more or less at the same level in November followed by decline from December till February. The seasonal rhythm indicated by relative abundance of different maturity stages occurring around the year, coincides with the above described cycle (Fig. 17).

Stage 2 males, include, developing virgins, recovering spent, and late developing, though available through out the year, increase sharply in abundance from January till April. Thereafter, a gradual progession through maturity stages 3 and 4, characterized by active spermatogenesis, to the ripe (stagge 5) was observed. Ripe males exuding milt observed in the month of September through October-November upto the end of December. The spent fish (stage 6) appear first time in the samples in the end of October; is observed till early January with dominence in November (Fig. 17).

**Female:**

As is evident from fig. 18, standard gonad weight (Gs) has lowest value in February till May; afterwards it increases sharply and doubles

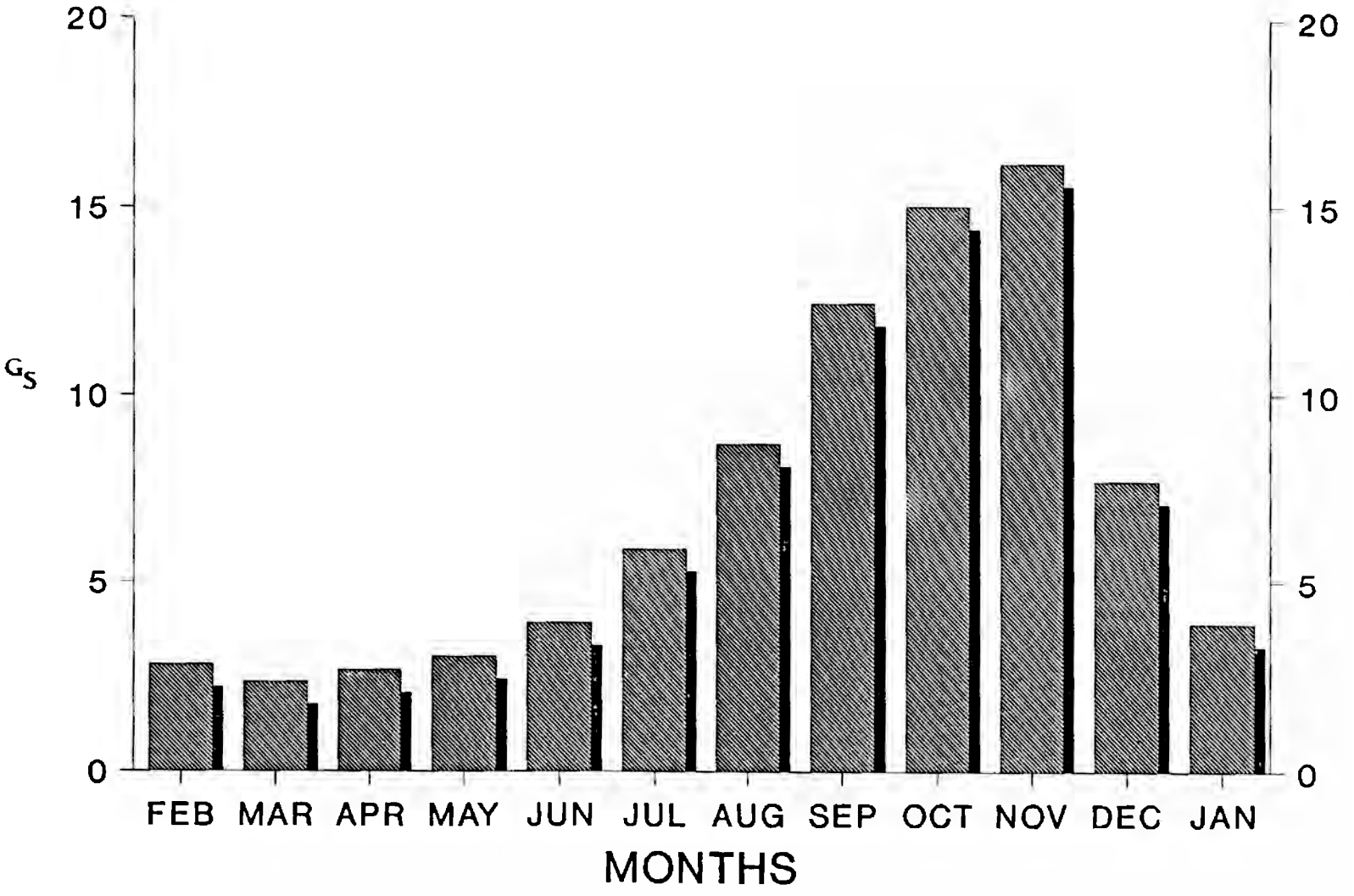



FIG. 15. Monthly variation in standard gonad weight (Gs; g) of male L. calcarifer.

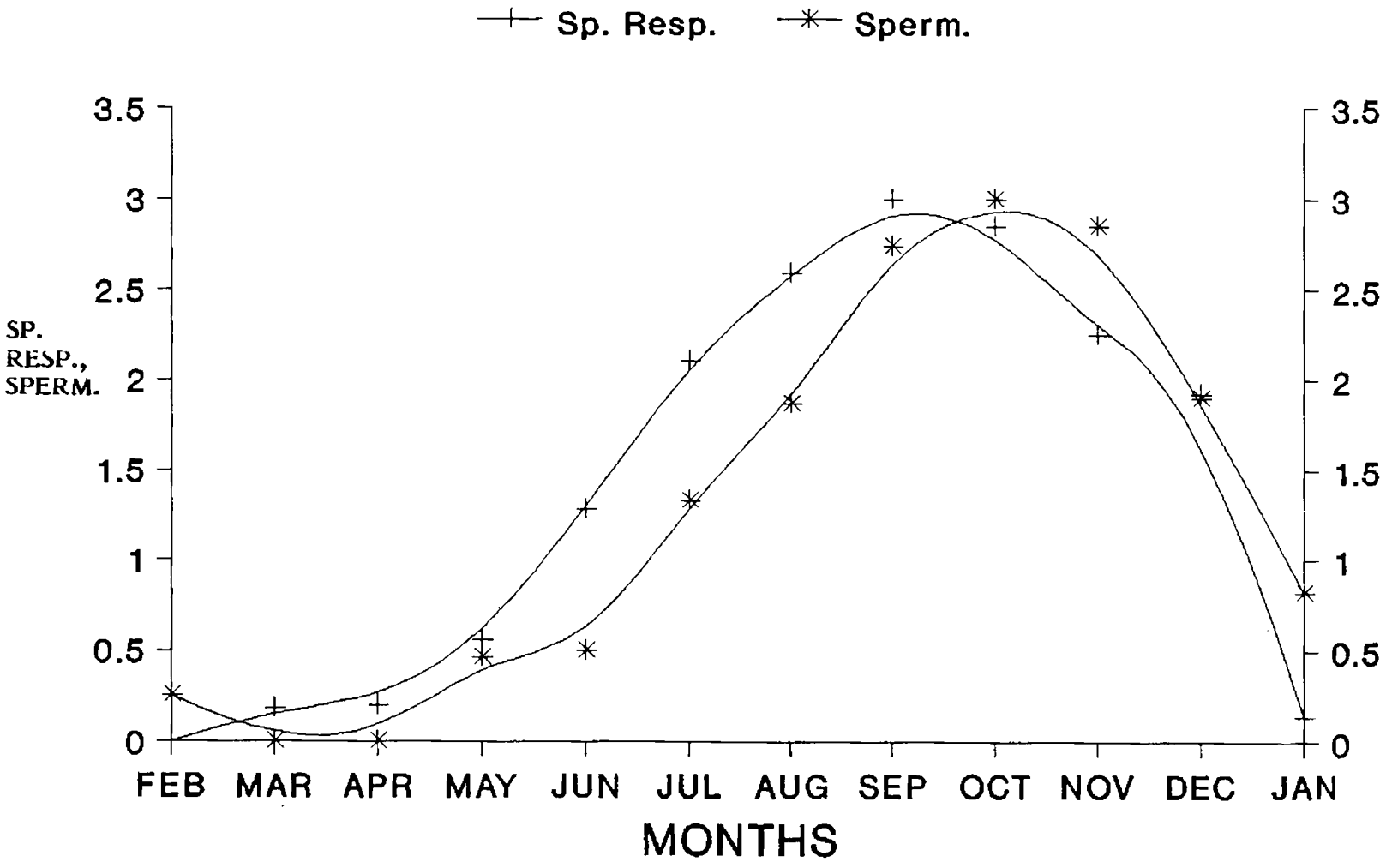



FIG. 16. Monthly variations in spermatogenic response (Sp. Resp.) and quantity of spermatozoa (Sperm.) of male L. calcarifer.

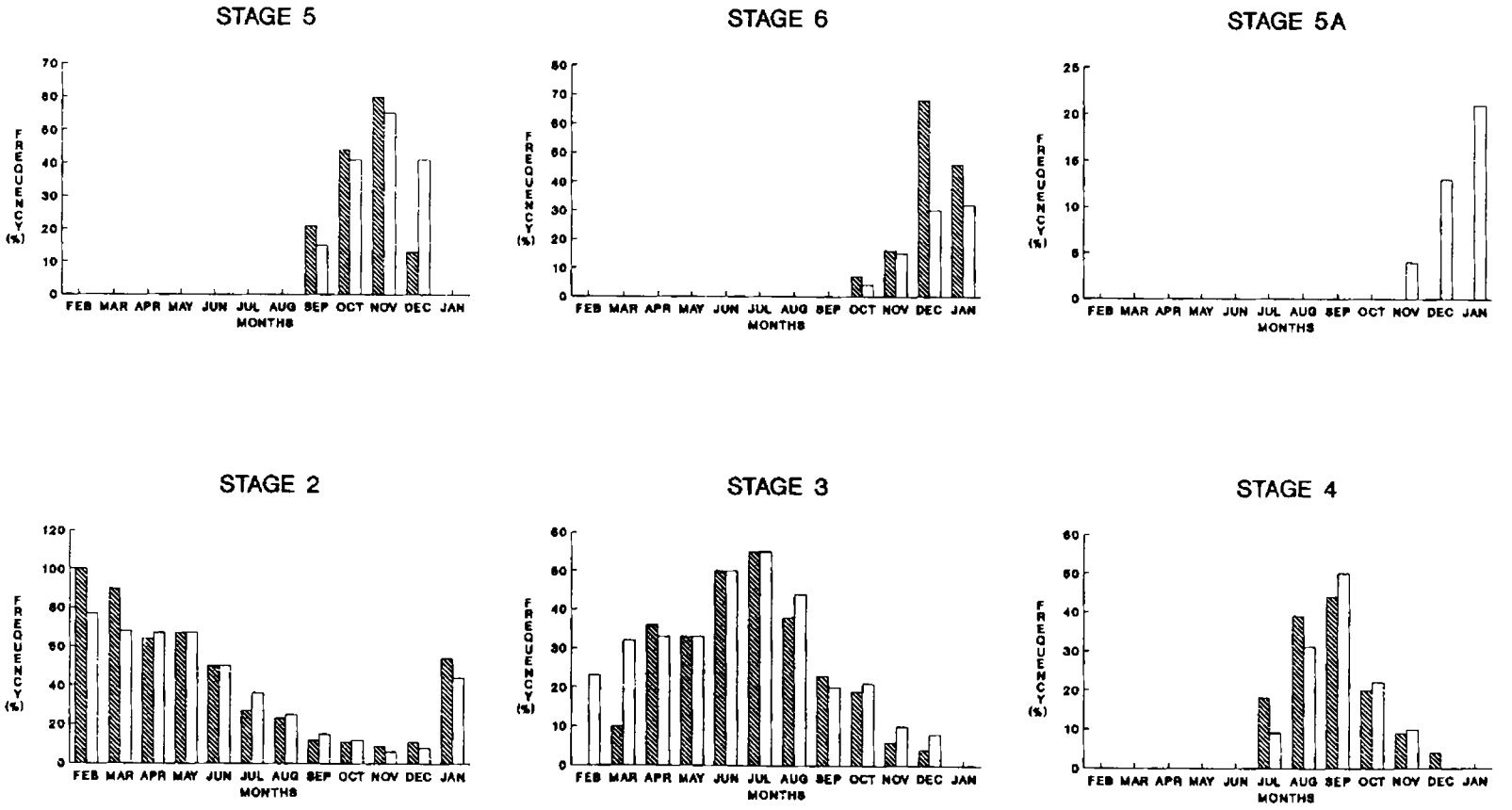



FIG. 17. Monthly variations of different maturity stages of male (staked bars) and female (empty bars) L. calcarifer

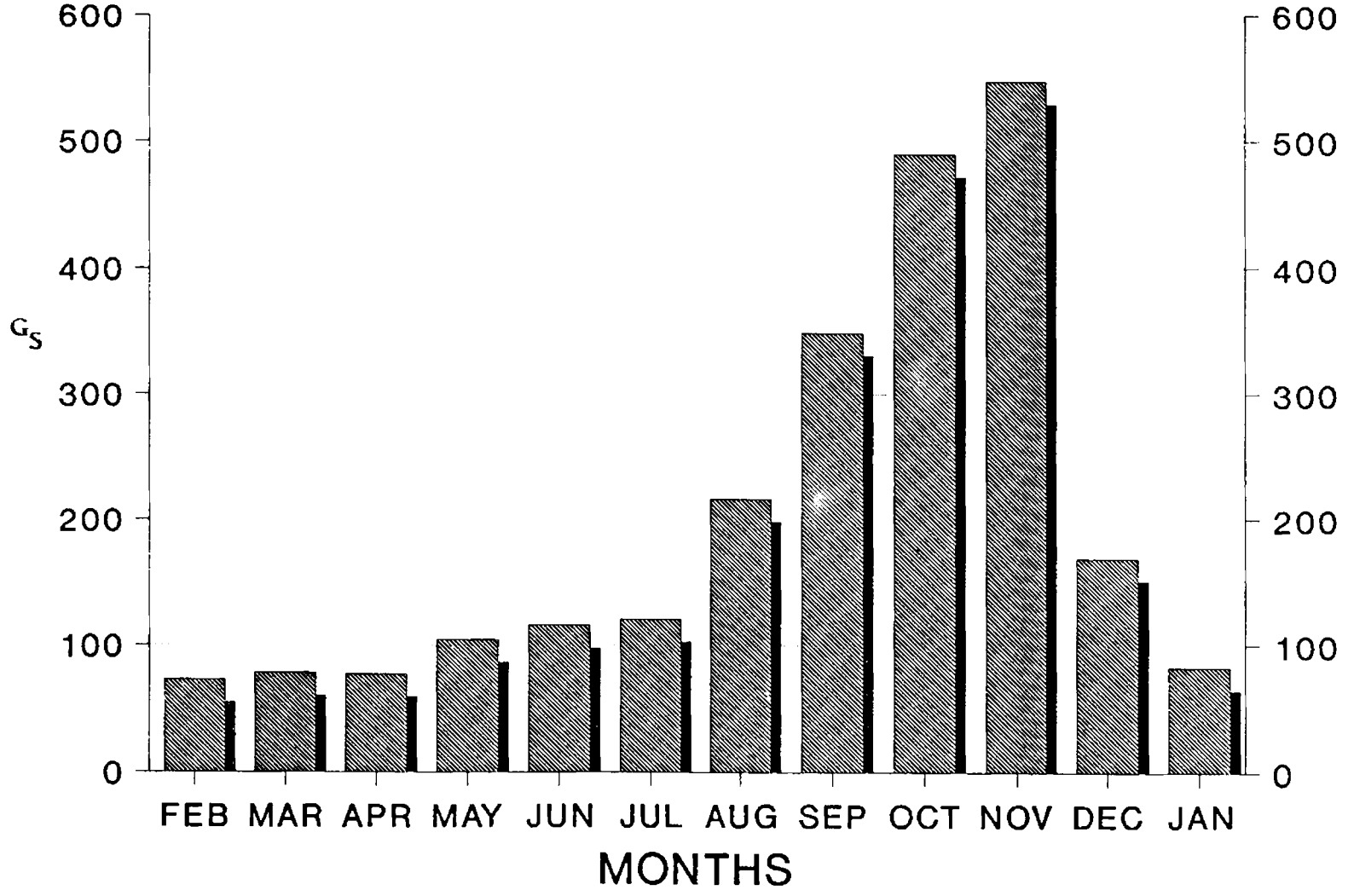



FIG. 18. Monthly variation in standard gonad weight (Gs;g) of female L. calcarifer.

between June and August; reaches its peak value in October and November. Gs value drops sharply in December to reach weight in February.

Mean diameter of the largest oocytes rises sharply between June and September and maintaines high level till November followed by abrupt fall in December till February (Fig. 19).

The L. calcarifer females with ovaries in recovering and maturing phases (stage and 3) are observed as early as in February (Fig. 17). However, the females possessing vitellogenic oocytes (stage 4) start appearing in July only. The ripe females are observed in late September till December with peak in November. The spent fish are encountered in late October only, available till early January. Further, a considerable difference between relative abundance of ripe males and females is pointed out from Fig. 17 as in December, the proportion of ripe females (stage 5) was 41 percent as compared to only 13 percent ripe males.

Fig. 20 reveals that 22.8 percent of the fish (stage 2 and 3) pass the season without ovarian recrudescence. The mature and ripe specimens (stage 4 and 5) contribute 41.9 percent; however the proportion of spent fish, which represent actual spawning, is 22.7 percent. The proportion of the ovaries which ripen but fail to spawn being 12.5 percent appear quite high, little more than half of the spent fish. Ham and Leesan (1961); Harris (1986) described the process as involution, involving ordered sequence of degenerative changes through which the ovary regresses from its peak vitellogenic activity to resting stage if conditions favourable for spawning doesn't oocur.

## SPAWNING SEASON

The observed gonadal cycle of L. calcarifer reveal that the spawning season extends from mid October to December with peak in November in the region under study. The breeding seems to be synchronized with rains as the spent fish were recorded only after the onset of wet season.

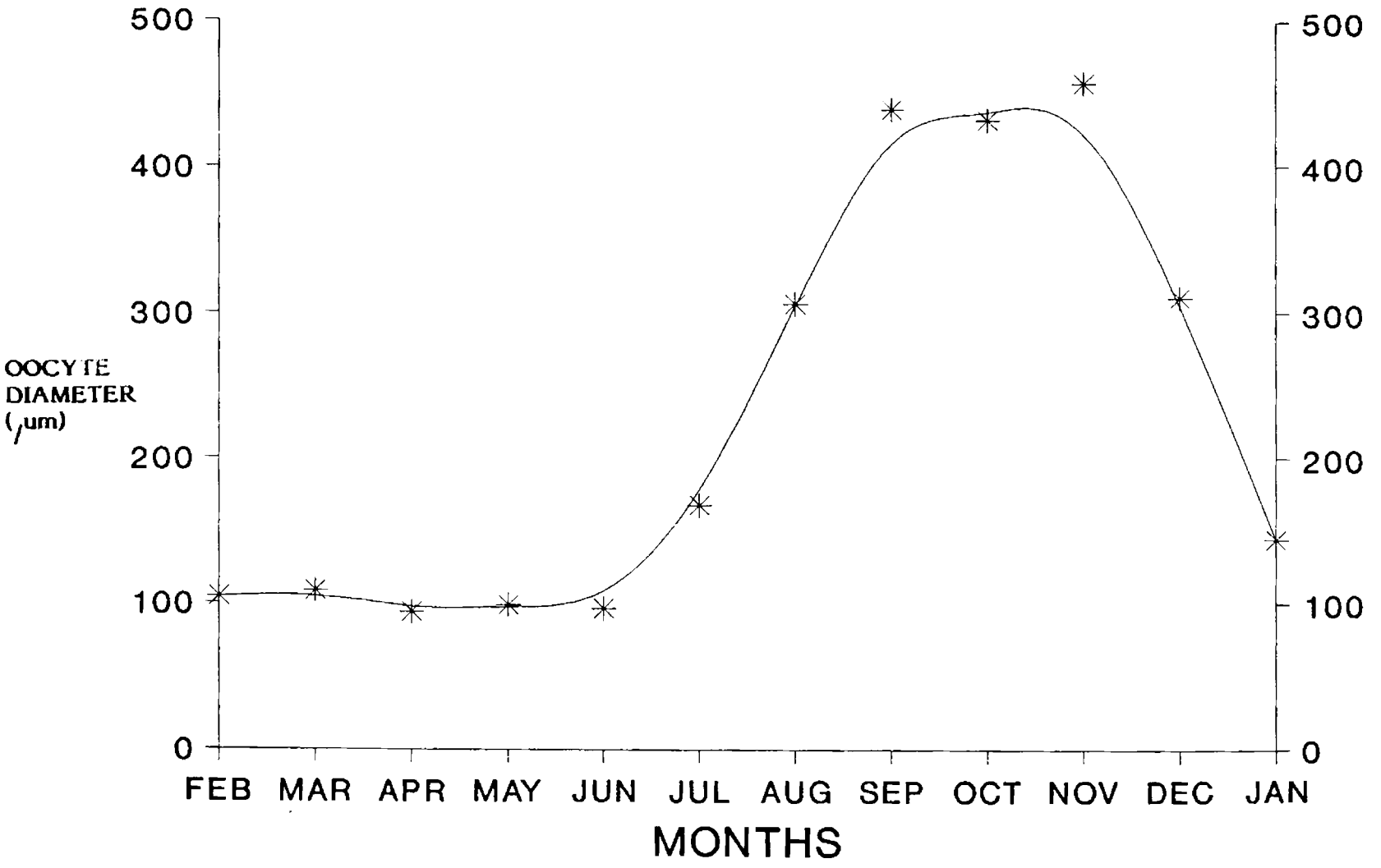



FIG. 19. Monthly variation in largest oocyte diameter (μm) in female L. calcarifer.

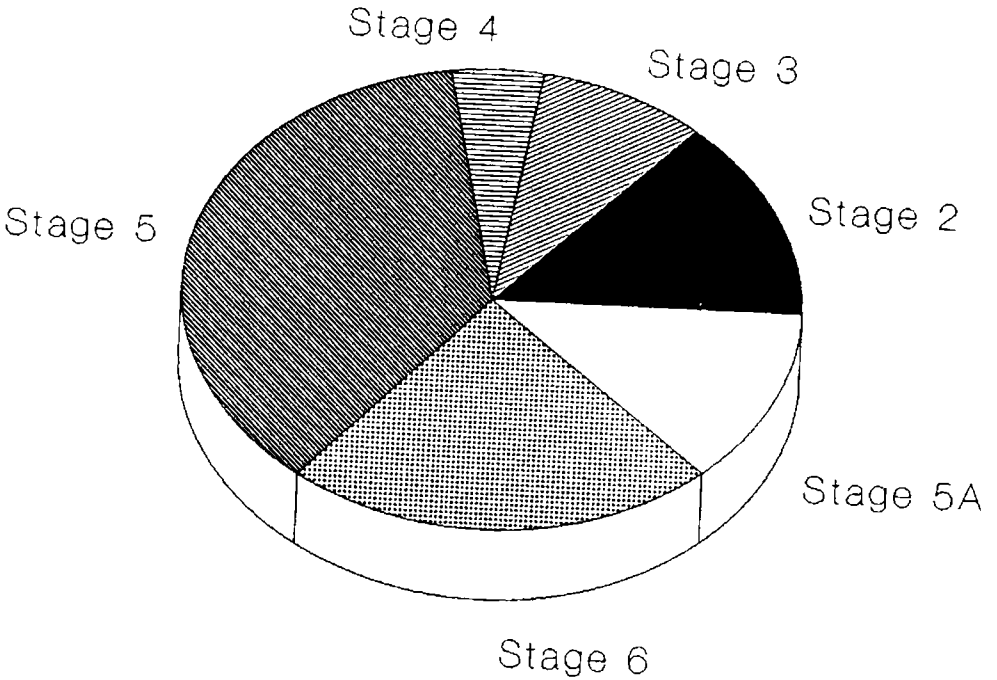



FIG. 20. Frequency distribution of different maturity stages in the sample of 184 female L. calcarifer collected during the months of October to December.

## DISCUSSION

The present observations indicate that the most of the male sea bass mature after attaining the length in the range 600 to 650 mm. Jhingran and Natarajan (1969) reported minimum size at maturity to be 425 mm in Chilka lake (no sex stated, persumbaly male), whereas smallest mature male recorded by Patnaik and Jena (1976) was of 505 mm TL. Wongsomuk and Manevongsa (1974) used smallest ripe male of the length 570 mm, for artifical spawning in Thailand. Size at maturity for males in Papua New Guinea lies between 510 to 700 mm (Moore, 1980). Davis (1982) observed the most of the males maturing in the length range of 700 to 750 mm. and 600 to 650 mm. in the northern territory and Gulf of Carpentaria respectively in Australia.

The observed relative difference in the sizes of the sexes in L. calcarifer indicate deviation from gonochoristic reproductive mode in favour of protandric hermaphroditsm. Patnaik and Jena (1976) encountered the smallest mature female of length 700 mm in India. Wangsomnuk and Manevongsa (1974) used the females of 640 to 850 mm length in induced breeding experiments, but there have not been any reference in the literature from Asia suggesting the occurance of sex inversion. Davis (1987) mentioned the prevalence of gonochorism among the sea bass population from Songkhala Lakes, Thailand. However, studies on sea bass in northern Australia and Papua New Guinea have identified that the fish is protandric hermoprodite (Moore, 1979; Moore and Reynolds, 1982; Reynolds and Moore, 1982; Russel and Garret 1983, 1985; Davis, 1982, 1985a, 1987; Grey, 1987).

Davis (1982, 1987) advocated sex ratio/length relationship as a useful tool to predict the size at which most of the fish are changing sex, corresponding to the point of inflection on the curve. This can be used as an objective mean for comparing different populations. During the current study, point of inflection was found to be at 885 mm, TL; slightly larger (approximately 6 per cent) than the mean length (835 mm, TL) of the transitional males detected. Moore (1979) reported length range of a few transitional males detected to be 910 to 990 mm. (Mean length

950 mm) in Papua New Guinea. Davis (1982) fitted Gompterz curve to sex ratio/length data of, Reynold (1978) from Papua New Guinea and observed the point of inflection at 986 mm TL, almost 5 percent larger than the mean length reported by Moore. In the northern Australia and south eastern Gulf of Carpentaria, the point of inflection was calculated to be at 1002 mm. and 936 mm respectively. Davis (1984a) discovered a sexually precocious population in the northeastern Gulf of Carpentaria where point of inflection was found to be at 535 mm TL.

Longhurst (1965) observed two types of females in protandric Galcoides decadactylus. Digyny is just opposite to diandry reported among certain protogynous (females change to males) species of Scaridae, Labridae (Reinboth, 1980). Sea bass population was found to be digynous, comprising two types of females, primary and secondary. Similar observations were reported by Moore (1979)., Davis (1982, 1987). Moore (1979) coined the possibility that some males may not undergo the sex change however, he could not collect any large size male. During the present study, one male specimen of total length 1275 mm, was encountered, confirming the possibility of such variations. Similar variations have been reported in protandrous Sparus longispinis (Kinoshita, 1936).

L. calcarifer displays seasonally synchronized annual breeding habits. Gonadal cycle of the fish reflects that the spawning commences around mid October and extends upto December with peak in November. The spawning appear to be synchonized according to the rains. No spawning was recorded before the onset of monsoon.

In India, spawning of sea bass was reported during winters in Sunderbans (Naidu, 1939); October and November on the basis of availability of ripe specimens and large number of fingerlings (60-80 mm) (Rao, 1964; Shetty et al. ,1965) in Mahanadi estuary, July to August in Junput from the occurrance of post larvae (De, 1971). Gopalakrishnan (1972) collected the fry of L. calcarifer from Kulpi on Hooghly river and Thakuran as well as Matlah rivers during May to October. In the Hooghly-Matlah estuarine

system, juveniles (6-9 mm) from May to July (Ghosh, 1973) and larvae (4-7 mm) from April to June (Mukhopadhyay and Varghese, 1978) have been observed. Rao and Gopalakrishnan (1975) observed the fingerlings (50-70 mm) in the northern sector of pulicat lake in May-June and advanced fry (22-52 mm) during July to September in Chilka lake (Kowtal, 1977).

Barlow (1981) reported primary and secondary spawning seasons corresponding to the northwest monsoon (August to october) and weaker southeast monsoon (February to June) in Thailand. Ruangpanit (1987) observed peak season of spawning from April to September in Songkhla lake, Thailand, though it continues throughout the year.

In Queesland, spawning period extends from October to March (Garrett, 1987); the peak occurring during November and early December in northern stock (Davis, 1985a; Russell and Garrett, 1985) whereas from late December to early January in southearlyeast coast water (Garrett, 1987). Dunstan (1959) established two spawning peaks, November and January in central Queensland waters. In Papua New Guinea spawning starts at the beginning of and continues through the summer monsoon season from November to March (Dunstan, 1962) and from October to February (Moore, 1982). In the northern territory of Australia breeding commences in September and continues until February whereas in the Gulf of Carpentaria the spawning period extends from late December to March (Davis, 1985a, 1987).

The present study indicates the breeding period of sea bass in Tuticorin area of comparatively shorter duration while most of the reports suggest that the fish has a prolonged breeding season. Dustan (1959); Moore (1982) suggested that the differential arrival of mature fish on the spawning grounds from the landlocked freshwater habitats, when flooding occurs late in the rainy season, to be responsible for exstended nature of the breeding season.

The observed fecundty estimates agrees with the earlier reports depicting the enormous egg producing capacity of L. calcarifer. (Dunstan,1959;

Wongsomnuk and Maneewongsa, 1974; Patnaik and Jena, 1976; Moore, 1982; Davis, 1984b) It is one of the most fecund fishes reported in the literature, with maximum estimated egg numbers upto 46 x $10^6$ (Davis, 1984b). The fecundity was found to increase with the size of the fish, which agrees with the trend observed in most of the fishes (Beverton and Holt, 1957; Davis, 1977b;Greeley et al 1987) .As defined for L. niloticus (Ogutu-Chiango, 1988) the actual reproductive potential of L. calarifer will depend upon the number of eggs that are fertilized and hatch, the environmental conditions to which the offsprings are exposed and the growth rate of the offsprings. High fecundity is an adaptation to counter the impact of harsh environmental conditions to which eggs are exposed and is quite prevalent among the teleosts which do not show any parental care.

In L. calcarifer, special significance is attached to the high fecundity as limited number of large females can maintain adequate recruitment (Davis, 1984b). Being a protandric hermaphrodite, the females have been observed to constitute small proportion of mature population (Moore, 1979; Davis, 1982, 1987) and egg production in some fish may not start till the age of eight years (Davis, 1982). Though current study reveal a dissimilar situation where sex-ratio favours females, yet importance of high fecundity deserves recognition as only a small propotion of mature females appear to participate in the spawning activity.

All the fish do not undergo gonadal recrudescence every year as observed during the present study, is in aggreement with earlier reports of Dustan (1959); Moore (1982) and seems to be a part of the normal process. However, high proportion of females undergoing involution appears to be an unhealthy feature, since it indicates the lack of suitable opportunities for spawning.

The degeneration of vitellogenic follicles especially mature yolky oocytes is of common occurence in fish ovary during prespawning, spawning and post-spawning periods and has been studied extensively in numerious species of teleosts (Browning, 1973; Babu and Nair, 1983; Guraya et al., 1975, 1977; Saidapur, 1978, Hunter and Macewicz, 1985). The degeneration

of ripe ovaries is an effective response to mobilse and relocate large energy investments rather then turning it into waste, if conditions conducive for spawnig do not occur (Ham and Leesan, 1961). Besides, it esures that young ones hatch out only when conditions are favourable for their survival (Wooton 1982; Lam, 1983;Guaraya, 1986).

In a protandric hermaprodite, the sex ratio is usually in the favour of males (Shapiro, 1984; Warner, 1988). Moore (1982) observed that the males outnumber the females participating in the breeding event. Several males probably fertilize the eggs of a single female, ensuring successful fertilization of all the eggs released (Garrett et al., 1987; Mackinnon, 1987). Obviously, it will necessitate males to constitute larger proportion of mature population as noted by Moore (1979); Davis (1982). The low fertilizing capacity (due to less proportion of males) of the population can consequently keep the adequate opportunities of spawning away from the mature female spawners and probably can be one of the prime factors inducing involution among gravid females (Trippel and Harvey,1990). The emerging facts about the role of chemical cues in reproduction further support this inference (Liley 1982; Pandey, 1984). Stacey and Hourston (1982) found a phermone in the milt of Clupea herrengus that trigger spawning in the gravid females.

The population structure at any given time is the net resultant of the various environmental forces acting on it (Bal and Rao,1984). The sex ratio, observed, seems to be a visible artefact in the population structure, might have appeared due to continuous exposure to certain unfavourable environmental conditions over a due course of time. This gives a prima-facie evidence of selective loss of males. Fig. 21, depicts the various stages of life history of L. calcarifer on the basis of the present observations.

The reproductive strategy of sea bass is synchronized so as to provided the advantage of food rich and predator free supralittoral habitats formed during rainy season (Moore, 1982; Moore and Reynolds 1982; Davis, 1987). Moore (1982) suggested that the access to these wetlands is through salt water bridges formed during the spring tide too shallow for the passage of any large fish. Davis (1985a) observed that the juveniles ascend many

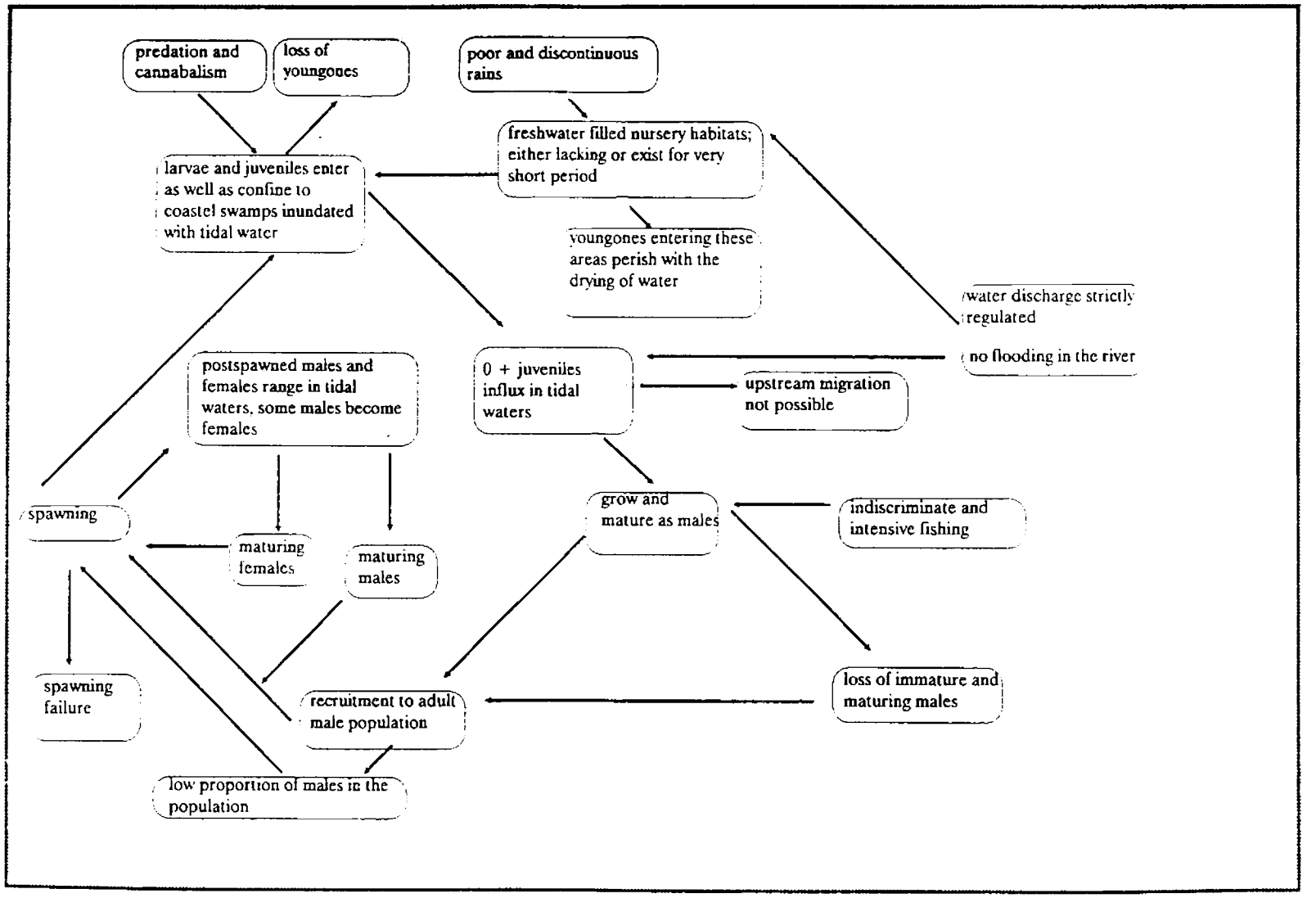



FIG. 21. Schematic representation of life history of L. calcarifer population off the Tuticorin coast (Gulf of Mannar).

kilometers up the river to flood plains if such areas are not available near the coast. These nursery habitats appear critical to the life cycle of the fish. Russell (1987) suggested a close positive relationship between juvenile nursery areas and sea bass catches in Queenland and attributed the decline in the catches partly to the destruction of these wetlands due to urban and agricultural developments. Moreover, young ones were found unable to make use of alternative habitats for nursery purpose in such circumstances

As described earlier, Tuticorin is regarded as a dry area. Due to low water discharge in the river and meagre local rain falls, nursery habitats are virtually not formed or may exist temporarily in the form of small pools. Since the rainfall is mostly discontinuous (Fig.4), the young ones finding access to these supralittoral areas are more likely to perish with the drying of the water. Mangroove swamps adjacent to the estuary and creeks have been observed to be the major nursery grounds for young fish. But these swamps may not provide predator free environment being regularly inundated with tidal waters. The prominent predatory fish species occurring are Therapon sp. and Psammoperca waigiensis. Besides this, the presence of grown up sea bass juveniles of earlier spawnings may make the environment hostile for the juveniles from the subsequent spawnings, as sea bass are highly cannibalistic, capable of consuming sea bass of half of its own size (Davis, 1985b).

Juveniles remain in these swamps until they attain length of fifteen to twenty centimetres. The fish of length twenty centimeters and more are distributed in the mouth as well as streams of estuary, creeks and other inshore coastal water areas (Kasim, personal communication). There is no evidence of availability of sea bass of any size in the freshwater stretch of the river. Probably meagre flow of water may not make upstream movement possible. Several authors have reported sea bass to be a catadromous migrant (Chacko, 1956; Dunstan 1959; Moore and Reynolds 1982; Reynolds and Moore 1983, 1985; Davis 1982, 1985a, 1987; James and Marichamy, 1987). Creation of dams and weirs across the rivers have been responsible for the exclusion of L. calcarifer from much of its historical range along the eastern coast of Queensland (Pearson, 1987). Grey (1987)

documented generalised life history of L. calcarifer in Australia and mentioned that the young of the year migrate towards the upper reaches of the river and move downstream after attaining sexual maturity as males (600 to 700 mm TL; 3 to 4 years age).

The freshwater phase in the life history probably may not be a prerequisite for attaining the sexual maturity, as the population under study appear to pass all the stages in the saline water only. However, upstream migration is likely to provide scope for escaping from intensive fishing pressure with indiscriminate gears, being operated in the coastal areas. The long time interval till the sea bass attain the size of sexual maturity (600 to 650 mm TL) can make it prone to fishing mortality without even spawning once in life time. As reported earlier, that capture fishery of L. calcarifer, in India, is mostly sustained with immature fish aged less than two years (Kasim and James, 1987). As the fish first mature as male, any mortality prior to maturity will have direct bearing on the recruitment to adult male population and perhaps responsible for the observed low relative abundance of males in the population. Reinboth (1980) argued that the heavy, even partially selective fishing of sex inverting species with smaller and larger individuals of different sexes could lead to highly shrewed sex-ratios, even to endanger the survival of certain populations. The difference in the sizes in ambisexual species is the main aspect of their biology which relates to the fishery management in capture fisheries. Increase in spear-fishing of larger Serranids (protogynous) in the Red Sea has been reported to cause management problems due to selective elimination of males (Fishelson, Op. cit. Reinboth, 1980). Thompson and Munro (1974, op. cit. Reinboth, 1980) observed females contributing seven times more to the sex ratio in intensely exploited population of protogynous Ephinephelus guttatus. Drop in female population of L. calcarifer in Papua New Guinea from 27 percent in 1973 to 13 percent in 1978 has been attributed to selective exploitation of larger fish (Anon, 1978; op. cit, Davis, 1982). Unless the size at sex change is adaptable, such exploitations can possibly cause rapid decline in population. However, there is no evidence of change in size at sex inversion in any of the examples mentioned above (Reinboth, 1980; Davis, 1982).

The Tuticorin area is not known to sustain regular fishery of sea bass and does not exhibit any specific fishing effort too. Despite its good market price, there is a lack of interest among fishermen towards this species, mostly due to low probability of capture. This tendency may be able to reduce the intensity of exploitation of larger individuals. However, for the population under study, late transformation of sex is desired. It is beyond the scope of the current data to assess whether size at sex inversion has undergone any change over the period of time in the absence of any previous information.

## SECTION II

## GAMETOGENESIS

As in higher vertebrates, basic and complementary task of the gonads of teleosts is to produce viable gametes i.e. sperms and eggs, required for successful reproduction with an aim to perpetuate the species. Teleosts exhibit an amazing range of diverse reproductive strategies, which allow them to distinguish amongst vertebrates. Besides, gonochorism being the predominent mode, numerous examples of ambisexuality, including simultaneous hermaphroditism and functional sex inversion (protogynous as well as protandrous) are found scattered in different orders (Atz, 1964). Absence of a dual origin of the gonad has been suggested to account for such a widespread intersexuality among teleosts. The gonad lack medullary tissue and correspond only to the cortex of other vertebrates (Hoar, 1969). The natural diversity of chromosomal conditions in teleosts with respect to the phenotypic expression of sex is unparalleled among vertebrates (Gold, 1979). The natural diversity of chromosomal conditions in teleosts with respect to the phenotypic expression of sex is unparalleled among vertebrates (Gold, 1979). As a consequence of these diversities, fishes provide remarkable specimen for studies into various problems concerning sex determination as well as differentiation (Yamamoto, 1969; Reinboth, 1982).

The complexity of reproduction is reflected in the wide range of gonadal structures found among teleosts especially ambisexuals; exhibiting diverse patterns of organization of heterologous germinal elements (Reinboth, 1983; 1988; Brusle, 1987; Brusle-Sicard and Reinboth, 1990). Irrespective of the topographical organisation, the germ cells and somatic cell elements constituting the gonad are fundamentally similar with respect to morphology as well as development.

Organization of teleostean testis has been distinguished into unrestricted spermatogonial/lobular type and restricted spermatogonial/tubular type. (Grier, 1981; Billard et al., 1982) the lobular type is typical of most of the teleosts while tubular type is restricted to atheriniformes.

The development of male germ cells is cystic in teleosts,(Nagahama, 1983). Following the terminology used in mammals, two forms of morphologically distinct spermatogonia type A and B have been described in several teleosts, Oryzias latipes (Michibata, 1975; Grier, 1976; Shibata and Hamaguchi 1988); Salmonids (Billard, 1984). It has been suggested that the spermatogonia type A are stem cells, seperated from each other by cytoplasm of the sertoli cells; which give rise to spermatogonia type B, organized in the germinal cysts. Spermatocytes, produced in the process, are destined to undergo meiosis and give rise to haploid spermatids. Spermatids, through spermiogenesis differentiate to produce specialized cells, spermatozoa, having suitable architecture to perform basic functions viz. activation of egg and transmission of paternal genome into the egg (Afzelius, 1970). Divergent modes of reproduction are reflected in the sperm morphology as well as spermiogenesis, which may differ considerably even among closely related species (Grier, 1981).

Oogenesis, dynamic process of oocyte development, exhibits events common to all teleosts, in principle. These include oogonial poliferation (Tokarz, 1978); nucleolar multiplication (Brusle', 1980); formation and dispersal of a Balbiani's vitelline body (Guraya, 1979; Coello and Grimm, 1990) formation of cortical alveoli as well as vitelline envelope and accumulation of yolk (Anderson, 1968; Selman et al, 1986, 1988) and oocyte maturation (Masui, 1985; Greeley, et al., 1986a). Extensive use of various improved techniques of histochemistry, biochemistry, autoradiography and electron microscope has played significant role in recent times, still precise information concerning different aspects of oogenesis at subcellular and molecular level stands meagre (Guraya, 1986; Selman and Wallace, 1989).

Recent years have witnessed increasing interest in teleost reproduction because of its close relationship with development of aquaculture. Thorough knowledge of cellular and molecular events related to fish reproduction can be instrumental in propagating better techniques of induced breeding, cryopreservation of gametes and genetic engineering.

Aquaculture potential of L. calcarifer does not need to be reemphasized. However, it is important to mention that gametogenesis of this fish has been described at a very preleminary level (Davis, 1982). The present section is devoted to the description of cellular changes associated with the development of gametes in L. calcarifer in the light of both optical as well as electron microscopic observations.

## RESULTS

### TESTES

The testes of L. calcarifer are paired, elongated, strap like organs. Dorsally, each testes is composed of thick tunica albuginea consisting of smooth muscles, connective tissue, spermducts and blood vessels. ( Plate Ia ). The Spermducts variable in number run dorsolaterally throughout the length of the testis. Towards the ventral side, testis consists of irregularly branching seminiferous lobules packed with germinal cysts. Thin septa originating from tunica albuginea, ramify forming the thin lobular walls.

Each lobule is completely surrounded by a thin basal lamina ( Plate Ib ). Inter lobular space consists of flat, spindle shaped myoid cells, arranged in single to multiple layers. At ultrastructure level, myoid cells are characterized by the presence of densely packed filaments in the cytoplasm and occasional pinocytotic vesicles near the plasmalemma. Zone between myoid cells and basal lamina consists of connective tissue containing collagan fibres. Occassionally, connective tissue is also visible, continuous in between the myoid cells.

Interstitial areas, are mostly angular spaces, arising due to compact arrangement of the lobules. Leydig cells ususally present in these areas, are of irregular shape, distributed single or in clusters ( Plate Ic ). Fine structure of Leydig cells demonstrate elongated nucleus with dense chromatin along the envelope; surrounded by granular cytoplasm containing mitochondria with tubular cristae, ribosomes and lipid bodies ( Plate Id ) .

## PLATE I

a. Light micrograph showing organization of testis with thick tunica albuginea (TA), sperm duct (SD)' on dorsal side (D). Seminiferous lobules (Sl) surrounded by lobular walls (lw) present towards ventral (V) side. Ventral furrows (Vf) are also visible. X 40.

b. Electron micrograph of Interlobular wall (ILW) consisting of myoid cells (MC), connective tissue (CT) and basal lamina (arrow heads). X 4700.

c. Light micrograph of interstitial space (I) containing cluster of Leydig cells (Ld). The seminiferous lobules are packed with spermatozoa (SZ). X 400.

d. Electron micrograph of Leydig cell with elongated nucleus surrounded by cytoplasm containing abundant lipid droplets (LD) and mitochondria (arrow). X 6600.

**PLATE I**

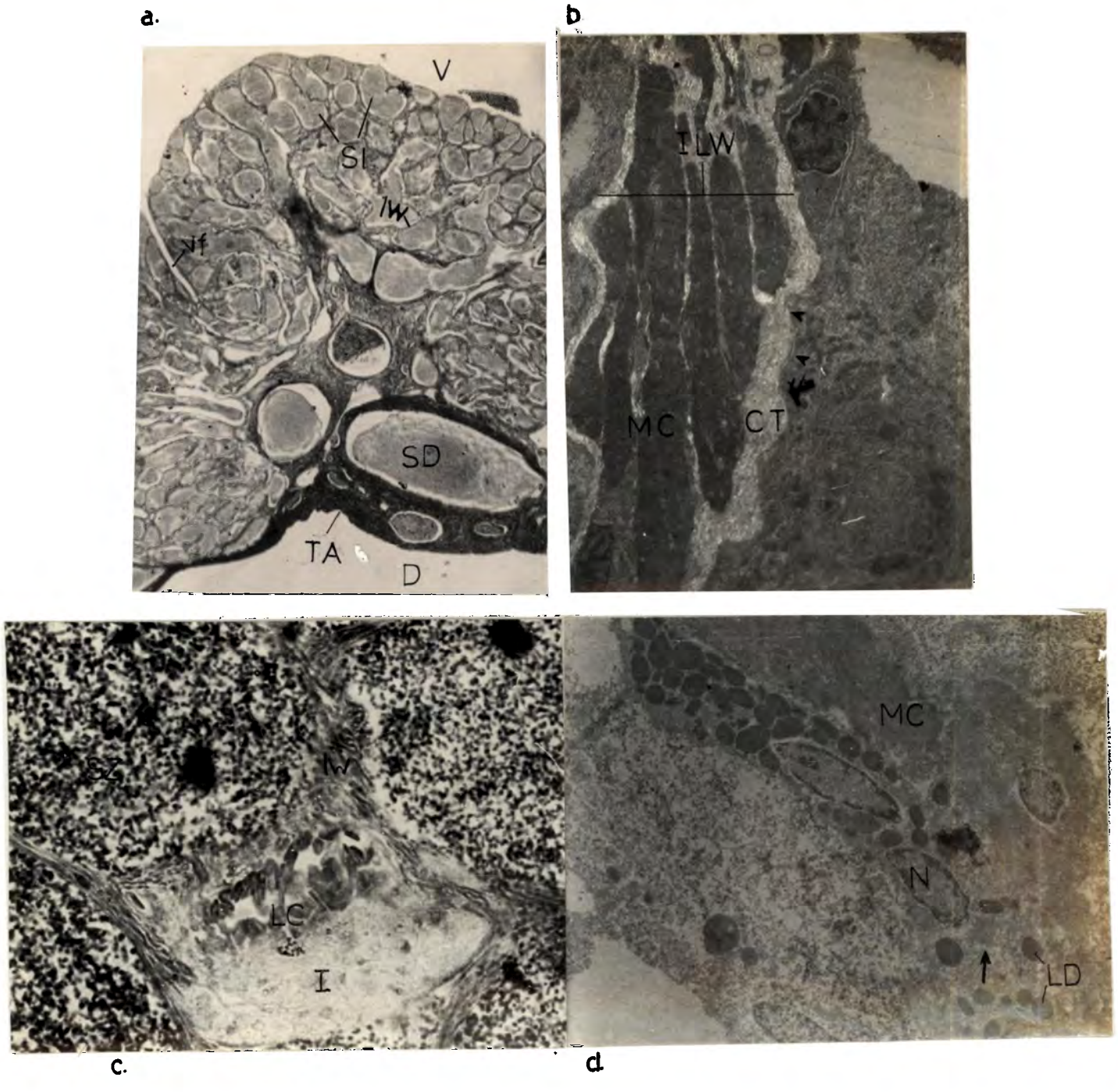


Table 5. Salient characteristics of various male germ cell types in L. calcarifer.

| | Primordial germ cells | Spermatogonia type A | | Spermatogonia type B | Primary spermatocytes | Secondary spermatocytes | Early spermatid | Late spermatid |
|---|---|---|---|---|---|---|---|---|
| | | type Aa | type As | | | | | |
| Topography | clusters | form layers around lobules | Isolated | in cysts | in cysts | in cysts | in cysts | in cysts |
| Association with sertoli cells | | Individually surrounded by sertoli cells | Individually surrounded by sertoli cells | sertoli cells delimit cyst | Sertoli cells delimit cyst | sertoli cyst delimit cyst | sertoli cells delimit cyst | sertoli cells delimit cyst |
| Cell shape | irregular | ovoid | ovoid | ovoid | ovoid | ovoid | ovoid | spherical |
| Cell size Length (µm) | 9.8-11.2 | 11.5-13.8 | 11.5-13.8 | 8.5-10.4 | 6.8-8.5 | 5.3-6.4 | 4.0-4.6 | 2.8-3.2 |
| Cell size Width (µm) | 8.0-9.4 | 10.0-12.8 | 10.0-12.8 | 7.8-9.0 | 6.5-7.6 | 4.6-5.5 | 2.9-3.4 | (diameter) |
| Nuclear shape | near spherical | near spherical | near spherical | ovoid | ovoid | near spherical | spherical | kidney shaped |
| Nuclear size Length (µm) | 6.1-6.8 | 7.1-7.8 | 7.1-7.8 | 5.2-6.8 | 3.8-4.7 | 3.2-3.8 | 2.5-2.8 | 1.4-1.6 |
| Nuclear size Width (µm) | 5.0-5.5 | 6.3-7.0 | 6.3-7.0 | 3.4-4.8 | 3.4-4.1 | 2.6-3.1 | (diameter) | 1.1-1.2 |

Inside the lobular compartment, two types of cells can be distinguished, sertoli cells and germ cells. Sertoli cells are somatic cells found in close association with the germ cells. Sertoli cells contain a nucleus, mostly notched, with dense chromatin. The cytoplasm contain ribosomes, mitochondria rough endoplasmic reticulum and lipid bodies. Lipid bodies are more abundant during recovering spent testis ( Plate IIIb ).

## SPERMATOGENESIS

During the present study, seven stages of germ cells were recognised on the basis of light and electron microscopic evidences (Table 5).

### 1. Primordial germ cells (PGCs)

PGCs are only observed in the newly differentiating testis. These cells, found in clusters of variable sizes, are characterized by polygonal shape and a highly basophilic nucleus ( Plate IIa,b ). PGCs proliferate mitotically and arrange in the form of single layer around newly forming lobules, prior to their transformation into type A spermatogonia ( Plate IIb ).

### 2. Type A Spermatogonia (GA)

Light and electron microscopic evidences distinguish two forms of type A spermatogonia: Type Aa and As.

**Type Aa Spermatogonia** (GAa):

GAa are seen arranged in the form of a layer of cells, seperated from each other by strands of sertoli cell cytoplasm. Lobular configration of the testis is still not fully established ( Plate IIc,d; IIIa ). GAa are more or less oval cells withregular outline, found in developing virgin as well as recovering spent testis.

Fine structure of the GAa ( Plate IIIb ) reveal abundance of cell organelles in the cytoplasm, including ribosomes, mitochordria, usually

## PLATE II

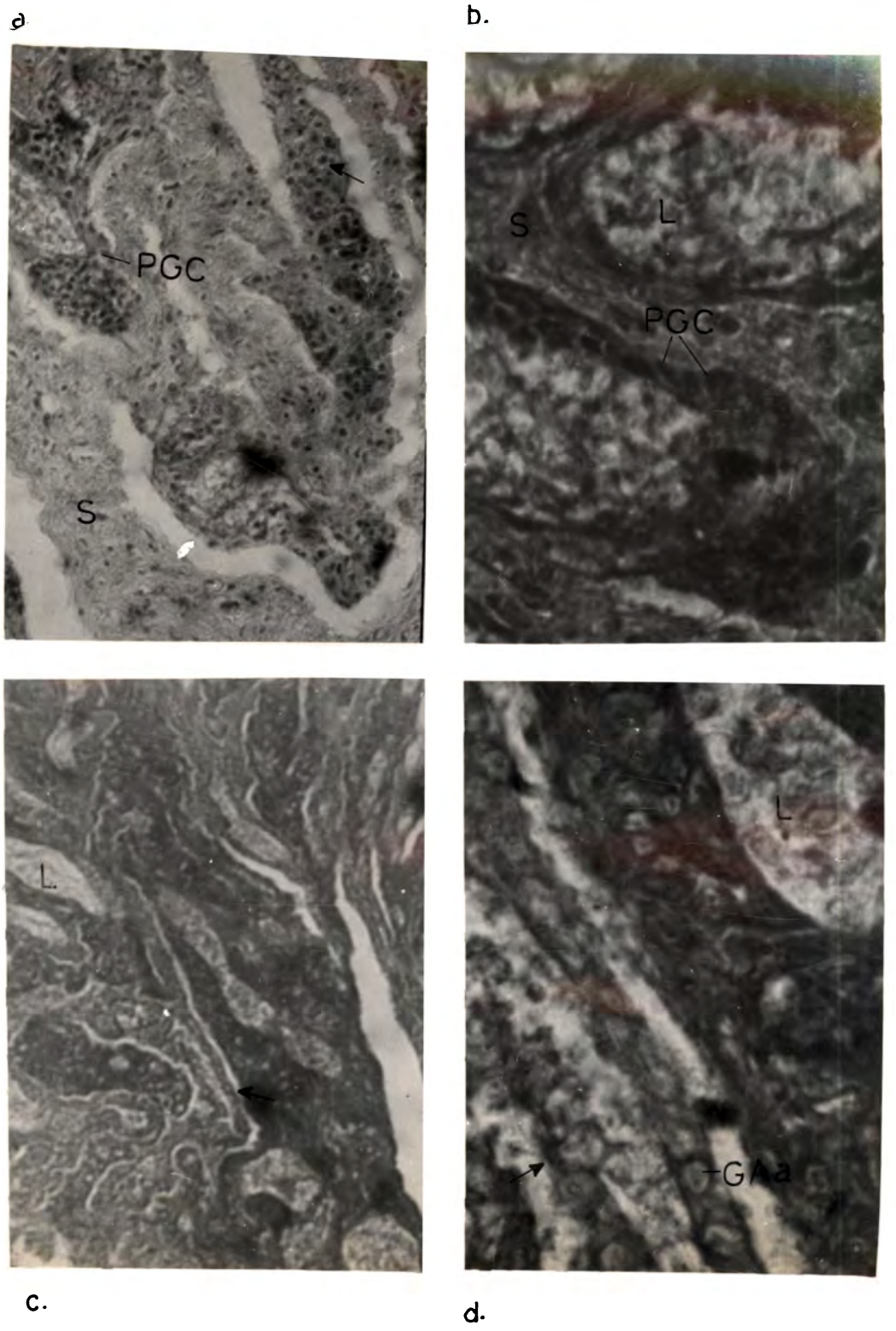
a. Light micrograph exhibiting clusters of primordial germ cells (PGC) in the stroma of newly differentiating testis. Arrow indicate mitotic figure. X 4,00

b. Light micrograph showing newly forming lobules (L) surrounded by layer of primordial germ cells. X 1,000.

c. Light micrograph os layers of spermatogonia type Aa(GAa). X 4,00.

d. Magnified view of spermatogonia type Aa(GAa). Arrow indicate sertoli cell nuclei. X 1,000.

**PLATE II**



## PLATE III

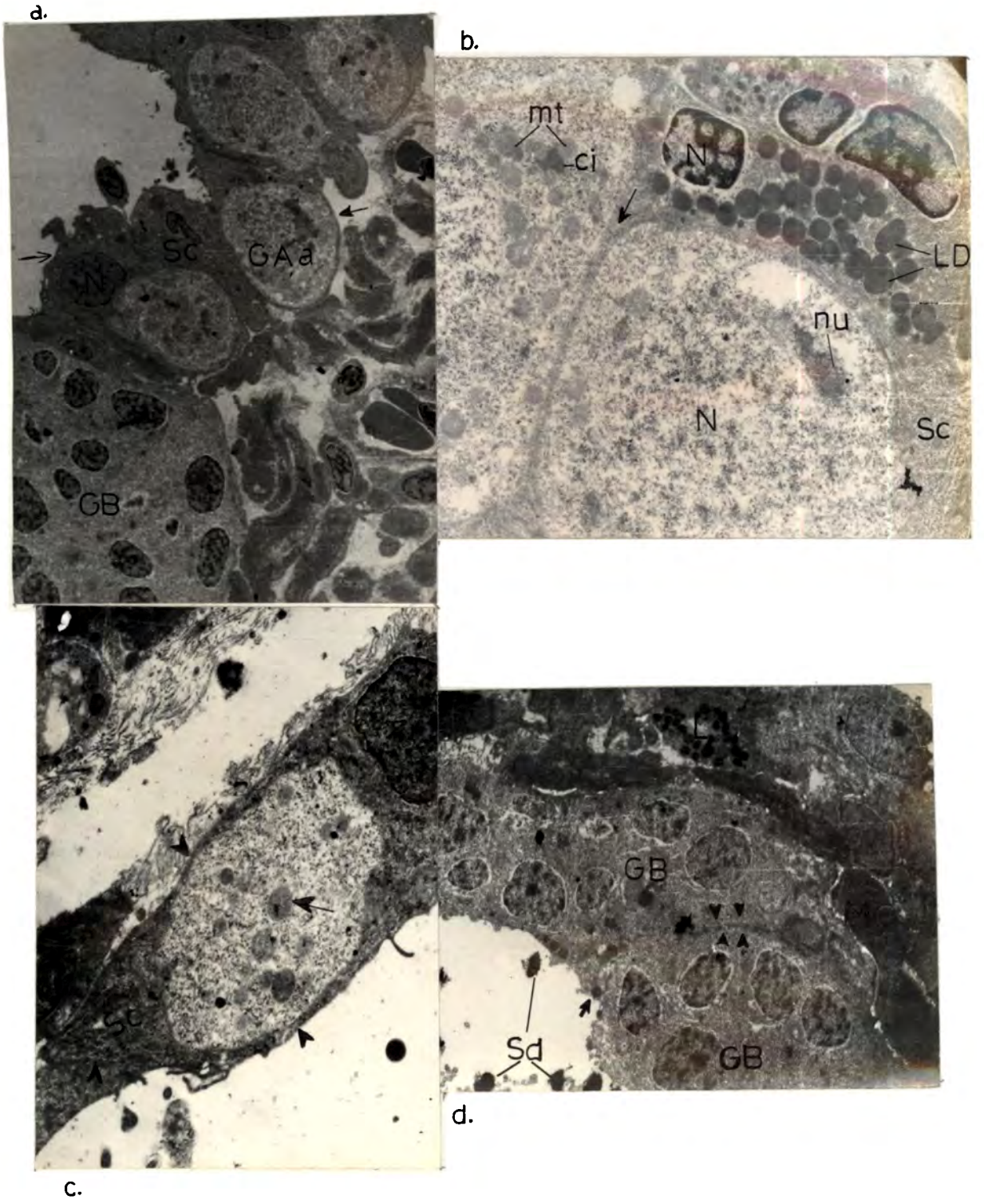
a. Electron micrograph showing spermatonia type Aa(GAa), individually surrounded by sertoli cell (Sc). Arrows indicate boundaries of sertoli cells. Germinal cyst containing spermatogonia type B (GB) is also visible. X 3,200

b. Electron micrograph of spermatogonia type Aa (GAa) seperated by intervening strand of sertoli cell cytoplasm (arrow). Sertoli cell (Sc) cytoplasm contain abundant lipid droplets. GAa cytoplasm is rich in mitochondria (mt) with intermitochondrial cement (Ci) and nuage (nu). X 9,600

c. Electron micrograph of spermatogonia type Aa (GAs) enclosed within the cytoplasmic strands of sertoli cells. Cytoplasm contains few mitochondria (arrow). X 6,800

d. Electron micrograph of peripheral portion of a seminiferous lobule showing cysts of spermatogonia type B (GB) bounded by sertoli cell cytoplasm (arrowheads). Lipid droplets ae also visible (arrow). Sperm heads (Sd) in the adjoining cyst indicate completion of spermiogenesis. X 4,000.

**PLATE III**



spherical and associated with intermitochondrial cement, nuage and rough endoplasmic reticulum. The cell organelles are distributed towards the cell pole. The nucleus is large, eccentric in location. The nuclear chromatin is finely distributed in the nucleoplasm. Lobules in early maturing testis (stage 3) also contain spermatogonial cells similar to the GAa cytologically but found isolated just next to basal lamina.

**Type As Spermatogonia (GAs)**

GAs are observed as isolated cells, enclosed with in the plasmalemma of sertoli cells, in the peripheral areas of lobules in mature (Stage 4) as well as ripe testis ( stage 5). Cytoplasm of these cells is lightly electron dense with scarce free ribosomes and mitochondrian. Nuage and intermitochondrial cement is mostly lacking ( Plate IIIc ).

**3. Type B Spermatogonia (GB)**

GA divide mitotically several times to form GB organised in the germinal cyst delimited by the cytoplasm of sertoli cells ( Plate IIIa,d; IVa). GB are smaller then GA with dense cytoplasm endowed with abdundant free ribosomes mitochondria, rough endoplasmic reticulum, intermitochondrial cement as well as nuage. Some mitochondria are elongated. Centrioles can also be identified. Intercellular bridges are not visible. Nucleus is usually oblong containing dense chromatin often found in clumps close to nuclear envelope. Nucleoli, usually fibrous component, are present in the nucleus ( Plate IVb,c ). GB divide mitotically several times within the cyst before transforming into primary spermatocytes.

The germ cells within a germinal cyst develop synchronously, however, all the cysts in a lobule may not progress similarly,(Plate IIIa)as depicted by the presence of a cyst containing GB, among the GAa; whereas in the plate IIId cysts containing GB are present while neighbouring cysts have already completed spermiogenesis.

## PLATE IV

a. Electron micrograph showing part of a germinal cyst containing spermatogonia type B. Nuclei (N) surrounded by cytoplasm containing mitochindria (mt) and intermitochondrial cement (C1) are also seen. Arrowhead indicate boundary of the cyst. X 8,600

b. Electron micrograph of part of the GB. Nucleus (IV) contains nucleolus (nu) with pars fibrosa (pf). Nuclear chromatin is in the form of dense clumps (arrow). Mitochondria and centriole are visible in the cytoplasm. X 23,000

c. Electron micrograph of two nuclei of spermatogonia type B (GB), apposed to each other showing the origin from same parent cell. Arrow indicates incomplete cytokinesis during the division. X 23,000.

**PLATE IV**

a.

b.

c.

**4. Primary spermatocytes (PSC)**

Primary spermatocytes are smaller cells with highly basophilic nuclei destined to undergo meiosis ( Plate Va ). PSC are characterized at ultrastructure level with varied chromatin complex structures. Spermatocytes contain oval nuclei with chromatin resembling that of parent cells (GB) in preleptotene stage. Chromatin in leptotene nucleus is more or less uniformly dense, with the appearance of interwoven fine threads ( Plate Vb ). Typical tripartite structure of synaptonemal complex is distinct during pachytene stage ( Plate Vc ). Cytoplasm of PSC contain abundant free ribosomes, mitochonclria, intermitochondrial cement is observed till pachytene stage. Intercellular bridges are visible. Occassionally bundles of microtubules can be found close to nuclear envelope. First meiotic division of primary speomatocytes give rise to secundary spermatocytes.

**5. Secondary spermatocytes (SSC)**

SSC are much smaller cells with very scant cytoplasm surrounding an intensely staining basophilic nucleus ( Plate Vd ). Cytplasm contain ribosomes, occasionally grouped as polyribosomes, mitochondria and centrole. Nuclear chromatin is in the form of dense clumps ( Plate Vla,b ). The secondary spermatocytes undergo maturation division (meiosis II) to produce spermatids.

**6. Spermatid (SD)**

Young spermatids, enclosed with in the germinal cyst are undifferentiated cells with a nearly spherical, centrally positioned nucleus containing irregularly dispersed coarse chromatin granules ( Plate Vlc ). Spermatid cytoplasm contain free ribosomes, polymorphic mitochondria, golgi body and centrioles. Certain electron dense bodies are also visible often along the circumference of the nucleus. Spermatid metamorphose into highly specialized spermatozoa through a specific process, spermiogenesis, involving concurrently occurring events viz. condensation of nuclear chromatin, development of flagellum, formation of midpiece and expulsion of excess cytoplasm.

## PLATE V

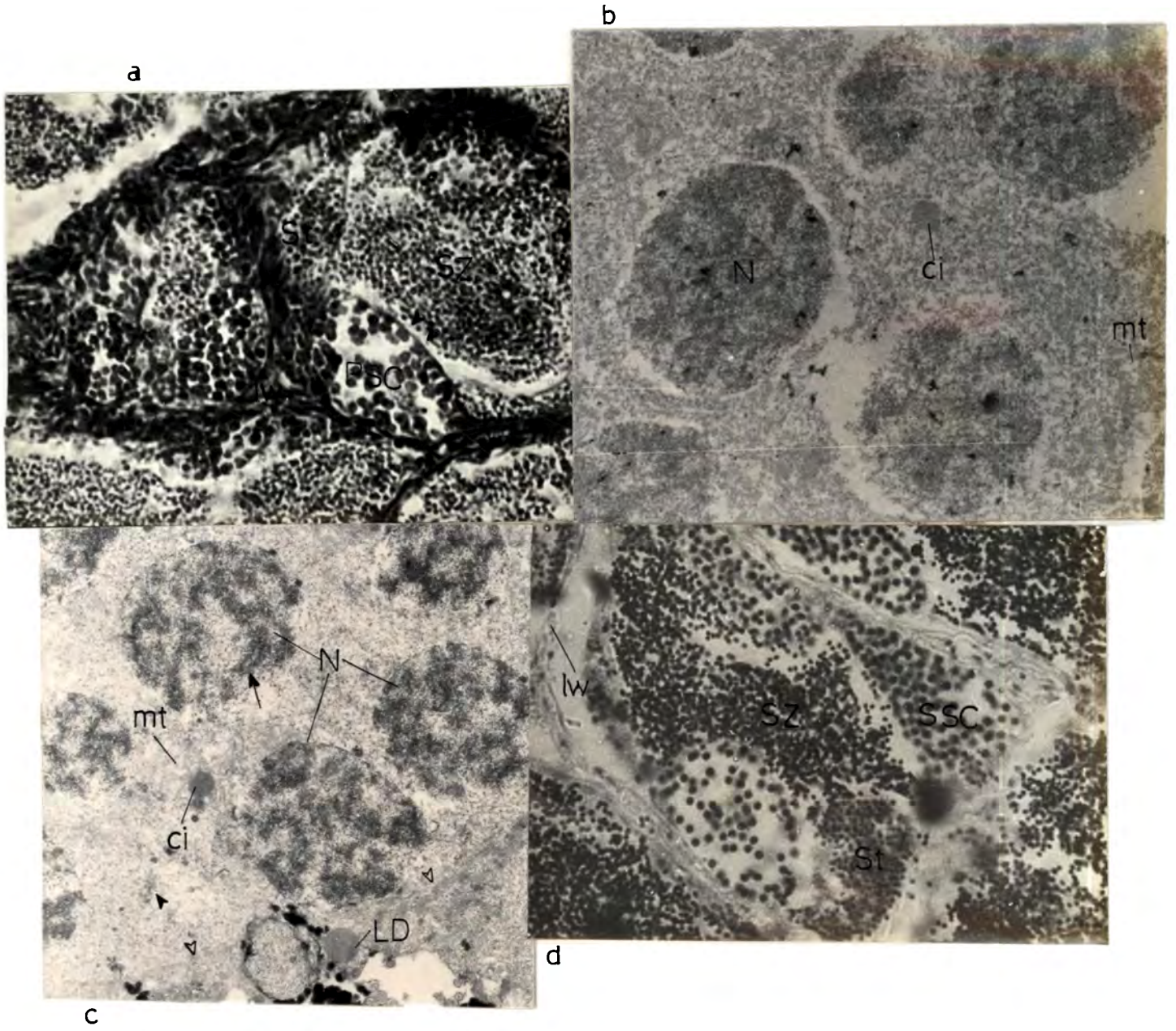
a. Light micrograph of stage 3 testis showing germinal cyst containing primary spermatocytes (PSC). Lumencontain spermatozoa (SZ). Arrow head indicates boundary of germinal cyst. X 100.

b. Electron micrograph showing a group of primary spermatocytes with leptotene nuclei (iv). Mitochondria (mt) and intermitochondrial cement (ci) are present in cytoplasm. X 9,000.

c. Electron micrograph of pachytene spermatocyte depicting cytoplasm containing mitochondria (mt) associated with intermitochondrial cement (Ci). Mitrotubules (boundary) germinal cyst is also visible. X 7,300.

d. Light micrograph of a seminiferous lobule showing cyst of secondary spermatocytes (SSC) and spermatid (St). The lumen of lobule contains spermatozoa (SZ). X 100.

**PLATE V**



## PLATE VI

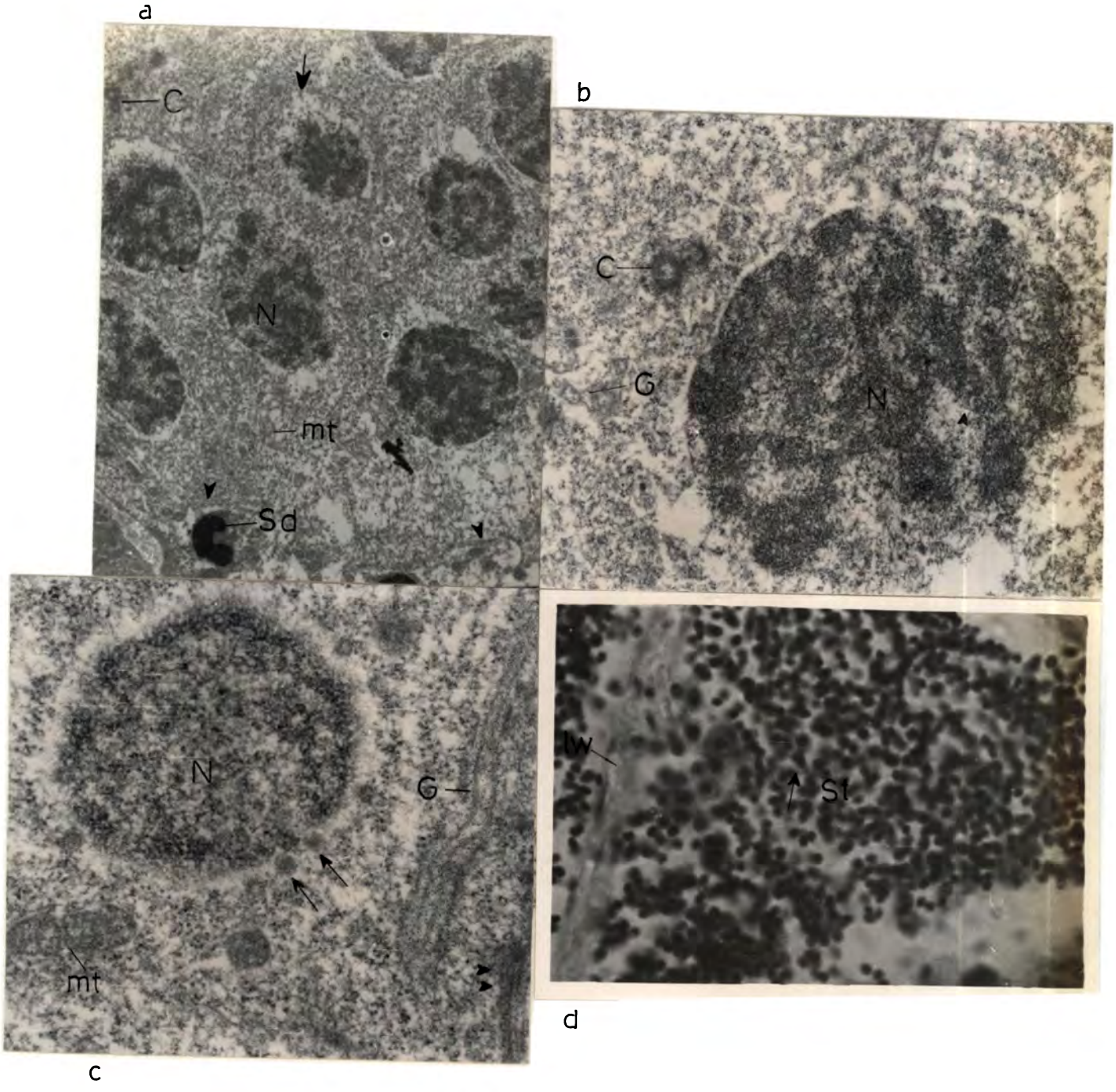
a. Electron micrograph of a group of secondary spermatocytes Arrow heads indicate boundary of the germinal cyst and the arrow shows the boundary of spermatocyte cell. Cytoplasm contain mitochondria (mt) and centriole (c). Note the dense clumps of nuclear chromatin in the nuclei (N). X 14,600.

b. Electron micrograph of a part of secondary spermatocyte showing nucleus (N) with dense clumps of nuclear chromatin (arrow head). Golgi body (G) and centriole (C) are visible in the picture. X 20,000.

c. Electron micrograph of a young spermatid containing nucleus (N), surrounded by cytoplasm with polymorphic mitochondria (mt) and glogi body (G). Arrow indicates dense bodies circumferential to nucleus. Arrow heads show area of junction between spermatid and sertoli cell. X 24,000.

d. Light micrograph of a group of spermatids (St) exhibiting kidney-shaped nuclei (arrow head). X 1, 000.

**PLATE VI**



Spermiogenesis is divisible into two discrete phases with respect to changes in nuclear chromatin.

**(i) Homogenization of chromatin:**

Chromatin granules become finer in size and homogenously distributed imparting uniform moderately, electron dense appearance to the nucleus of the spermatid (Plate VIIa). Centriolar complex with proximal centriole (PC) anterior to distal centriole (DC) comes to lie adjacent to flattened side of nuclear surface, where the nuclear wall invaginates to form articular fossa (AF) with scalloped boarder. Distal centriole acts as kinetosome and gives rise to axoneme, oriented perpendicular to the plasma memberane. Nuclear shape no longer remains spherical but kidney shaped with caudal AF pole broader as well as flattened and anterior round pole.

**(ii) Condensation of chromatin:**

**a. Early condensation stage:**

Chromatin granules increase in size and can be individually resolved ( Plate VIIb ). The process appear to commence at the caudal pole and proceed to other end evident from a low density area at the anterior side. Nuclear wall in this zone is seen partially fragmented, probably undergoing disintigration and reconstruction.

Electron micrograph ( Plate VIIb ) reveal that the distal centriole is flanked by electron dense satellites; perpendicular to intercentriolar axis, a plate is visible between the two centrioles. The plate is linked to both centrioles with fibres. Mitochondria are also visible adjacent to centriolar complex.

**b. Late condensation stage:**

During this stage chromatin aggregates to form large electron dense globules. It appears that the chromatin acquire fibrous texture which thicken and condense to form globules. The fibres are usually present for a short duration. The globule formation in usually seen more intense

## PLATE VII

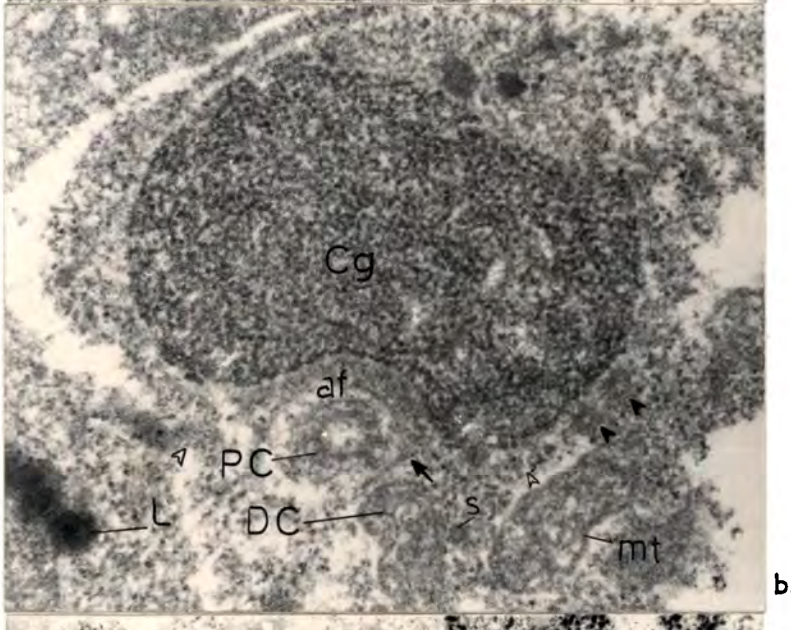
a. Electron micrograph showing spermatid (N) nucleus with homogenous chromatin. Articular fossa (af) has scalloped boarder. Fibres are also visible in the granular chromatin (arrrow head). X 46,000.

b. Electron micrograph of spermatid nucleus at early chromatin condensation stage showing enlarging chromatin granules (Cg). Proximal centriole (PC) is present in the articular fossa. Between proximal centriole and distal centrioles (DC), hoizontal plate is visible (arrow). Satellite around distal centriole (s) is also seen. Arrowheads indicate dense bodies circumferential to nucleus. Mitochondria (mt) are also visible close to the nucleus. X 40,000.

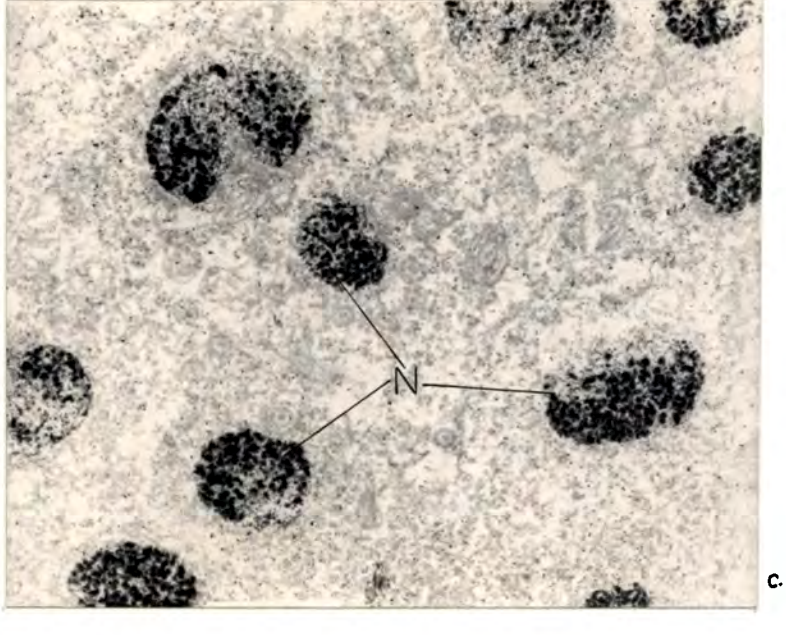
c. Electron micrograph of a group of spermatids with nuclei (N) at late condensation stage. X 8,600.

**PLATE VII**


a.




b.




c.

at the caudal end ( Plate VIIc, VIIIa ) indicating the onset of the process from this pole of the nucleus. Progressively the dense chromatin globules grow and fill inside the nuclear envelope. ( Plate VIIIb,c ). Globules are packed loose initially ( Plate VIIIc,d), undergo compaction during final stages and gradually become difficult to resolve individually. Occassionally, some spaces may be left, appearing as electron lucent areas in the highly electron dense spermatozoon head. ( Plate VIIIf ).

Concurrent to the chromatin condensation process, lagellum increases in length and is articulated inside the articular fossa. Mitochondria are relocated to form a ring around the articular fossa, the constituent are spherical, with granular matrix and a few cristae, positioned in the shallow depressions in the caudal region of the nucleus ( Plate VIIIe ). Plasma memberane make a loop seperating mitochondria from the proximal part of the flagellum and encovers posterior half of the mitochondria.

During the spermiogenesis, the cell volume is reduced considerably and excess cytoplasm is expelled in the form of packets which float freely in the cyst lumen ( Plate VIIIc ) and are phagocytysed by sertoli cells. This makes the plasma memberane to closely adhere to the nucleus in the mature spermatozoon ( Plate VIIIc).

## 7. Spermatozoa (SZ)

Mature spermatozoa is divisible into head, midpiece ,and tail. Head is doom shaped with highly electron dense nucleus having a keyhole shaped articular fossa at the posterior end for anchoring the flagellum ( Plate VIIIb ). Mid piece is constituted by ring of eight to ten mitochondria Tail consists of a central axoneme encovered by plasma memberane sheath. The axoneme is characterized by typical 9 + 2 pattern of microtubules. The nine peripheral microtubule doublets are attached individually to outer plasma memberane as well as two central micrortubules by small fibrils. The central microtubules are also held together with fibrils ( Plate IXa ).

## PLATE VIII

a. Electron micrograph of a kidney shaped nucleus (N) of spermatid at late condensation stage. Note the globule(g) formation is more intense towards caudal end with articular fossa (af). Chromatin fibres (arrrow) are also visible. X 33,000.

b. Electron micrograph showing spermatids at the end of sperm development. Plasma memberane (arrowhead) is closely apposed to nucleus in the sperm head (SH). Cytoplasmic extrusions are visible in the cyst lumen. X 8,600.

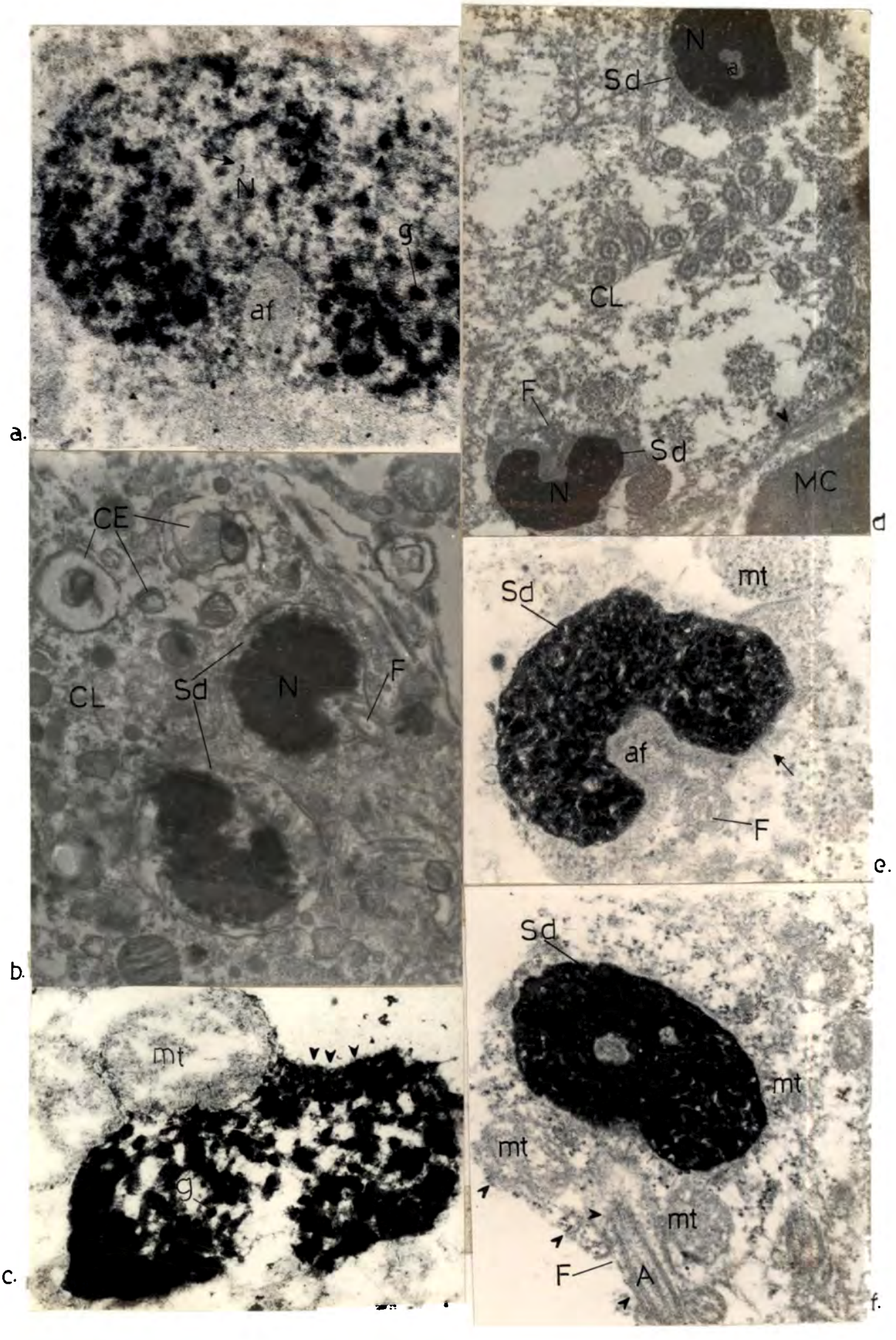
c. Electron micrograph of the sagittal section through caudal end of spermatid nucleus showing depressions (arrows) to accommodate mitochondria . X 33,000.

d. Electron micrograph showing sperm heads (SH) in the cyst lumen. Note the keyhole shape of articular fossa (af) in the longitudinnal section. X 8,000.

c. Electron micrograph of longitudinal sagittal section of sperm head (SH), Note the globules are loosely packed. Arrow indicates nuclear envelope continuous inside the articular fossa (af). Flagellum (F) in transverse section is also visible. X 26,800.

f. Electron micrograph of sagittal section of mature sperm passing through caudal end of sperm head. Note the electron-lucent area in the head (arrow) though globules are more compact. Mitochondria (mt) form a single layer surrounded by plasma membrane posteriorly, which loops (arrowheads) to seperate mitochondria form flagellum (F). Axoneme (A) is also visible. X 26,000.

**PLATE VIII**


a.
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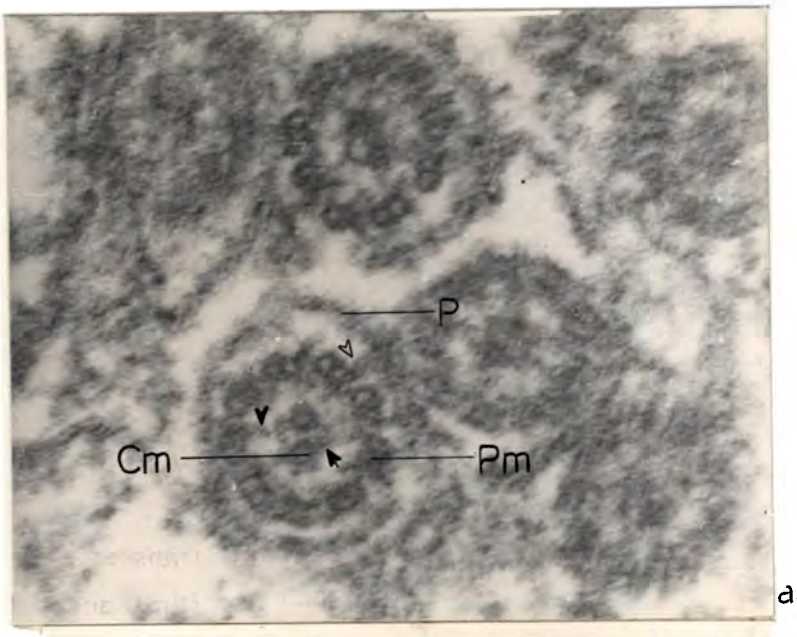
## PLATE IX

a. Electron micrograph showing transverse section of flagellum showing nine outer fibre doublets (Pm) and a pair of inner fibres (Cm). Inner fibres are joined by fibres to each other (arrow) and outer fibres (arrowhead). Outer fibres are joined (arrowhead; unfilled) to plasma memberane (P). X 96,000.

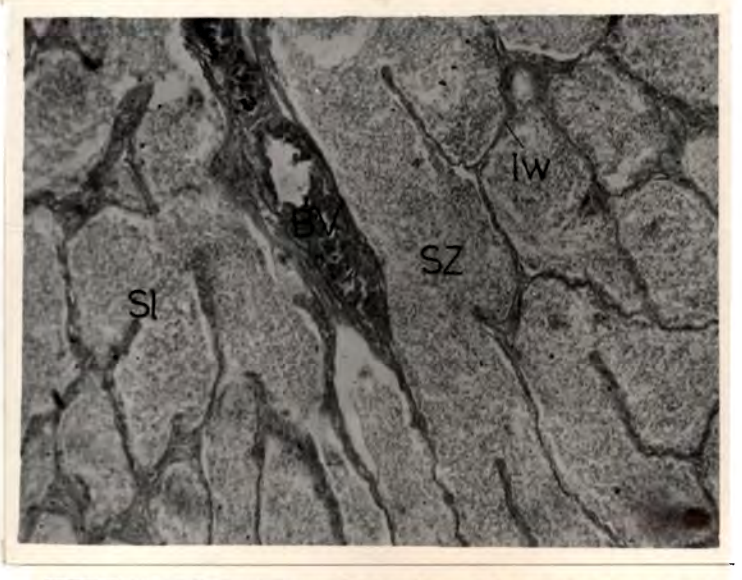
b. Light micrograph of lobules containing clamps of spermatozoa (SZ) in a mature testis . X 100.

c. Light micrograph showing loubles (L) packed with spermatozoa (SZ) in a running ripe testis. Blood vessel (BV) is also visible. X 100.

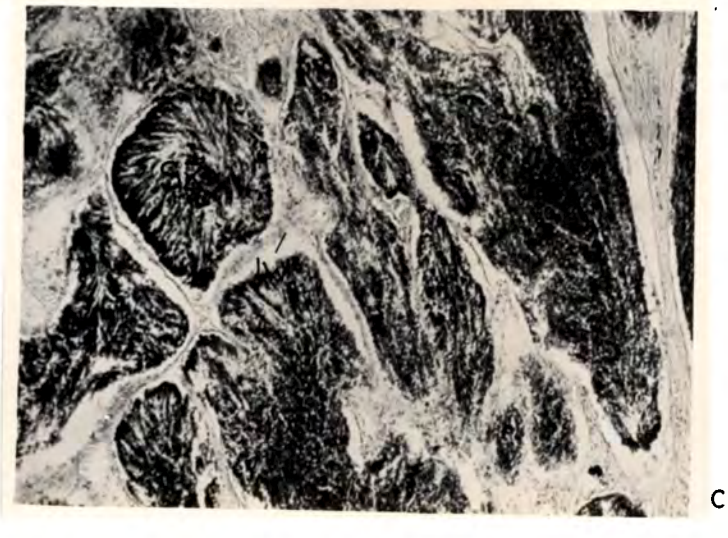
**PLATE IX**




a.




b.



c.

Mature spermatozoa are released from germinnal cyst into the lumen of lobule. Initially, the spermatozoa are seen as clumps with their tails held together (Plate IXb) Gradually, individual spermotozoa seperate out and are packed in lobules of ripe testis. (Plate IXc). During spawning phase, lobules and sperm ducts are filled with milt, does not have spermatogenic cyst. However, Plate Xa; reveal the exception to this, from the testis of a large sized males body weight (19.5 kg) described earlier. Active spermato-as well as spermiogenesis is evident. After the spawning, the lobules contain residual spermatozoa and spermaducts are mostly empty. (Plate Xb). After the spent stage, the testis revert to recovering spent to resume the cycle for next spawning season. Post spawned testis undergoing sex inversion can be recognized histologically, by cytoplasm; may be very few in number, initially (Plate xc). Gonads of L. calcarifer do not appear to contain heterologous germinal elements simultaneously and are dimorphic. The starp like testis reorganize completely to transform into cylindrical ovary. The transitional gonads cannot be identified macroscopically.

## OVARIES

The newly formed ovaries are deep red in colour due to high vascularization and are packed with oocytes of diameter less then 80 µm as well as clusters of primordial cells; which distinguish it from recovering spent ovaries (stage 2); starting point of new ovarian cycle after spawning.

## OOGENESIS

The development of oocyte occur in ovigerous lamella. Different stages of oocyte can be observed distributed randomly in the stroma (Plate XIa). Various stages of development upto the formation of egg are described below.

### Primordial germ cells

Primordial germ cells are small polygonal cells, occuring in clusters in the newly developing ovary (Plate XIIa) and characterized by large strongly basophilic nucleus. These cells proliferate mitotically(Plate XIIb).

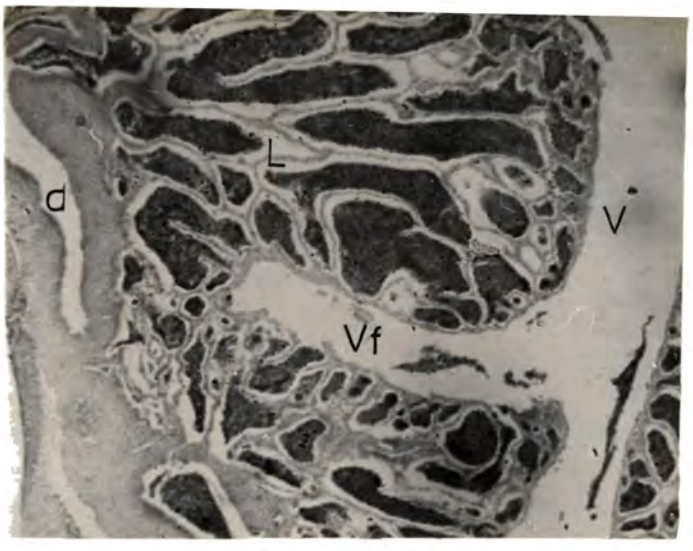
## PLATE X

a. Light micrograph of the lobules which contain residual spermatozoa in a spent testis. X 100.

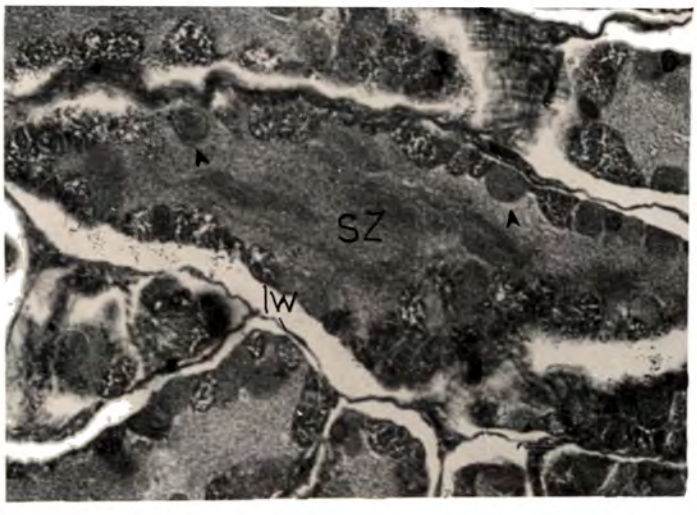
b. Light micrograph passing through lobules of testis from a large size male (19.5 Kg body weight). Lumen of the lobule is packed with spermatozoa (SZ) and cysts (arrowhead) are also seen along the lobular wall (lw). X 100.

c. Light micrograph of lobules of a post-spawned testis undergoing sex inversion. Note the early oocytes (arrow) with strongly basophilic cytoplasm. X 100.

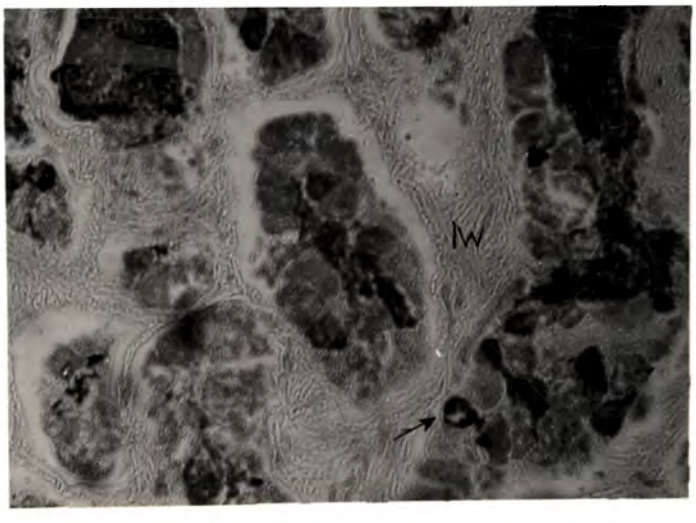
**PLATE X**




a.




b.

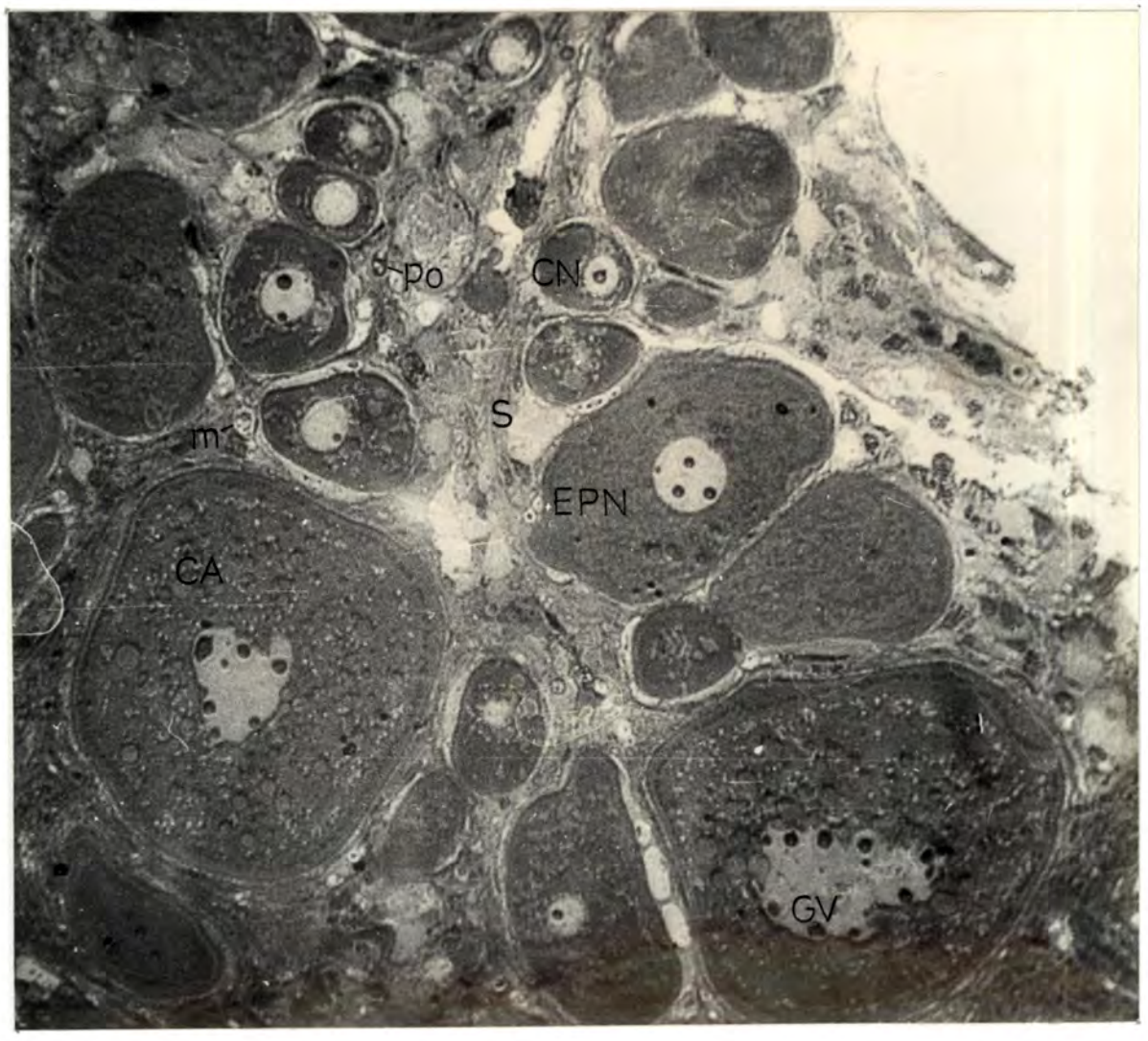



c.

## PLATE XI

a. Light micrograph showing random arrangement of oocytes in stroma (S) of ovigerous lamellae. Population of previtellogenic oocytes (EPN-early perinucleolus; CN- chromatin nucleolus stage) co-exist with the oocytes entering secondary growth phase (CA-cortical alveoli stage). Meiotic (m) and post meiotic (po) stage oocyte are also visible. X 120.

**PLATE XI**




a

## PLATE XII

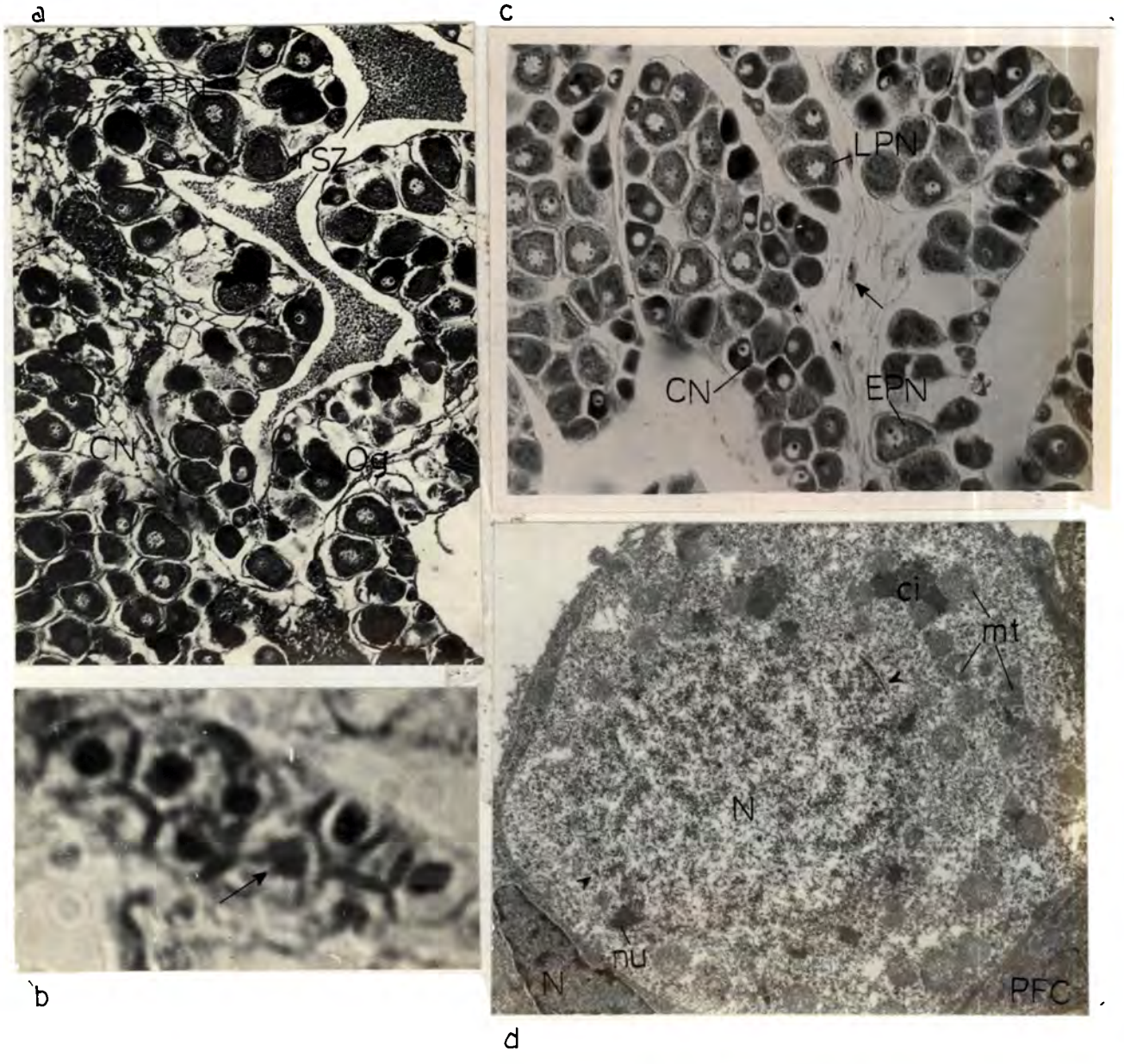
a. Light micrograph of newly differentiated ovary showing abundant clustes of primordial germ cells (arrow) along with early previtellogenic oocytes. X 140.

b. Light micrograph of a cluster of primordial germ cells indicating mitotic figure (arrow). X 1400.

c. Light micrograph of newly formed ovary at more advanced stage then that in (a). Note less abundence of primordial germ cell clusters (arrow). The oocytes are predominently at perinucleolar stages. X 140.

d. Electron micrograph of an oogonium showing nucleus (N) with a distinnct nuclear envelope (arrowheads). Cytoplasm has polar distribution of cell organelles containing mitochondria (mt) with associated cement (ci) and nuage (nu). Pe follicle cell (PFC) with dense nucleus (N) is also visible. X 13,200.

**PLATE XII**



Gradually, with the advancement of growth, primordial germ cells decline in abundance ( Plate XIIc).

**Oogonia (approximately 10 /um)**

Oogonia are oval or nearly spherical cells with regular outline ( Plate XIId ). Usually oocur isolated or in small clusters, scattered in stroma of ovigerous lamellae. Oogonia are characterized by large nucleus to cell ratio (N/c=0.50 to 0.62).Nucleus is large and oval in shape. Nucleoplasm appear electron-lucent containing small clumps of chromatin more near the nuclear envelope. Nuclear envelope is smooth, regular and formed of two layers.

Cytoplasm contain spherical mitochondria, free ribosomes and endoplasmic reticulum. Aggregates of nuage material are frequently observed isolated or in association with mitochondria. Cell organelles are normally polar in distribution.

Oogonia are usually contiguous with neighbouring germ cells or somatic cells, prefollicle cells. Prefollicle cells are characterized by an irregular outline and cytoplasm more electron dense then oogonia. Nucleus can be oval or elongated with dense clumps of chromatin.

**Primary growth Phase:**

**Chromatin nucleolus oocytes (10-25 /um diameter)**

Cells are oval characterized by increasing amount of cytoplasm as well as cell organelles and decreasing nucleus to cell ratio (N/C=0.42 to 0.50).

Nucleus is large, oval shaped more or less eccentric location and contain single nucleolus (Plate XIa, XIIIa,b ). Chromatin is fine, granular and uniformly dispersed in the nucleoplasm. Occassionally, patches of dense granules are seen scattered. Nuclear envelope has partial interruptions and form outpockets, released in the ooplasm as vesicles. Nuclear envelope becomes more regular in the later stages.

## PLATE XIII

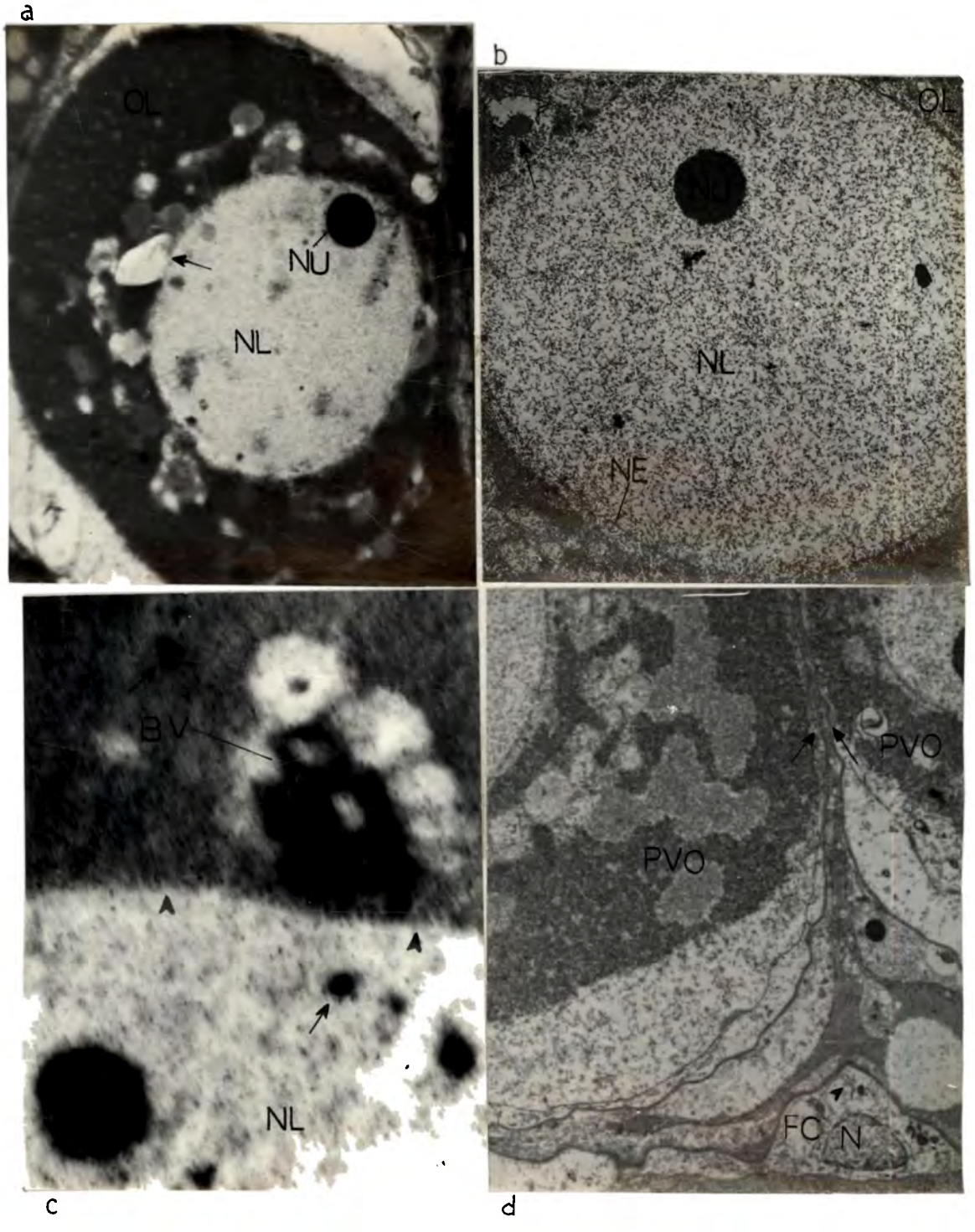
a. Electron micrograph of an early chromatin nucleolus stage oocytes showing highly dense ooplasm with tightly packed ribosomes. Nucleus contains moderately dense nucleoplasm (NL) and single nucleolus (NU). Nuclear envelope show outpocketing and interruptions (arrow). X 5,500.

b. Electron micrograph of the nucleus of a chromatin nucleolus stage oocyte, showing single compact nucleolus (NU) in the nucleoplasm (NL). Nuclear envelope (NE) is not regular; arrow indicate a body in the ooplasm, appear to be an extruded nucleolus. X 6800.

c. Electron micrograph showing early stage of Balbiani's vitelline body (BV) originating close to nuclear envelope in chromatin nucleolus oocyte. X 15,000.

d. Electron micrograph of the area between two adjacent previtellogenic (PVO) oocytes. Basal lamina are closely apposed (arrow). Follicle cell (FC) of another oocyte is visible with central nucleus (N) and intercellular filaments (arrowhead) in the cytoplasm. X 7,300.

**PLATE XIII**



Cytoplasm is strongly basophilic and electron dense especially in early stages ( Plate XIIIa ), tightly packed with ribosomes. Electron-density reduce with the increase in cell size during growth. Circum-ferential to nuclear envelope is a low density area of cytoplasm. Dense aggregates of nuage material are also seen scattered along the nuclear envelope ( Plate XIIIb ). The Balbiani's vitelline body originite, during late chromatin nucleolus stage, adjacent to outer nuclear envelope as a dense mass of nuage, associated with spherical mitochondria. The reticulate configuration of the central mass cannot be resolved distinctly at this stage ( Plate XIIIc ). Lightly staining patches, containing mostly mitochondria are confined to perinuclear ooplasm at this stage ( Plate XIIId).

The oocytes are contained with in the definite follicles, with mono-layer of a few follicle cells extending their processes between oolemma and basal lamina ( Plate XIIId).

Cytoplasm is less electron dense then prefollicle cells. Basal lamina of two adjacent follicles are usually mutually apposed. Thecal elements are still lacking.

**Early perinucleolar stage (25 - 80 $\mu$m)**

Oocytes are round or oval shaped with nucleus to cell ratio 0.40 to 0.50. The cells contain large sized, more or less spherical in shape and centrally located, nucleus ( Plate XIa ). Nuclear envelope is regular, smooth and consists of two layers, with a perinuclear space in between (Plate XIVa; XVa ). The nuclear envelope is interrupted by nuclear pores surrounded by a dense spherical ring, annulus. The structure of the pore complex is more clear in tangential plane ( Plate XIb; XVb ). In the center of the pore complex dense dot is also visible.

This phase is characterized by multiplication of nucleoli of unequal size. The number of nucleoli range from five to eight, with a tendency to arrange along the inner nuclear envelope. Nucleoli containing vacuoles and composed of exclusively fibrillar component are also seen close to

## PLATE XIV

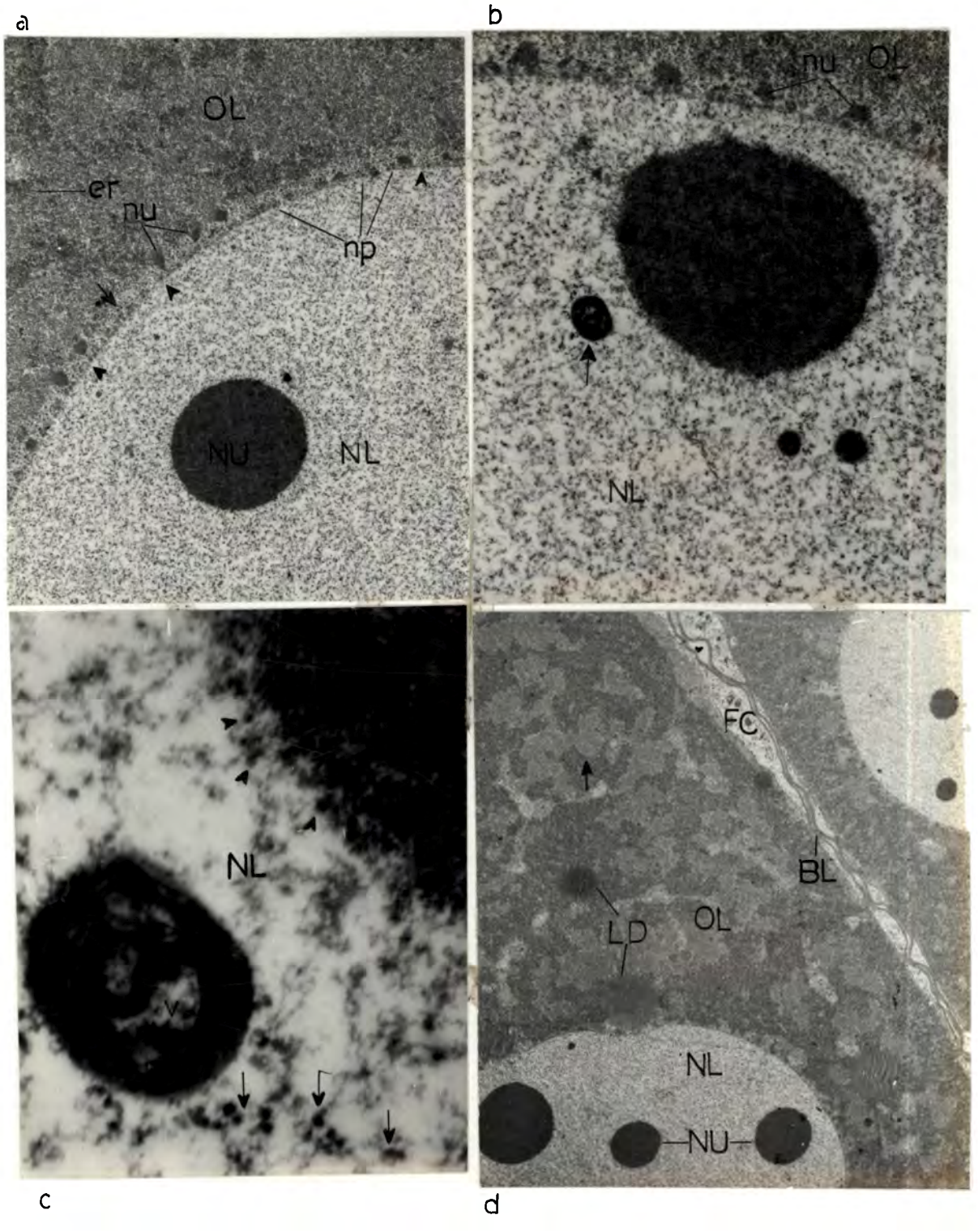
a. Electron micrograph of the perinuclear area of the early perinucleolus stage oocytes showing a compact nucleolus (NU). Nuclear envelope (arrowheads) has a number of nuclear pores (np). Nuage (nu) material is present along the circumference of nucleus in the ooplasm. X 7,400.

b. Electron micrograph of nuclear area of early perinucleolar oocytes showing large compact nucleolus (NU) along with small vacuolated nucleoli (arrrow). Nuage (nu) material is visible close to the nuclear envelop in the ooplasm (PL). X 6,500.

c. High power electron micrograph showing small nucleolus with vacuole (V). Note the fibrillar composition of this nucleolus in contrast to granules (arrowheads) present in large compact nucleolus (NU). These granules resemble to that scattered (arrows) in the nucleoplasm (NL). X 93,000.

d. Electron micrograph of adjacent early perinucleolus stage oocytes. Note the presence of a few randomly distributed lipid droplets (LD) in the ooplasm (OL). Follicle layer (F) is surrounded by basal lamina (BL). Gaps are arising between the basal lamina ( BL ). The ooplasm contain low electron density patches (arrow). X 3,300.

**PLATE XIV**



## PLATE XV

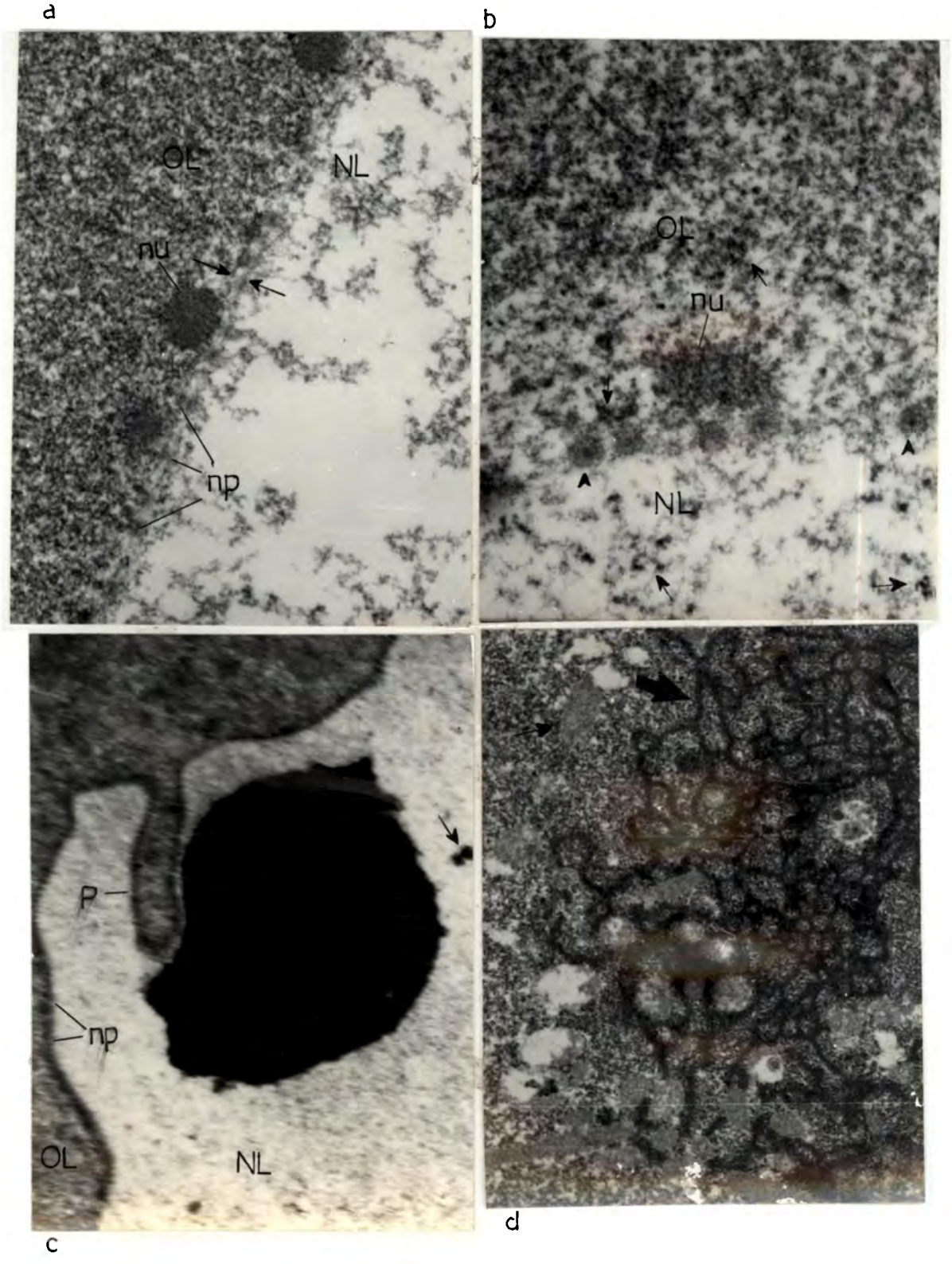
a. Electron micrograph showing double-walled structure of nuclear envelope (↠ ↞) and nuclear pores (np). Note the presence of dense ring, annulus ,around the nuclear pores. X 1,15,500.

b. Electron micrograph depicting nucleo-cytoplasmic interaction. The granules (arrows) found in nucleoplasm resemble those found close to nuclear envelope, nuage material and ooplasm. Nuclear pore complex (arrowheads) in tangential plane show dense circular annulus with a central dense dot. X 60,000

c. Electron micrograph depictinng precise contact between nuclear envelope and nucleolus (NU) through formation of process (P). Nucleolar material pass out in the ooplasm (OL) through nuclear pores (np.). Nucleolar fragments are seen scattered in the nucleoplasm. X 10,000.

d. Electron micrograph showing a part of Balbiani's vitelline body at late perinucleolar stage. Note the reticulate configuration of nuage and/or nucleolar material (➡) Mitochondria associated with the body have no attached ribosomes ( ). X 16,500.

**PLATE XV**



large compact nucleoli ( Plate XIVb,c ) of fibrillo-granular composition. Nucleoplasm is electron-lucent with scattered granules similar to that found in nucleolus (Plate XIVc) These granules pass out in the ooplasm through nuclear pores ( Plate XVb ). Occassionally, nuclear envelope invaginate to establish precise contact ( Plate XVc ) with large nucleoli appear to causing erosion of nucleolus and facilitating passage of nucleolus.

During this stage dense clumps of nucleolar particles, 'nuage', are seen scattered along the outer nuclear envelope in abundance (Plate XIVa,XVa)

The Balbani's vitelline body migrate in the outer ooplasm and is more differentiated consisting of a regular network of nuage material ( Plate XVd ). The mitochondria are observed in between the interices or adjacent to the structure and lack ribosomes.

The lightly staining patches, confined to perinuclear ooplasm in the preceeding stage, are scattered through out the area (Plate XIVd). These patches consist of clusters of mitochondria similar to those present close to Balbiani's vitelline body.

Follicle layer is more organized ( then ) in the previous stage. The association between adjacent oocytes show detachment at certain points.

**Late Perinucleolar stage (70 - 120 /um).**

The oocytes at this stage are round, with a large spherical nucleus with a diameter ranging from 35 to 45 /um (N/C 0.36 - 0.44). The multiple nucleoli are organized in the peripheral nucleoplasm. Nuclear envelope is regular and smooth in outline (Plate XVa

Cytoplasm exhibit high basophilia as well as electron density. The nuage material is still seen accumulating along the outer nuclear envelope.

Balbiani's vitelline body move towards periphery and disintegrates in the late stages. Light staining patches widely scattered in the early perinucleolar stage gradually disappear. At this stage, ooplasm is richy endowed with cell organelles distributed throughout from perinuclear to cortical area.

Oocyte begin to elaborate microvillar processes towards the overlying follicle cells ( Plate XVIb ), though, follicle cells do not extend microvilli yet. Follicle cells do not show any marked changes. In the gaps between the adjacent basal lamina, thecal elements start appearing to form a continuous theca around the oocyte.

**Secondary growth phase**

At the end of primary growth phase, a group of oocytes, out of the whole population, become distinct by inclusion of components characteristic of secondary growth phase.

**Cortical alveoli stage (120 - 270 /um)**

The oocytes exhibit appearance of three components (a) cortical alveoli (b) lipid droplets (c) vitelline envelope.

Cortical alveoli are the small vesicles, observed through out the peripheral ooplasm ( Plate XVIc ). At ultra structure level they appear as irregular shaped electron lucent structures containing loosely filled granular material ( Plate XVIIa

Lipid droplets, which start appearing in the perinucleolar stages, increase tremendously in number and occupy the whole area within the oolemma. ( Plate XVIc).

Under optical microscope, vitelline envelope can be resolved as a thin band immediately outside the oolemma ( Plate XVc ). At the ultrastructure level, it is distinguished with the appearance of homogenous

## PLATE XVI

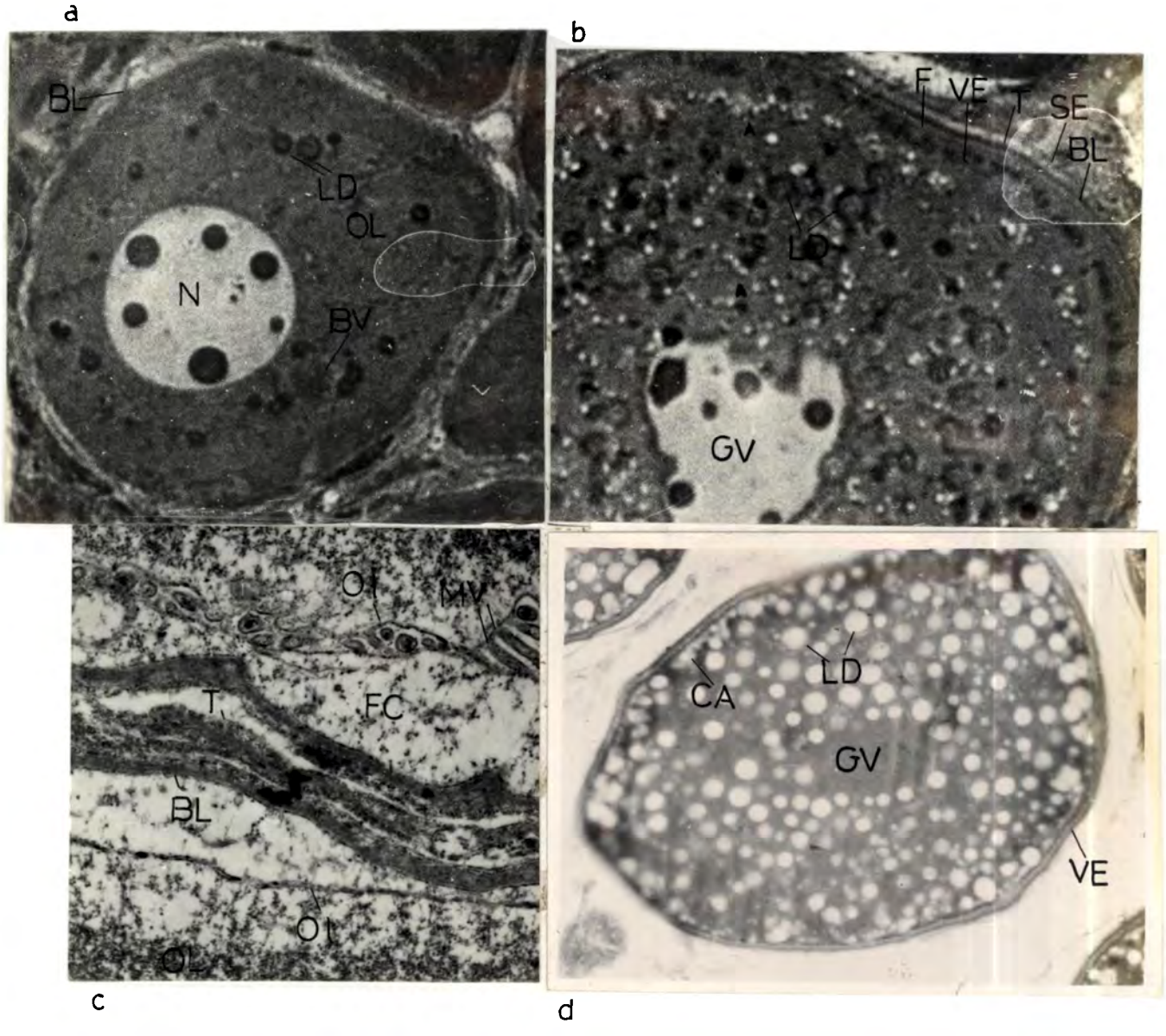
a. Light micrograph of a late preinucleolar stage oocyte showing the yolk nucleus (YN). Lipid droplets (LD) are seen cattered in the ooplasm (OL). X 500.

b., Electron micrograph of surrounding layers in late perinucleolar stage oocyte. Follicular layer (F) is surrounded by basal lamina (BL). In the gap between, two basal lamina, thecal (T) material is present. Oocyte has started extending microvillar processes (MV) towards follicle cells. X 12,000.

c. Light micrograph of a part of cortical alveoli stage oocyte. Germinal vesicle (GV) has peripheral nucleoli (NU). Number of Lipid droplets (LD) is increased. Cortical aleoli (arrowheads) are randomly distributed but more concentrated in peripheral ooplasm. Oocyte is surrounded by vitelline envelope (VE), follicular layer (F), basal lamina (BL) and thecal layer (T), and surface epithelium (SE). X 340.

d. Light micrograph of early vitellogenic oocyte with a distinct vitelline envelope (VE). Abundant lipid droplets (LD) are randomly distributed. X 140.

**PLATE XVI**

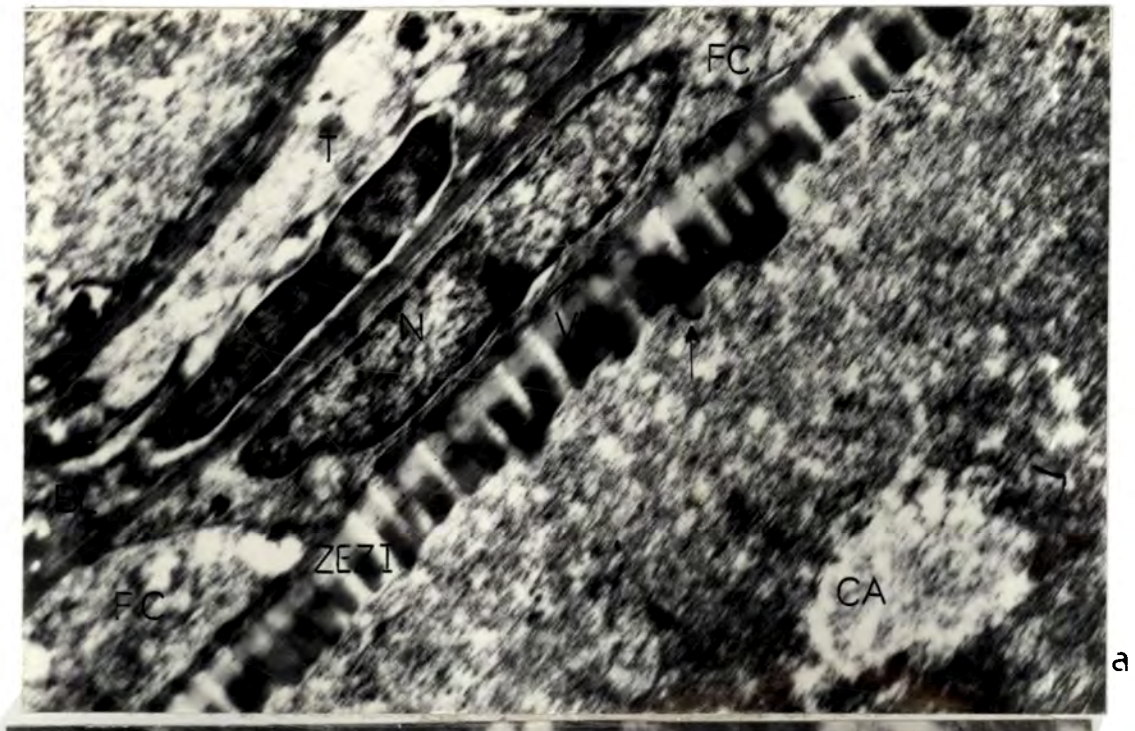


## PLATE XVII

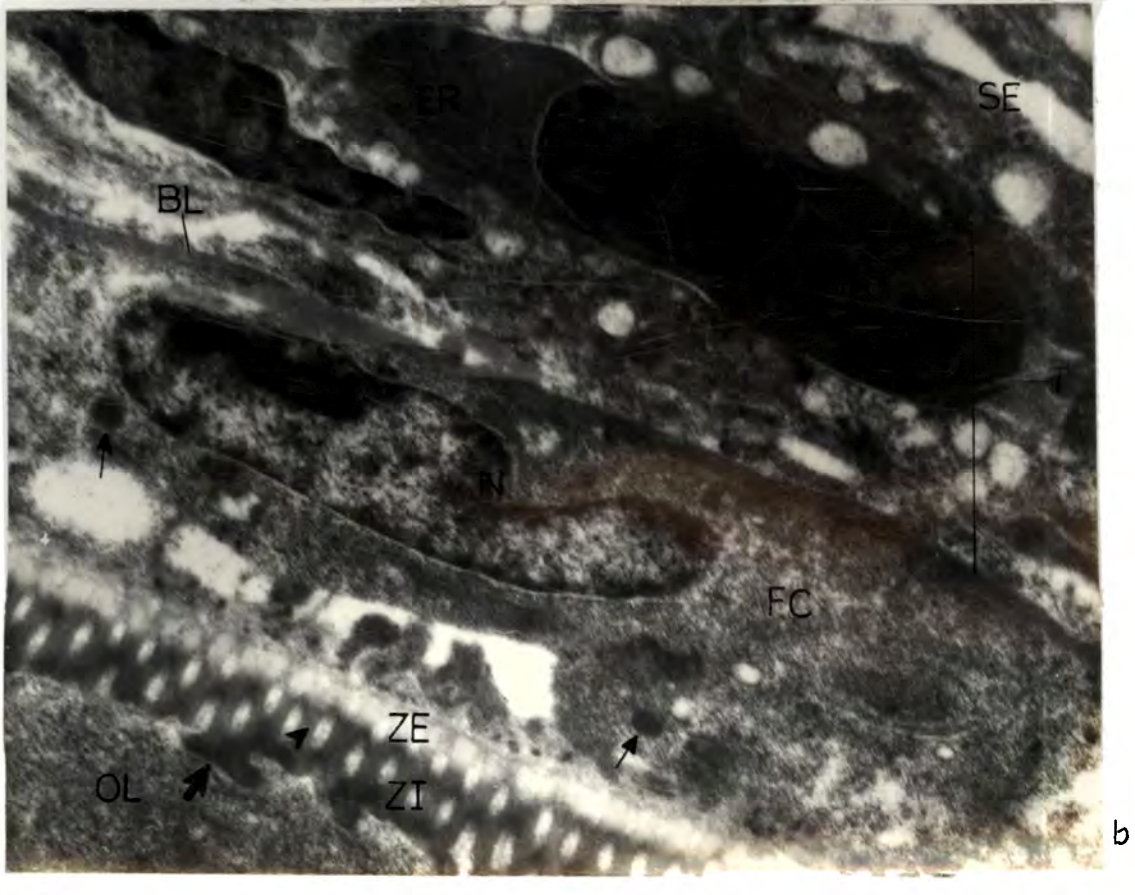
a. Electron micrograph depicting vitelline envelope (VE) with bilaminar structure external (ZE) and internal layers (ZI). Pinocytotic (arrow) activity has commenced. Dense material (arrowhead) (appears to be yolk precussor) passed into oocyte. Endoplasmic reticulum (er) is present. Outside vitelline envelope, follicle cells (FC) have typical squamous appearance with elongated nucleus (N). Note the irregular shape of cortical alveoli (CA) filled with electron-lucent material.. X 8,000.

b. Electron micrograph showing vitelline envelop (VE) in tangential plane with pore canals (arrowhead). Pinocytotic vesicles are visible (arrow). Follicle cells (FC) have more cuboidal appearance. Outside basal lamina (BL) is a thecal layer (T) showing blood vessel (indicated by erythrocyte; E). Dense bodies found close to blood vessel resemble to those found in follicular cell. X 10,200.

**PLATE XVII**




a




b

material deposited between the oocyte microvillar processes of width 4.8 /um. The vitelline envelope attains bilaminar appearance with external (ZE = 2.2 /um) and internal (ZI = 2.6 /um) layers ( Plate XVIIa ). Microvillar processes continue to lengthen as the vitelline envelope thicknes. The oocyte surface start exhibiting pinocytotic activity ( Plate XVIIa).

Follicle cells exhibit typical squamous appearance, contain elongated nucleus with dense granular chromatin. The cytoplasm contain mitochondria and rough endoplasmic reticulum. Some dense structures, probably yolk material is present in the follicle cells particularly at the boundary with oocyte ( Plate XVIIa ). The follicle cell processes also eloborate through the perforations in the vitelline envelope.

**Vitellogenesis (260 - 500 /um)**

This phase is characterized by accumulation of exogenously derived yolk in the form of yolk spheres accounting for majority of the growth of the oocyte. From the population of cortical alveoli oocytes, a clutch of oocytes is recruited which undergo vitellogenesis. The oocytes in this clutch develop synchronously.

Early vitellogenic oocytes (260 - 380 /um) contain many developing yolk spheres in the peripheral as well as interior ooplasm. The cortical alveoli are easily resolved below the vitelline envelope. The lipid droplets increase in size as well as number ( Plate XVId ). Vitelline envelope thickness ( Plate XVId, XVIIb ) and appear striated due to the presence of pore canals. These pore canals appear oblong in cross section. The microvilli traverse through these pore canals ( Plate XVIIb).

As the oocyte growth proceeds, the yolk spheres become more heterogenous in size. The late vitellogenic oocyte (380 - 500 /um) is characterized by accumulation of mature yolk spheres. The lipid droplets, appearing more electron - lucent, increase in number as well as size and are heterogenously distributed. The cortical alveoli become difficult to resolve under optical miscroscopy due to accumulation of yolk spheres.

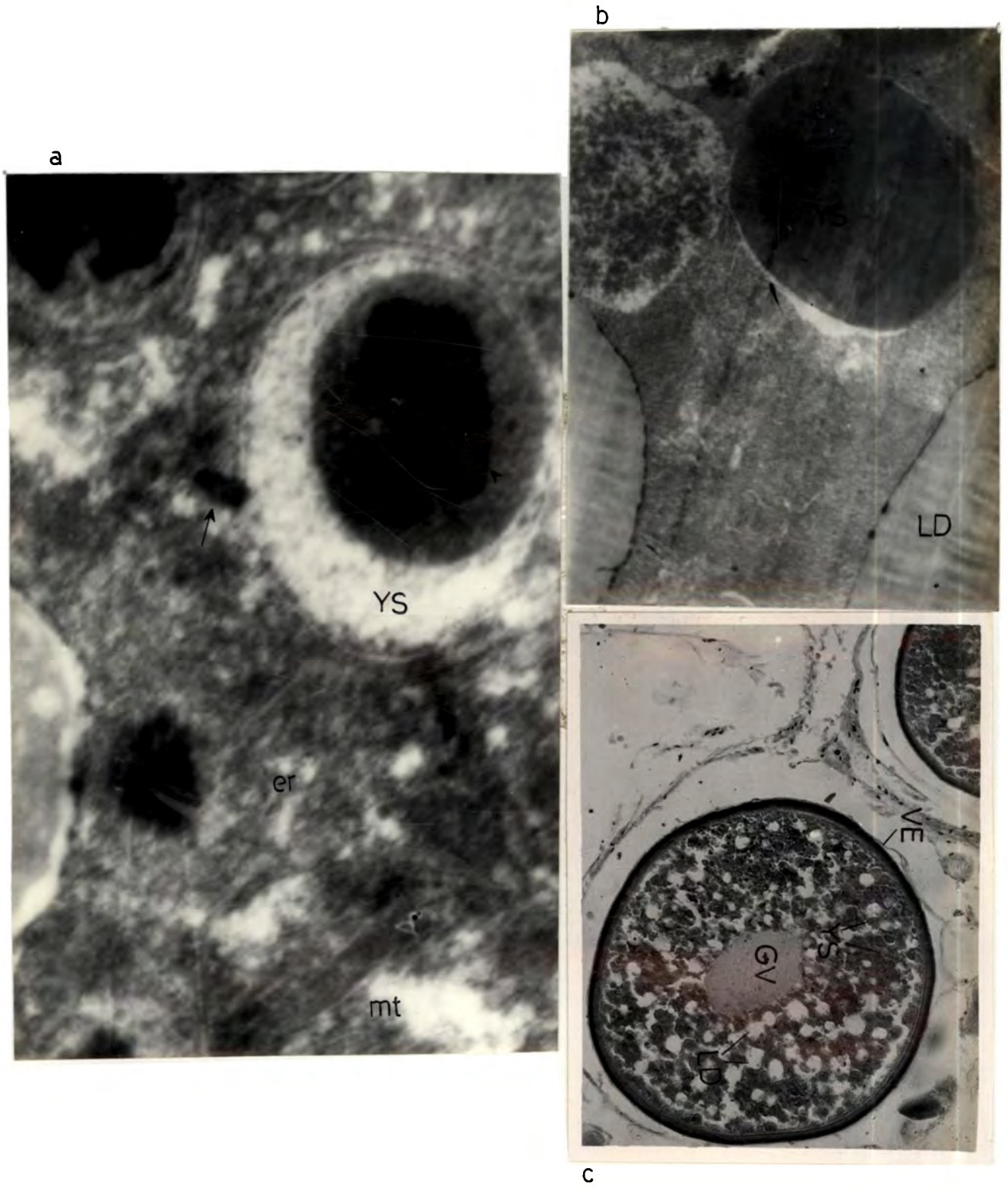
## PLATE XVIII

a. Electron micrograph showing transitional yolk spheres(Ys). Note the central highly dense material surrounded by area of low electron density in a fluid filled double walled vesicle. Dense bodies referred in earlier micrographs is seen passing into yolk sphere (arrow). X 12,600.

b. Electron micrograph of a mature yolk sphere (YS). Lipid droplets (LD) are also seen. X 8,000.

c. Light micrograph of a oocyte after completion of vitellogenic growth, compactly filled with yolk spheres (YS). X 140.

**PLATE XVIII**



Bilaminar, vitelline envelope increase in width ( 7.8 /um; ZE = 2.6 /um; ZI = 5.2 /um) and become architecturally complex with apparent radiated appearance. High power optical microscopy can resolve the pore canals as well as beaded configuration of internal layer of vitelline envelope, ( Plate XIXa ), which is more precise under the electron microscope. Microvillar processes traverse through these pore canals ( Plate XIXb).

During vitellogenesis, oocyte surface continues to display intense pinocytotic activity. The pinocytotic vesicles are filled spherical bodies (Plate XVII most likely to be associated with sequestration of yolk precursor material and appear scattered in the peripheral ooplasm as small dense structures of irregular shape. These structures are incorporated into developing yolk spheres; fluid filled memberane bound structures ( Plate XVIIIa ) Ultrastructure of early vitellogenic oocyte depict the presence of transitional yolk spheres. The transitional yolk spheres exhibit distinct heterogenous electron density ( Plate XVIIIa ). The yolk is processed and condense, to become homogenously electron dense in the mature yolk spheres ( Plate XVIIIb ) and pack the interior of the oocyte ( Plate XVIIId )

Follicle cells surrounding vitellogenic oocytes contain mitochondria, rough as well as smooth endoplasmic reticulum, ribosomes in the cytoplasm. Nucleus is elongated with a conspicuous nucleolus present occasionally. The dense bodies are present in the cytoplasm more distinct at the boarder with vitelline envelope. ( Plate XVIIb ). Outside the basal lamina, thecal elements form a continuous layer and are surrounded by a layer of surface epithelium (Plate XVIIb)

**Oocyte maturation (450 to 700 /um).**

During this stage, oocyte resumes first meiotic division. The germinal vesicle migrate towards periphery and breaks down.

The lipid droplets distributed throughout the ooplasm start aggregating and coelsc to form a single oil globule (Plate XIXc).

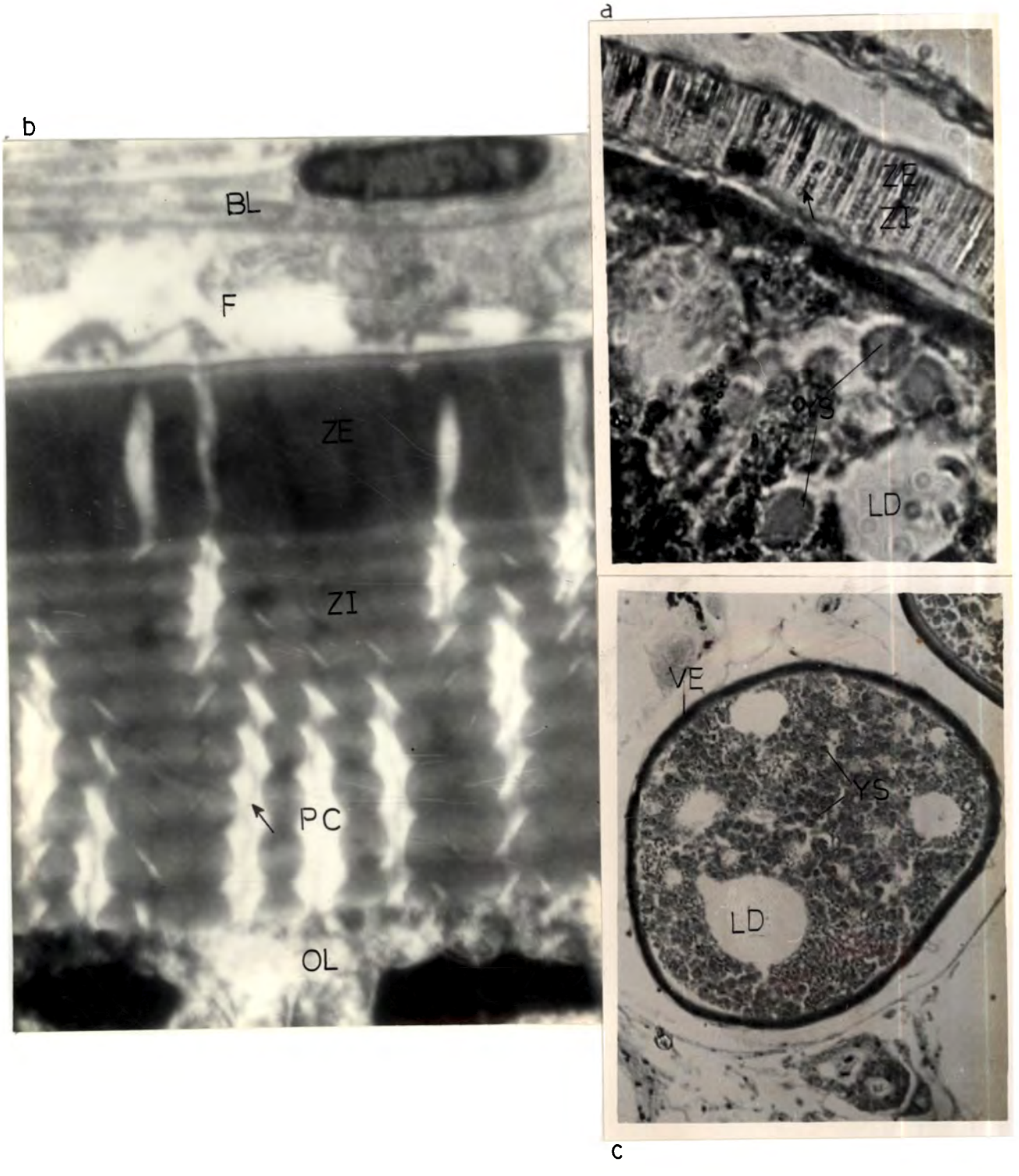
## PLATE XIX

a. Electron micrograph of vitelline envelope from fully grown oocyte showing pore canals (arrrow) with microvillar process. X 13,000.

b. Light micrograph showing magnified view of vitelline envelope by fully grown oocyte and pore canals (arrow). Yolk spheres (YS) and Lipid droplets (LD) are also visible. X 1,400.

c. Light micrograph of oocyte undergoing maturation. Note the lipid droplets (LD) joining to form a single oil globule. X 140.

**PLATE XIX**



Yolk spheres are compactly packed, resulting in peripheral displacement of ooplasm to a thin rim. Oocyte size increases considerably. The vitelline evelope is highly compact with internal layer exhibiting brick wall appearance. Pore canals are not visible ( Plate XXa ). The cortical alveoli are present below the vitelline envelope and does not show any evidence of significant morphological alteration during the oocyte growth at ultrastructural level.

The follicular layer becomes stretched due to increase in oocyte size and forms wide intercellular spaces, may get detached at some places from the oocyte surface. These brilliant yellowish coloured ripe eggs (650 to 700 µm diameter) are close to ovulation; and most likely to be shed in the single spawing. Spent ovary has collapsed follicles and residual oocytes.

## ATRESIA OF VITELLOGENIC OOCYTES

Follicular atresia is a degenerative process responsible for the loss of oocytes from the ovary. The atresia was found to affect either whole oocyte mass, subject to the failure of spawning (Section 1) or residual oocytes, left in the post spawned ovary. The process is divided into four stages:

### a stage:

In the initial stage of atresia, owing to the shrinkage of oocyte, a gap arise between follicular layer and vitelline envelope. Vitelline envelope become wrinkled and crack before disintegrating at several places Plate XXa ). Germinal vesicle disappears; yolk spheres shrink and become irregular and start liquifying at periphery. Follicular layer undergo hypertrophy and follicle cells start invading yolk mass.

### b stage:

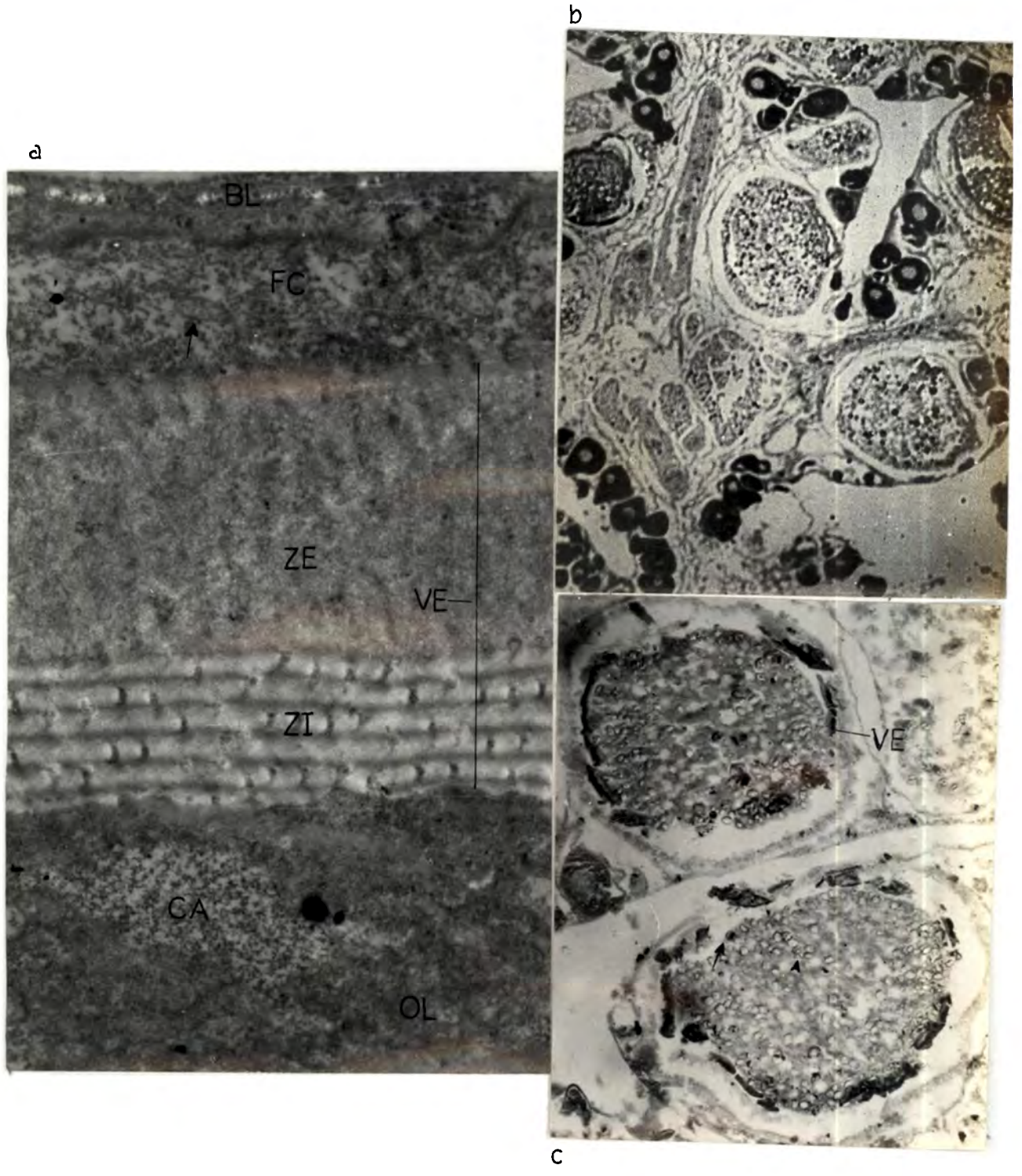
Vitelline envelope completely breaks (Plate XXC ). The yolk from periphery continue to liquify and pass into follicle cells, while central portion still remain crumbled mass of yolk. Blood capillaries also grow in close vicinity of the deggenerating oocyte.

## PLATE XX

a. Electron micrograph of vitelline envelope undergoing compaction during maturation. Note the brick-walled appearance of internal layer (29). Cortical alveoli (CA) is similar to those found in early stages. X 13,000.

b. Light micrograph of atretic oocytes; at stage a (a) and stage b (b). X 80.

c. Light micrograph showing oocytes at the stage b of atresia; the vitelline envelope (VE) is broken into pieces, yolk is gradually liquifying (arrowhead) and a phagocytic cell (arrow) is also visible. X 140.


a
BL
FC
ZE
VE
ZI
CA
OL
b
VE
c

**c stage:**

Follicle cells make aggregate in the interior of the oocyte and yolk globules turn into fluid mass ( Plate XXIa ) and gradually whole oocyte is resorbed. The follicle layer with thecal cells remain in the atrium. A light yellow pigment appear in these cells.

**d stage:**

Yellow pigment is still visible. Finally, phagocytic follicle cells and thecal cells degenerate leaving fibrous mass surrounded by stromal elements. This mass disappear after sometime.

## INTERSTITIAL (SPECIAL THECAL) CELLS

Interstitial cells are spherical or oval shaped cells embedded in the stroma of ovigerous lamellae in the recovering spent ovaries (Plate XXIb). The cells are characterized by a centrally located nucleus surrounded by cytoplasm containing abundant spherical mitochondria with cristae, ribosomes and memberane bound dense bodies besides large spheres, seems to be filled with secretory substance (Plate XXIIa, b). Smooth endoplasmic reticulum appear in the form of whorls ( Plate XXIIb).

## DISCUSSION

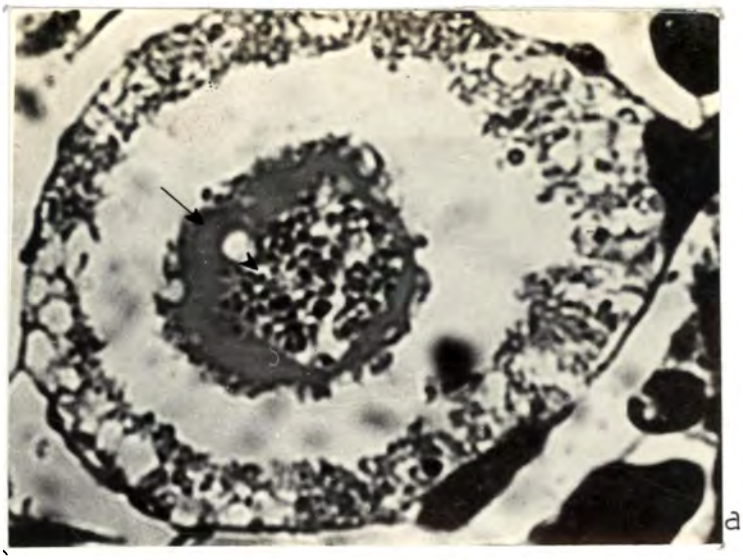
Recent ultrastructural studies have clarified the architecture of teleostean testes. Testes of L. calcarifer are of 'lobular' or 'unrestricted type', found in most of the teleosts (Grier, 1981; Billard et al., 1982). The lobules are surrounded by contractile myoid cells, described in some teleosts ( Ruby and McMillan, 1975; Grier et al, 1980). Myoid cells, arranged in single to multiple layers, appear to form common boundary wall exterior to the basal lamina of adjoining lobules, ratner than lining the individual lobules as reported in sticklebacks (Ruby and McMillan, 1975).

## PLATE XXI

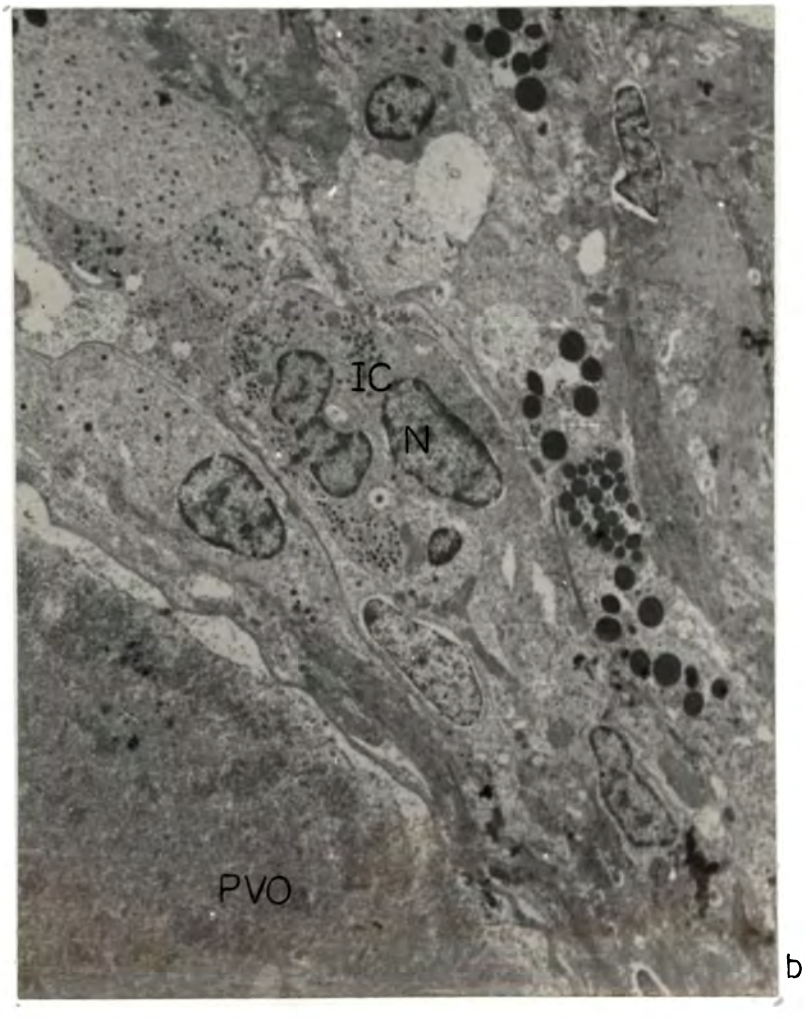
a. Light micrograph of oocyte at stage **c** of atresia. Note the vitelline envelope is completely degenerated. Yolk is converted into liquid mass (arrow); invaded by phagocytic cells (arrowhead). Blood vessels (BV) surround the oocyte. X 400.

b. Electron micrograph showing interstitial cells (IC) embedded in the stroma, surrounding a previtellogenic oocyte (PVO). X 5750.

**PLATE XXI**



a



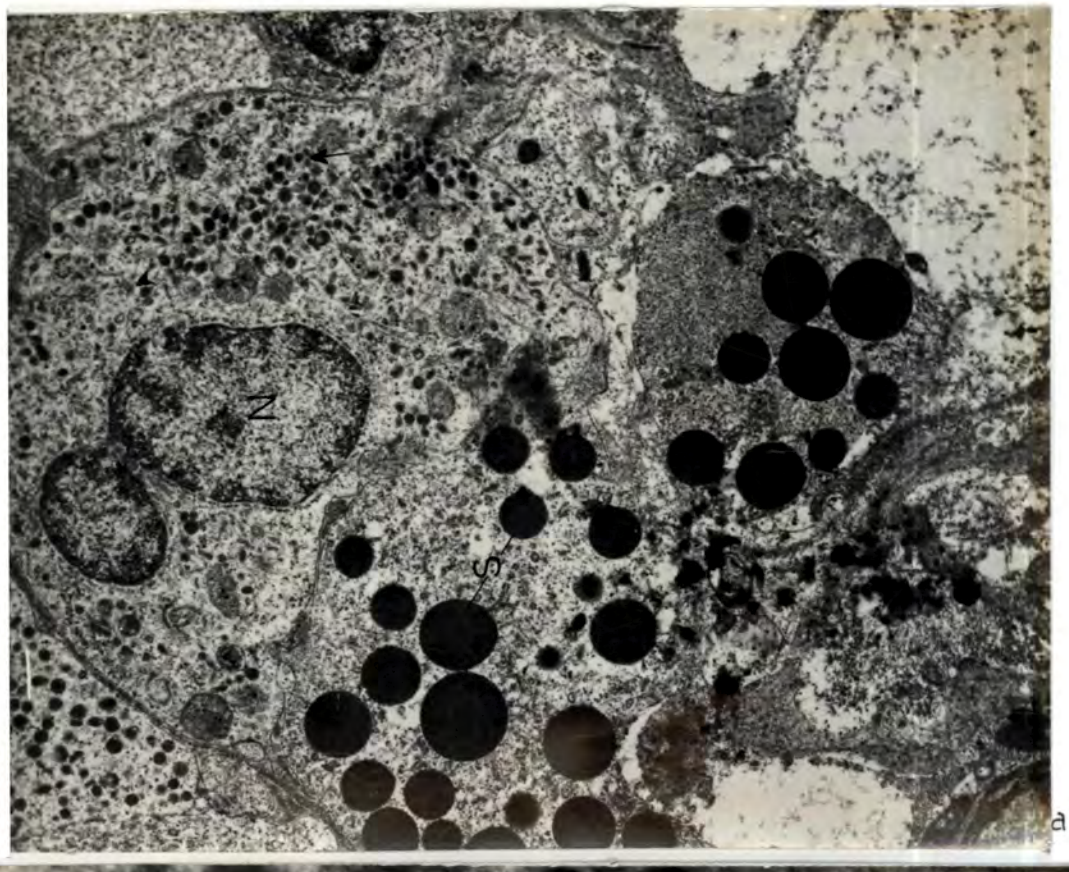

b

## PLATE XXII

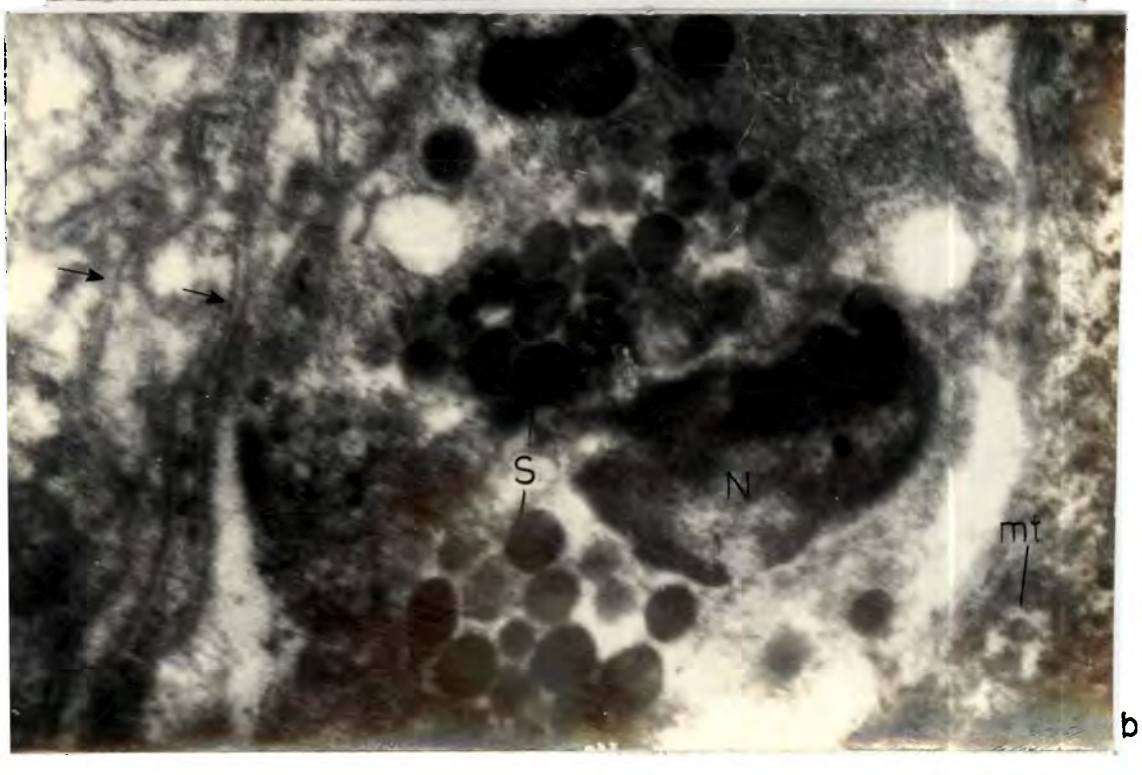
a. Electron micrograph of an interstitial cell containing a nucleus (N). The dense bodies are indicated by arrow. Large electron dense spheres (S) appearing like secretory material are visible. Arrowhead indicate intracellular filaments. X 15,000.

b. Electron micrograph of an interstitial cell depicting electron dense spheres (S) and whorls of endoplasmic reticulum (arrow). X 31,000.

**PLATE XXII**




a




b

Leydig cells found in the interstitium appear to be associated with steroidogenesis in L. calcarifer. These cells have been regarded as homologous to Leydig cells of mammals and have been described to have steroidogenic role in several teleosts (Nayar and Sunderaraj, 1970; Hoar and Nagahama, 1978; vanden Hurk et al.,1978; Grier et al, 1980; Mishra and Pandey, 1984; Harris, 1986; Elizabeth, 1987).

During the present study, Leydig cells were found to contain lipid bodies in early maturing stage. Leydig cells have been regarded to vary in abundance, distribution and lipophilia seasonally, though variations are not clearly understood (Grier, 1981). Lofts et al (1966), observed lipid in the leydig cells only during the height of the reproductive season in Fundulus heteroclitus. Grier (1981) mentioned disappearance of lipids in Leydig cells in Mugil cephalus as reproductive recrudescence commences. Several authors have observed absence of lipids in many reproductivity active teleosts (Nicholls and Graham, 1972; Guraya, 1976).

Interior of seminiferous lobule contain cells and sertoli cells. Sertoli cells are intralobular somatic cells (Billard et al., 1982) observed to be involved with the typical function of phagocytosis of residual bodies and cast off by developing spermatids, reported in several teleosts(Nagahamm, 1983). Phagocytosis has been deemed as a criterion for justifying sertoli cell homology between mammals and teleosts (Grier and Linton, 1977).

Development of germ cells is 'Cystic'; characteristic of teleosts (Nagahamma, 1983). The boundaries of germinal cysts are delimited by plasmalemma of sertoli cells.

In principle, the sequence of events upto the formation of spermatozoa is broadly common among all the vertebrates. In teleosts, with cyclic breeding habits, the mode of spermatogeniai renewal at the end of spawning season is yet not certain; as this group of vertebrates lacks permanent germinal epithelium (Ruby and McMillan, 1975). In the following text, specific emphasis has been put forward towards this aspect of spermatogenesis.

In L. calcarifer, GAa observed, are individually enclosed within the plasmalemma of sertoli cells, justifying these to be two forms of type A spermatogonia. GAa are most abundant and organized in a layer in testis at developing virgin/recovering spent stage. Rich endowment with organelles particularly ribosomes, mitochondria intermitochondrial cement as well as nuage suggest the cells to be metabolically active. Brusle' and Brusle' (1978) in Liza auratus; Billard (1984) in Poecilla latipinna; Elizabeth (1987) in Mugil cephalus found spermatogonia containing abundant cell organelles in contrary to the scarcity observed in Oryzias (Satoh and Egami, 1973); Garassius (Remacle, et al., 1977) and Platypoecilus (Ruso and Pisane, 1973). As the spermatogenesis progresses, most of the GAa, pass into successive stages and existing cells no more remain proliferative, probably elucidates the sparse distribution of these cells in early maturing testis (stage 3). However, the cells appear active owing to presence of abundant organelles. It is quite probable that significance of retaining activity may be to provide spermatogonia if necessity occur during early stages. Inability to locate any cell comparable to GAa while lobules are packed predominently with spermiogenic cysts and spermatozoa is in support to the above interpretation. In contrast, during these stages, the cells with very few organelles indicating low metabolic activity; GAs are only observed. Presence of only one form, either GAa or GAs at particular maturity stage, prompts to consider more appropriate that the GAa and GAs are the active and resting phases of the same cell, rather than two different types of cells. In other words GAa are the active spermatogonia whereas GAs are the stem spermatogonia, remaining dormant in the lobules. Regarding the origin of GAs, it is likely that some GAa does not transform into GB and may be retained as stem cells, as described in mammals (Elias, 1978). It is yet not possible to comment, whether during spermatogonial proliferation itself certain cells are destined to the role of stem cells. In any case, existence of dormant spermatogonia is evident. Ruby and McMillan (1975) reported that spermatogonia are found in the interstitium in sticklebacks, where initial development of cyst occurs. Sertoli cells pass into interstitium and engulf spermatogonia. Shreshtha and Khanna (1976) observed resting

spermatogonia in the testis of Schizothorax plagiostomus. Grier (1981) mentioned the occurrence of spermatogonia in ripe testis of Esox lucius, Ictalutus punctatus and Perca flavescene. Pierantoni et al. (1990) observed two types of primary spermatogonia in the post spawned testis of Gobius paganellus, however, the functional significance was not explained.

Brusle'(1982) reported the presence of latent primordial germ cells as well as spermatogonia in Liza auratus and held PGCs responsible for the renewal of testicular cycle. Similarly, Billard (1984) coined the possibility of PGCs remaining in adult testis and contributing actively to spermatogenesis. Shibata and Hamaguchi (1988) have contradicted such possibility in Oryzias latipes.

In L. calcarifer, PGCs were observed only in newly differentiating testis, never in adult stage. It is quite probable that functional significance of PGCs, to provide spermatogonia for spermatogenesis may be confined upto the initiation of the cycle first time in life. Thereafter, GAs appear to be responsible for renewal of spermatogenic cycle in recovering spent testis, for the ensuing spawning season. The stem cells appear to become active and proliferate to give rise to GAa, once lobules have been emptied off the stock of spermatozoa of preceeding cycle, pointing out that the presence of spermatozoa can have inhibitory effect on the spermatogenic activity, as reported in Salmonids (Billard et al., 1982).

Type B spermatogonia are organised in germinal cyst. In each cyst, spermatogonial division as well as further spermatogenesis proceed synchronously.

Primary spermatocytes are smaller cells characterized by clumps of chromatin and formation of synaptonemal complexes. Intermitochondrial cement and nuage present till this stage, is usually considered to be unusual feature for teleosts (Brusle' 1982; Billard, 1984). In the sequence of events, primary spermatocytes undergo first meiotic division to produce smaller haploid, secondary spermatocytes. Secondary spermatocytes after second meiotic division give rise to spermatids.

Transformation of spermatids to mature spermatozoa involve a synchrony of concurrently occurring processes. Despite a lot of similarities, interspecific discrepencies regarding the events associated with spermiogenesis are prevalent among teleosts (Stanley, 1969; Mattei, 1970; Grier, 1975, 1976; Brusle', 1981; Billard, 1983a; Huan-Lou and Takahashi, 1989; Jones and Butler, 1988a; Sprando and Russell, 1988).

Mattei (1970) defined two types of spermiogenesis, 'Type A' and Type B' in teleosts. The common steps between the two groups are (1) the young spermatids (2) axoneme growth and migration to condensing nucleus (3) migration of mitochandria. Type A spermiogenesis differs from Type B, because of additional step of nuclear rotation, necessitated due to tangential placement of axoneme with respect of nucleus. Spermiogenesis in L. calcarifer owing to the absence of nuclear rotation appears to be of Type A.

The present study reveals a remarkable pattern of chromatin condensation. Fine chromatin granules, initially increase in size and appear to acquire fibrous texture before condensing finally to form globules. The aggregation of these globules does not result in uneven contour of sperm head as reported in Oreochromis niloticus (Huan-Lou and Takahashi, 1989) which exhibits comparable pattern of spermiogenesis. Brusle' (1981) observed sperm head of L. auratus as clump of electron dense granules formed from the fusion and enlargement of smaller granules. In Oncorhynchus tshawytscha and Salmo gairdneri (Billard, 1983) nuclear chromatin aggregates into thick fibres which become compact and homogenous in the head of mature sperm. Certain cyprinid teleosts have been observed to have nuclear chromatin as fine fibres and granules distributed homogenously as well as compactly in the sperm head (Fribourgh et al., 1970; Baccetti, et al., 1984).

Both early and late phases of chromatin condensation start at the caudal end and gradually proceed towards other side. Huan-Lou and Takahashi (1989) observed that the formation of globules is random in O. niloticus. The polarity has been reported in Oligocottus maculosus(Stanley, 1969), and Lepomis macrochirus (Russell et al., 1988), though both the species show divergence as compared to L. calcarifer in overall process.

During spermiogenesis, nuclear volume appear to reduce considerably, probably to pack the condensed chromatin compactly in the nuclear envelope. This process is accomplished by partial fragmentation and reconstruction of nuclear envelope. Similar phenomenone has been observed in several teleosts (Stanley, 1969; Sparando and Russell, 1988). Billard (1983a) suggested that the release of nuclear and cytoplasmic material from transforming spermatids may control spermatogenesis by regulation of sertoli cell function.

Teleost spermatozoa display high degree of interspecific polymorphism (Billard, 1970, Nicander, 1970; Afzelius, 1978; Poirer and Nicholson, 1982; Billard, 1983; Baccetti et al., 1984; Jones and Butler, 1988b) reflecting varied modes of reproduction (Grier, 1981). Such morphological heterogenity does not allow to have a generalized model of spermatozoa for this group of vertebrates.

L. calcarifer spermatozoa having a small round head without acrosome an indistinct midpiece with a few mitochondria and a long flagellum; adhere to group I (carps and pike) of the three major groups outlined by Billard (1970) according to gross morphological specialization. However, at the fine structure level, every species displays certain unique features without any respect for categorization (Jones and Butler, 1988b).

The lack of acrosome in the teleost spermatozoa corresponds to the presence of micropyle in the oocyte (Nicander, 1970). Head of L. calcarifer spermatozoa consists of a nucleus packed densely with chromatin globules. Occassional electron lucent areas observed, are rare among teleosts and have not been assigned any functional significance but arise accidently during chromatin condensation (Jones and Butler, 1988b).

A deep caudal articular fossa for anchoring flagellum and mitochondrial ring surrounding proximal portion of flagellum are not uncommon features of teleost spermatozoa. However, precise keyhole-shaped articular fossa and nuclear depressions to accomodate mitochondria observed in L. calcarifer spermatozoa are distinct from other species but for Platichthys flesus (Jones and Butler, 1988b). Interestingly, P. flesus and L. calcarifer

have different types of spermiogenesis; the former belong to Type B (Jones and Butler, 1988a).

Nicander (1970) suggested that the teleost species have low or high mitochondrial collar if the fertilization is internal or external respectively. L. calcarifer with external fertilization and low mitochondrial collar fits with this classification.

The tail of L. calcarifer spermatozoa has an axoneme, with typical 9 + 2 microtobular pattern; ensheathed by plasma memberance. The plasma memberane does not form any lateral ridges or fins as observed in O. maculous (Stanley, 1969); S. gairdneri (Billard, 1983); O. niloticus (Huan-Lou and Takashashi, 1989). These lateral ridges have been related to the efficiency of tail beat (Nicander, 1970).

In conclusion, though at ultrastructure level, L. calcarifer spermatozoa exhibit certain distinct features, however, morphologically it agrees well with that of primitive type of spermatozoa, retained by the species having external mode of fertilization (Franzen, 1970; Jones and Butler, 1988b).

Testes of L. calcarifer does not have any non-functional female tissue as observed in many protandric hermaprodites (Frick, 1979; Brusle-Sicard and Reinboth, 1990). The testes undergo complete reorganization during sex inversion and transition is completed shortly after spawning. Question arises concerning origin of female germ cells. At the ultra structural level, spermatogonia and oogonia exhibit resemblence to a large extent, indicating the possibility of the cells to be bipotential (Reinboth, 1982). Recently, Shibata and Hamaguchi (1988) demonstrated that bio-potentiality is maintained upto several mitotic generations of spermatogonia type B (GB) in Oryzias lalipes. However, PGCs are another cells worth significant attention. PGCs are undifferentiate cells found in clusters only in newly differentiating testis as well as ovary, never found in any other

stage. This probably indicate that the PGCs reappear during the transition period and may transform to oogonia to initiate the ovarian cycle. Until quite recently, the origin of early germ cells has been a matter of speculation. Brummet et al. (1982) suggested that connective tissue cells give rise to germ cells in Fundulus heteroclitus. However, Begovac and Wallace (1987) pointed out the luminal epithelial cells as possible source of early germ cells in Syngnathus scovelli.

Dynamics of cytological events associated with the oogenesis in L. calcarifer parallel those observed in other teleosts. (Tokarz, 1978,Nagaharna 1983; Guraya, 1986; Selman and Wallace, 1989).

Oogonia, found through out the life span in most of the female teleosts are mitotically proliferating stem cells. Oogonia transform into oocytes which ultimately grow and mature to become egg. Discrepancies exist among teleosts regarding the period of oogonial proliferation as well as oogenesis, which may be continuous or cyclic (Tokarz, 1978). In L. calcarifer, it appears to be cyclic as the fish is a seasonal breeder (Section 1).

Location of prominent nucleolus/nucleoli distinguishes, the primary growth phase into chromatin nucleolus, early perinucleolus and late perinucleolus stages. (Guraya, et al., 1975, 1977; Tokarz, 1978; Wallace and Selman, 1981; Begovac and Wallace, 1988; Selman and Wallace, 1989). The chromosomes of chromatin nucleolus stage are arrested in diplotene of meiotic prophase and acquire lampbrush configuration. The presence of lampbrush chromosomes is considered to be ubiquitous feature though has been demonstrated in a few teleost species (Bara, 1960; Lehri, 1968, Baumeister, 1973, Monace et al., 1978; Selman and Wallace, 1989). Following this arrest, a period of extensive oocyte growth commences concomitant to foliculogenesis.

Ultrastructural evidence suggest prominence of nucleocytoplasmic interaction in the previtellogenic oocytes. Very high electron density of

early chromatin nucleolus stage oocytes may be due to tightly packed abundant ribosomes, which get more dispersed with the increase in cell size. It is significant to note the outpocketing and interruptions in the nuclear envelope, probably to facilitate the extrusion of nucleolar fragements related to immediate accumulation of ribosomes at very early stages (Guraya, 1986). Thereafter, the abundance ribosomes increase consistently, and appear to be intimately linked to nucleolar multiplication, characteristic feature of perinucleolar stages.

Origin of nucleoli is generally accepted to be from certain heterochromatic regions of chromosomes; the nucleolar organizers. (Baumeister, 1976). Apart from compact, large nucleolus of granullofibrillar composition; appearance of small vacuolated nucleoli may be related to high rate of RNA synthesis and surge of extrapolation to cytoplasm(Azevedo and Coimbra, 1980). Exclusive fibrillar composition of these nucleoli further support this interpretation; since rDNA as well as RNA synthesizing capacity is considered to be more in fibrillar component (Baumeister, 1976; Mirre and Stahl, 1978). Interestingly, none of the large nucleoli were seen to have vacuoles. Azevedo and Coimbra (1980) reported vacuolated nucleoli to be of large size in Xiphophorous helleri.

The nucleolar multiplication is considered to be refection of amplification of ribosomal genes (Monaco et al, 1981; Selman and Wallace, 1989). Oocytes of teleosts multiply their genes for 28S and 18A RNA from the beginning of their growth, however, coding for these RNAs preferentially starts after the onset of vitellogenic growth. Besides, the synthesis of 5S RNA and transfer RNA oocur during the whole course of oogenesis and stored in the cytoplasmic ribonucleoprotein (8S and 42S) particles, before the 5S RNA integrates in the ribosomes. (Wegnez et al., 1978).

During the previtellogenic growth, the dense aggregates, 'nuage' scattered along the nuclear envelope, persistently increase. The granules composing the nuage resemble to that found in the nucleolus and are seen

scattered in nucleoplasm. Nuage is considered to be a universal marker of germ cells (Wakley, 1976) and has been shown to have ribonucleoproteins (Riehl, 1978; Clerot, 1979; Azevedo, 1984).

Nuclear envelope can undergo modifications such as formation of blebs, temporary interruptions, outpocketing etc. depending upon the amount as well as size of the nucleolar material; to facilitate its passage (Scharrer and Wurzelmann, 1969 a, b, Guraya, 1986). During the present study, barring the outpocketing and interruptions in early chromatin nucleolus stage, prominent mode of passage was through nuclear pores. It is interesting to mention that the wavy characteristic of the nuclear envelope, as observed in Mugil auratus (Brusle, 1980) M. cephalus (Gopalakrishnan, 1991) and of many other teleosts (Guraya, 1986), was conspicuously lacking. Nuclear envelope remains more or less regular except occassional formation of contact with nucleoli or bulging due to the presence of lipid bodies adjacent to it.

Balbiani's vitelline body is a predominent cytoplasmic structure in the perinucleolar stage oocytes in many organisms (Guraya, 1979, 1986; Coello and Grimm, 1990). At ultrastructure level it consists of a network of nuage associated with mitochondria (Yamamoto, 1964, Ulrich, 1969, Wegmann and Gotting, 1971). Balbiani's vitelline body appear to be a centre for biogenesis of ribosomes and mitochondria. The inference agrees with the opinion that nuage play prominent role in the formation of these organelis. (Clerot, 1976; Hogan, 1978; Brusle, 1980; Guraya, 1986). The mitochondria associated with this body usually lack ribosomes, similar to those found in clusters appearing as low electron dense patches in the ooplasm of early perinucleolar stage. These patches disappear in the late perinucleolar stage probably indicate their dispersal in the ooplasm.

A conspicuous feature regarding the Balbiani's vitelline body of L. calcarifer, is the absence of cell organelles other than mitochondria in contrast to that reported in several other teleosts ( Upadhyaya, et al., 1978; Guraya, 1986; Begovac and Wallace, 1988).

Oocytes entering secondary growth phase are recognised by the appearance of small, irregular vesicles, mostly in the peripheral ooplasm referred as 'yolk vesicles' in the literature, ubiquitously associated with small teleostean oocytes (Khoo, 1979; Wallace and Selman, 1981, Nagahamma, 1983; Guraya, 1986). Yolk vesicles have never been depicted in full grown oocytes, generally characterized by large, cortical alveoli (Wallace and Selman, 1981) considered to be participating in cortical reaction during fertilization (Kobayashi, 1985). Recently, it is established that the yolk vesicles give rise to cortical alveoli (Selman et al., 1988). Surprisingly, cortical alveol typical of teleostean eggs has not be observed in L. calcarifer. In contrast, ultrastructural evidence reveal that the vesicles found in early vitellogenic oocyte are retained without any significant morphological alteration in the egg. Optical microscopy fail to resolve these small vesicles distinctly in the yolky oocytes rather indicated by a strong periodic acid-schiff's (PAS) reaction below the vitelline envelope. Inference from these observations suggest; the vesicles being non-contributors to the yolk accumulation, may be more appropriately be referred as cortical alveoli, (Selman and Wallace, 1989) and can be species specific with regard to distribution, size and structure.

In most teleosts, lipid bodies appear during cortical alveoli stage (Selman and Wallace, 1989); however, appearance of lipid bodies in early previtellogenic as observed in L. calcarifer oocytes cannot be surprising (Guraya, 1965; Shacklay and King, 1977; Ramadan, 1979). Lipid bodies multiply tremendously from cortical alveoli stage onwards; distributed randomly throughout the ooplasm. In certain teleosts, lipid bodies aggregate initially in perinuclear region and are pushed to peripheral ooplasm towards the end of vitellogenesis (Guraya, 1986; Begovac and Wallace, 1988).

Dramatic rise in oocyte size is primarily attributed to the surge of yolk accumulation to be utilized later for the nourishment of developing embryo and larvae;a generalized phenomenone to all viviparous non-mammalian vertebrates (Wallace, 1985). The intense pinocytotic activity at the surface

of vitellogenic oocytes is probably associated with influx of hepatically synthesized yolk precursor material (Guraya, 1986). The yolk precursors are incorporated into developing yolk spheres where it becomes mature yolk. Ooplasm of vitellogenic oocytes show abundance of transitional yolk spheres identified by zonation in electron density.

Present study does not provide direct evidence for the involvement of multivascular bodies in the formation of yolk spheres. The mulastivascular bodies have not been found associated with Balbiani's vitelline body as reported in some teleosts (Upadhyaya, et al., 1978, Begovac and Wallace, 1988). However, the presence of multivascular elements associated with some transitional yolk spheres does not permit to ignore the possibility that multivascular bodies may act as initial platform for the formation of mature yolk spheres. Till now, involvement of multivascular bodies in the processing of yolk has been demonstrated only in trout (Busson-Mabillot, 1984).

Folliculogenesis proceeds concurrent to oocyte growth. The oocytes at the end of primary growth phase lie in their definite multilayered follicles, composed of vitelline envelope, follicular layer, theal layer, surface epithelium.

Thick vitelline envelope is to provide mechanical protection to oocyte and embryos, moreover, its plasticity allow oocyte to grow without hampering the passage of nutrients (Brusle, 1985). Vitelline envelope in L. calcarifer as in many teleosts begin to form as deposition of material between microvilli, extending from oocyte, surface, at the time of cortical alveoli formation (Selman and Wallace, 1989). It undergoes development and differentiates to become architecturally complex with striated appearance as in most of the teleosts studied, though species specific morphological discrepancies exist (Droller and Roth; Anderson, 1967; Shackley and King, 1977, Tesoriero, 1977, 1978; Laale, 1980; Stehr and Hawks, 1983; Brusle, 1985; Bagovac and Wallace, 1988; Cotell et al., 1988). Exact nature of the cells contributing to the formation of vitelline envelope has been a matter of speculation. Hamazaki (1986) demonstrated role of liver in the

synthesis of glycoproteins for the inner layer in Oryzias latipes. Begovac and Wallace (1989) reported that the major vitelline envelope proteins in the pipefish oocytes originate within the follicle.

Follicular cells, forming a single layer, surrounding the vitelline envelope undergo morphological alterations during oogenesis. The ultra-structure evidence suggests the presence of abundant cell organelles, which appear to increase progressively in amount. Steroid synthesizing role of follicle cells appear quite probable as suggested in several teleosts (Hoar and Nagahama, 1978; Guraya, 1986).

Presence of intracellular filaments suggest that the follicle cells orginate from stromal epithelial cells (Begovac and Wallace, 1987). The cytoplasm of follicle cells, have small dense bodies and rough endoplasmic reticulum indicating the possible protein synthesis. The presence of similar dense bodies around the blood vessels in thecal layer point out the involvement of follicle cells in the transport of follicle cells (Brusle, 1985).

The post-vitellogenic oocytes of L. calcarifer measure 440 mm (average diameter). Several workers consider that prematuration oocytes lie in the size range of 400 to 500 mm and can be stimulated by hormonal administration (Nacario, 1985; Mckinnon, 1987). Davis (1982) reported oocytes of L. calcarifer more than 500 mm to be close to ovulation. In other words, the oocytes in this size range, become capable of undergoing resumption of meiosis, accompanied by several nuclear and cytoplasmic changes, collectively, forming one of the end stages of oogenesis; oocyte maturation. Steriod 17$\alpha$, 20$\beta$ dihydroxyprogesterne is considered to be the principle physiological maturation inducer in teleost oocytes (Fostier et al; 1973, Jalabert and Finet, 1986; Nagahama and Adachi, 1985; Finet et al., 1988; Inbaraj and Haider, 1988; Petrino et al. 1989a, b). During maturation, oocyte undergo resumption of meiosis, characteristic events are migration of germinal vesicle towards periphery and break down; chromosomes condense and proceed to first meiotic metaphase and extrusion of first polar body; remaining haploid set of chromosomes proceed to second meiotic metaphase

where they arrest once again. At this stage, the oocyte becomes mature and fertilizable; referred as egg. Aggregation of lipid bodies to form a single oil globules, during maturation indicated by present observation aggrees well with the previous reports (Kowtal, 1976; Roy et al., 1977; Moore, 1982). Concommitant to these changes, size of oocyte increase conspicuously probably due to rapid uptake of water (Grealey et al, 1986a; Selman and Wallace, 1989). Another prominent change oocurs in vitelline envelope, which undergoes compaction and becomes more homogenous as well as appears to retain bilaminar organization.

Spawning season of L. calcarifer coincides well with the onset of rains. (Section 1). It appears that the oocytes complete vitellogenic growth well before the rains, however, maturing follicles were only observed after the start of wet season. Ovulation occur at the end of maturation process (Selman and Wallace, 1989).

Ovulated egg of L. calcarifer measure around 700 to 800 µm (0.7 to 0.8 mm) (Kowtal, 1976; Moore, 1982). During the present study, the size of maturing oocytes was found in the range of 0.65 to 0.73 mm. These fish appear to be close to spawning thought not running ripe. Unfortunately running ripe specimen was not encountered. Problem in getting runninng ripe specimens, attributed to the very short duration of this stage, has been highlighted, besides L. calcarifer (Davis, 1982); in several other teleosts Sardina coerulea (Clark, 1934); Lates niloticus (Hopson, 1969); Stolephorus purpureus (Leary et al; 1975); Engraulis mordox (Hunter and Goldberg, 1980).

Follicular atresia of yolky oocytes is a common feature and has been studied extensively in several teleosts. (Guraya et al, 1975; 1977; Davis, 1977a; Babu and Nair, 1983; Hunter and Macewicz, 1985). Sequennce of events through which the oocytes are degenerated and resorbed is similar to that described in other teleosts (Saidapur, 1978; Guraya, 1986). The yellow pigment formed toward the end of process has been ascribed to a relative deficiency of lysosome in lipid digestion. These residual bodies formed due to the incomplete lysis, mainly consist of complex lipids, finally converted to lipofuschin (Lang, 1981).

## SECTION III

## BIOCHEMICAL CHANGES ASSOCIATED WITH GONAD MATURATION

Maturation of gonads is accompanied by profound changes in the chemistry of fish due to translocation of material and energy mainly from the somatic sources to gonads. Reproductive effort in fishes involves three important aspects, production of gametes, development of secondary sexual characters and exhibition of reproductive behaviour (Miller, 1984). Cyclic rhythms of gonadal development are reflected in the biochemical composition of somatic tissue, as reproductive efforts usually require mobilization of reserve resources. (Love, 1975). Under natural conditions, the constituents of the blood serum in fishes are homeostatically maintained, although subjected to minor fluctuations. But the spawning cycle in teleosts has been demonstrated to cause major alterations in the blood chemistry. This is a generalized concept applicable to both the sexes, however, the changes are more pronounced in females.

Physiological processes related to egg production are known to cause elevated levels of serum components especially proteins, lipids, protein bound phosphorous and calcium; the parameters associated with the appearance of vitellogenesis (Follette and Redshaw, 1968; Aida et al., 1973; Yaron et al., 1977; Hori et al, 1979; Ng and Idler, 1983; Gopalakrishnan, 1991). Vitellogenin, principle yolk precursor protein in all the oviparous vertebrates investigated so far; is a large calcium binding glycolipophosphoprotein molecule, (Wallace, 1985). It has been demonstrated in the serum of several teleosts (Plack et al., 1971; Emmersen and Petersen, 1976; Hara and Hirai, 1978; Hori et al., 1979; Campbell and Idler, 1980; Nath and Sunderaraj, 1981; Van Bohemen et al., 1981; Fujii et al., 1987; Hara et al., 1987; Copland and Thomas, 1988; Fremont and Riazi, 1988; Benfy et al., 1989a,b; Quinito et al., 1989) Vitellogenin is sequestered into developing oocytes and uptake has been found to be selective in Salmo gairdneri (Tyler, et al., 1988, 1990), responsible for the majority of oocyte growth.

Despite considerable interest generated towards research on L. calcarifer during the last few years, there has not been any attempt to study the physiological mechanisms related to gonadal recrudescence. The previous two sections of the present work have indicated that the gonads pass through sequence of dynamic changes prior to gametes are ready for shedding during spawning. The current section deals with changes in selected biochemical parameters and presents delineation from intragonadal variations towards the study of somatogonadal relationships. Due emphasis has been given to the parameters associated with vitellogenesis, the most conspicuous phenomenone in the reproductive physiology of egg laying vertebrates.

## RESULTS

### MALE

#### Muscle (Table 6)

Moisture contents in muscle tissue increase from the value of 68.31 percent (stage 1) to 73.16 percent (stage 5) and 74.68 percent (stage 6). Total protein contents rise from 20.67 percent (stage 1) to 22.43 percent (stage 2) subsequently declining upto 18.67 percent (stage 5). Total lipid level increase between stage 1 and 2, from 3.21 to 3.40 percent. Thereafter the level steadily decrease upto 2.31 percent (stage 6). Cholestrol contents start declining from stage 2(0.43 percent) onwards; and attain lower level at stage 6 (0.31 percent).

#### Liver (Table 7)

Moisture contents increase consistently from the level of 61.42 percent (stage 1) to 68.76 percent (stage 6). Total protein contents is maximum at stage 2 (15.36 percent), thereafter decrease till stage 5 (13.15 percent) followed by marginal recovery in the stage 6 (14.27 percent). Total lipid contents is highest at stage 2 (10.23 percent) however thereafter decline steadily till stage 6 (7.84 percent). Cholestrol contents from the level of 2.12 percent (stage 1) decline gradually to 1.40 percent (stage 6).

**Table 6 Variations in biochemical parameters (Mean ±SD) in muscle tissue at different stages of maturity of male L. calcarifer.**

| Maturity stages | Moisture contents (%) | Total proteins (%) | Total lipids (%) | Total cholestrol (%) |
|---|---|---|---|---|
| 1 | 68.31 ± 3.5 | 20.67 ± 2.5 | 3.21 ± 0.5 | 0.41 ± 0.02 |
| 2 | 68.47 ± 2.8 | 22.43 ± 2.0 | 3.40 ± 0.3 | 0.43 ± 0.02 |
| 3 | 69.63 ± 3.7 | 20.30 ± 2.3 | 3.14 ± 0.4 | 0.41 ± 0.03 |
| 4 | 71.47 ± 2.9 | 19.18 ± 2.8 | 2.86 ± 0.5 | 0.38 ± 0.02 |
| 5 | 73.16 ± 4.0 | 18.67 ± 2.0 | 2.82 ± 0.3 | 0.35 ± 0.03 |
| 6 | 74.68 ± 3.1 | 20.13 ± 1.9 | 2.31 ± 0.2 | 0.31 ± 0.05 |

Table 7 Variation in biochemical parameters (Mean ± SD) in liver tissue at different stages of maturity of male L. calcarifer

| Maturity stages | Moisture contents (%) | Total proteins (%) | Total lipids (%) | Total cholestrol (%) |
|---|---|---|---|---|
| 1 | 61.42 ± 2.8 | 14.30 ± 1.3 | 8.60 ± 0.2 | 2.12 ± 0.02 |
| 2 | 62.67 ± 3.1 | 15.36 ± 1.4 | 10.23 ± 0.16 | 1.86 ± 0.01 |
| 3 | 64.18 ± 4.0 | 14.14 ± 1.2 | 9.10 ± 0.12 | 1.72 ± 0.03 |
| 4 | 67.61 ± 4.1 | 13.36 ± 1.3 | 8.36 ± 0.2 | 1.56 ± 0.02 |
| 5 | 68.15 ± 4.2 | 13.15 ± 1.6 | 8.17 ± 1.8 | 1.51 ± 0.01 |
| 6 | 68.76 ± 4.1 | 14.27 ± 1.5 | 7.84 ± 1.3 | 1.70 ± 0.01 |

**Serum (Table 8)**

Total protein contents increase from stage 1 (7.14 g/100 ml) onwards till stage 4 (9.83 g/100 ml) subsequently declining in stages 5 and 6 (9.06 and 7.42 g/100 ml, respectively). Similarly, total lipid level rise from 0.86 g/100 ml (stage 1) to 1.24 g/100 ml (stage 4) subsequently declining upto 0.91 g/100 ml (stage 6). Total cholestrol level increase from 0.30 g/100 ml (stage 1) to 0.51 g/100 ml (stage 4), thereafter decreasing to 0.28 g/100 ml (stage 6) Protein bound phosphorous and total calcium level ao not show much fluctuations during testicular cycle.

**Testes (Table 9)**

Moisture contents decrease from the value of 76.37 percent (stage 1) to 72.81 percent (stage 4) thereafter has a sharp rise to 77.83 percent (stage 5) and 79.54 percent (stage 6). Total proteins increase gradually from 13.141 percent (stage 1) to 22.36 percent (stage 5) followed by decline to 13.86 percent (stage 6). Total lipid from the level of 1.43 percent (stage 1) increase to 3.61 and 3.51 percent (stage 4 and 5) thereafter declining to 1.23 percent (stage 6). Total cholestrol contents rise from 0.40 percent (stage 1) to 0.51 percent (stage 2) thereafter decline gradually to 0.19 percent (stage 6).

The data pertaining to changes in biochemical parameters was subjected to analysis of variance (Anova; two way with interaction) to test significant changes (i) between different tissues (muscle, liver and testes) at various stages of maturity (ii) between different stages of maturity in various tissues. Since the interaction between the two sources of variation (stage and tissue) was significant, the main effects were tested against the interaction. The results indicated that there is no significant variations in all the parameters except moisture contents between the stages whereas between the tissues the variation was significant ($P < 0.001$). Variation in moisture contents is significant between the stages and between the tissues (Table 10).

**Table 8 Variations in biochemical parameters (Mean ± SD) in the blood serum of female L. calcarifer.**

| Maturity | Total protein (g/100ml) | Total lipid (g/100ml) | Total cholestrol (g/100ml) | Protein bound phosphorous ( /ug/ml) | Total calcium (/ug/ml) |
|---|---|---|---|---|---|
| 1 | 7.14 ± 0.41 | 0.85 ± 0.05 | 0.30 ±0.02 | 4.8 ± 0.6 | 78.15 ± 2.8 |
| 2 | 7.46 ± 0.61 | 0.90 ± 0.08 | 0.34 ± 0.01 | 4.78± 0.8 | 78.20 ± 3.5 |
| 3 | 8.24 ± 0.33 | 1.09 ± 0.07 | 0.46 ± 0.03 | 4.77± 0.6 | 77.58 ± 4.7 |
| 4 | 9.83 ± 0.63 | 1.24 ± 0.04 | 0.51 ± 0.02 | 4.81 ± 0.7 | 78.18 ± 6.2 |
| 5 | 9.06 ± 0.80 | 1.07 ± 0.06 | 0.39 ± 0.03 | 3.78± 0.03 | 78.21 ± 5.1 |
| 6 | 7.42 ± 0.71 | 0.91 ± 0.02 | 028 ± 0.01 | 4.80± 0.5 | 78.16 ± 3.9 |
| Calculated F. Value | 68.16 | 47.81 | 36.63 | 1.076 | 7.218 |
| Remarks | P< 0.001 | P< 0.001 | P< 0.001 | N.S. | N.S. |

Table 9 Variations in biochemical parameters (Mean ± SD) in the testicular, tissue of L. calcarifer

| Maturity stages | Moisture contents (%) | Total proteins (%) | Total lipids (%) | Total cholestrol (%) |
|---|---|---|---|---|
| 1 | 76.37 ± 2.80 | 13.41 ± 1.40 | 1.43 ± 0.41 | 0.40 ± 0.06 |
| 2 | 74.21 ± 3.19 | 14.84 ± 1.20 | 1.72 ± 0.83 | 0.51 ± 0.08 |
| 3 | 73.30 ± 4.20 | 17.68 ± 1.40 | 2.48 ± 0.61 | 0.38 ± 0.07 |
| 4 | 72.81 ± 3.80 | 21.68 ± 1.14 | 3.61 ± 0.73 | 0.26 ± 0.04 |
| 5 | 77.83 ± 4.10 | 22.36 ± 1.20 | 3.57 ± 0.54 | 0.25 ± 0.03 |
| 6 | 79.54 ± 3.70 | 13.86 ± 1.50 | 1.23 ± 0.38 | 0.19 ± 0.06 |

**Table 10 Result of the analysis of variance of biochemical parameters between tissues and maturity stages in male L. calcarifer**

| Parameters | Source of variation | Calculated F values | Remarks |
|---|---|---|---|
| Moisture | Between tissues | 109.183 | $P < 0.05$ |
| | Between stages | 14.625 | $P < 0.001$ |
| | Interaction | 8.228 | $P < 0.001$ |
| Protein | Between tissues | 5.418 | $P < 0.05$ |
| | Between stages | 1.068 | NS* |
| | Interaction | 16.802 | $P < 0.001$ |
| Lipid | Between tissues | 21.461 | $P < 0.01$ |
| | Between stages | 1.348 | NS |
| | Interaction | 63.721 | $P < 0.001$ |
| Cholestrol | Between tissues | 168.921 | $P < 0.05$ |
| | Between stages | 1.760 | NS |
| | Interaction | 14.191 | $P < 0.001$ |

* NS = Not significant

Degrees of freedom (df) tissues = 2
stages = 5
interaction = 10

The variation in the composition of blood serum at different stages of maturity, was investigated by a one way analysis of variance applied seperately for each biochemical parameter (Table 8). The results show significant variation of all the parameters between different maturity stages except protein bound phosphorous and total calcium.

## FEMALE

### Muscle (Table 11)

Moisture contents in muscle tissue maintain more or less same level in stages 1 and 2 (64.25 and 64.63 percent respectively) followed by consistent increase till stage 6 (77.00 percent). Total protein contents increase form the value of 21.6 percent (stage 1) to 24.8 percent (stage 2), subsequently gradually falling to 16.8 percent (stage 5) before increasing again to 18.4 percent (stage 6). Total lipid contents increased between stage 1 (3.6 percent) and stage 2 (4.1 percent), thereafter declining till stage 6 (2.3 percent). The decline is conspicuous between stage 2 (4.2 percent) and stage 3 (3.3 percent). Cholestrol contents increase from 0.38 percent (stage 1) to 0.41 percent stage 2 followed by gradual decline till stage 6 (0.30 percent).

### Liver (Table 12)

Moisture contents in liver increase to the value of 72.40 percent (stage 4) from 64.61 percent (stage 1), subsequently drop marginally to 71.76 percent (stage 6). Total protein contents after increasing from 16.4 percent (stage 1) to 18.6 percent (stage 2) declined to consistently upto 11.8 percent (stage 4)followed by recovery upto 14.2 percent (stage 6). Total lipid level increase between stage 1 and 2, from 8.8 to 10.10 percent. Thereafter, the level decline steadily upto 5.1 percent at stage 6.

Cholestrol contents in the liver tissue, increase from 2.41 percent (stage 1) to 2.68 percent (stage 2), subsequently decline gradually to 1.8 percent (stage 4). The cholestrol level increase marginally to 2.1 percent (stage 5) before attaining the value of 2.0 percent (stage 6).

**Table. 11** **Variations in biochemical parameters (Mean ± S.D) in muscle tissue at different stages of maturity of female L. calcarifer.**

| Maturity | Moisture contents (%) | Total proteins (%) | Total lipids (%) | Total cholestrol (%) |
|---|---|---|---|---|
| 1 | 66.25 ± 2.5 | 21.6 ± 2.5 | 3.50 ± 0.3 | 0.38 ± 0.02 |
| 2 | 66.63 ± 2.5 | 24.8 ± 2.3 | 4.20 ± 0.34 | 0.41 ± 0.02 |
| 3 | 69.5 ± 1.5 | 19.1 ± 2.5 | 3.30 ± 0.3 | 0.38 ± 0.03 |
| 4 | 74.0 ± 2.5 | 17.5 ± 2.0 | 2.60 ± 0.2 | 0.35 ± 0.06 |
| 5. | 76.5 ± 1.5 | 16.7 ± 1.5 | 2.76 ± 0.06 | 0.33 ± 0.02 |
| 6 | 77.0 ± 2.0 | 18.4 ± 2.5 | 2.25 ± 0.3 | 0.30 ± 0.01 |

**Table. 12 Variations in biochemical parameters (Mean ± S.D) in liver tissue at different stages of maturity of female L. calcarifer**

| Maturity Stages | Moisture contents (%) | Total proteins (%) | Total lipids (%) | Total cholestrol (%) |
|---|---|---|---|---|
| 1 | 64.61 ± 2.7 | 16.4 ± 1.2 | 8.8 ± 0.11 | 2.41 ± 0.001 |
| 2 | 65.31 ± 3.0 | 18.6 ± 1.2 | 10.10 ± 0.09 | 2.68 ± 0.02 |
| 3 | 69.64 ± 4.1 | 14.4 ± 1.3 | 8.2 ± 0.008 | 2.20 ± 0.01 |
| 4 | 72.40 ± 4.00 | 11.8 ± 1.1 | 6.8 ± 0.12 | 1.80 ± 0.01 |
| 5 | 72.00 ± 4.1 | 13.4 ± 0.8 | 5.9 ± 0.11 | 2.10 ± 0.02 |
| 6 | 71.76 ± 4.1 | 14.2 ± 1.2 | 5.1 ± 0.10 | 2.00 ± 0.03 |

**Serum (Table 13)**

Serum protein contents decrease from 9.24 g/100 ml (stage 1) to 6.79 g/100 ml (stage 2); thereafter increase steadily to 11.01 g/100 ml followed by decline to 9.25 g/100 ml (stage 5) and 7.18 g/100 ml (stage 6). Similarly, total lipid contents decline from 0.98 g/100 ml (stage 1) to 0.86 g/100 ml (stage 2), subsequently rise to1.71 g/100 ml (stage 4) before finally decreasing to 1.56 g/100 ml (stage 5) and 1.12 g/100 ml (stage 6). Total cholestrol level decrease from the value of 0.39 g/100 ml (stage 1) to 0.26 g/100 ml (stage 2), thereafter steadily increase to 0.56 g/100 ml (stage 4) followed by gradual decline to 0.43 g/100 ml (stage 5) to 0.25 g/100 ml (stage 6). Serum protein bound phosphorous and calcium contents depict a similar pattern in relation to maturation. Serum protein bound phosphorous increase from the base level of 5.2 µg/ml (stage 1) to 52.3 µg/ml (stage 4). The paremeter decline to value of 27.4 µg/ml (stage 5) before finally reaching close to base level 6.7 µg/ml (stage 6). Serum calcium contents new exhibit steady upward trend from stage 1 (74 µg/ml) to a stage 4 (218 µg/ml), subsequently the level drops during stage 5 (148 µg/ml) and stage 6 (107 µg/ml).

**Ovary (Table 14)**

Moisture contents from high value (70.4 percent) at stage 1 decline till stage 4 (59.75 percent). The ovaries close to spawning (stage 5), exhibit sharp rise in moisture contents (66.5 percent); attaining even higher values just after spawning (73.00 percent). The total protein contents increase progressively from the initial value of 12.50 percent (stage 1) to 27.85 percent (stage 4) and 29.4 percent (stage 5) subsequently declining to 13.8 percent (stage 6). Ovaries at stage 1, has low lipid contents (3.9 percent), which rise gradually as the maturation advances till stage 4(15.6 percent), thereafter maintaining slight higher level at stage 5 (17.2 percent) decline sharply at stage 6 (5.8 percent). Total cholestrol contents exhibit a distinct pattern. Previtellogenic ovaries; stage 1 and 2, have high levels of cholestrol 1.01 and 1.28 percent respectively, which declines to 0.64 percent (stage 4) followed by elevation to 0.96 percent (stage 5) before finally dropping to 0.42 percent (stage 6).

**Table 13 Variations in biochemical parameters (Mean ± S.D) in the blood serum of female L. calcarifer.**

| Maturity | Total protein (g/100ml) | Total lipids (g/100ml) | Total cholestrol (g/100ml) | Protein Bound phos-porous (µg/ml) | Total calcium (µg/ml) |
|---|---|---|---|---|---|
| 1 | 9.24±0.30 | 0.98±0.035 | 0.39±0.035 | 5.2±0.8 | 74.00±4.6 |
| 2 | 6.79±0.41 | 0.36±0.05 | 0.30±0.05 | 8.6±1.2 | 92.00±5.3 |
| 3 | 9.85±0.75 | 1.60±0.04 | 0.52±0.04 | 32.4±3.6 | 161 ±7.8 |
| 4 | 11.01±0.9 | 1.71±0.04 | 0.56±0.04 | 52.3±7.6 | 218 ±6.7 |
| 5 | 9.25±0.9 | 1.56±0.09 | 0.43±0.05 | 28.8±2.1 | 148 ±4.9 |
| 6 | 7.18±0.75 | 1.12±0.02 | 0.25±0.03 | 6.7±1.6 | 107 ±4.2 |
| Calculated F.Value | 48.68 | 240.61 | 80.67 | 170.63 | 83.14 |
| Remarks | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |

**Table 14 Variations in biochemical parameters (Mean ± S.D) in the ovarian tissues of L. calcarifer.**

| Maturity | Moisture contents (%) | Total protein (%) | Total lipid (%) | Total cholestrol (%) |
|---|---|---|---|---|
| 1 | 70.40 ± 3.00 | 12.50 ± 1.2 | 3.90 ± 0.41 | 1.01 ± 0.06 |
| 2 | 69.20 ± 2.50 | 14.20 ± 1.24 | 5.21 ± 0.87 | 1.28 ± 0.06 |
| 3 | 63.25 ± 3.20 | 18.60 ± 1.61 | 10.43 ± 1.10 | 0.88 ± 0.10 |
| 4 | 59.75 ± 4.10 | 27.85 ± 2.21 | 15.60 ± 1.67 | 0.67 ± 0.09 |
| 5 | 66.50 ± 3.50 | 29.40 ± 2.30 | 17.25 ± 1.50 | 0.96 ± 0.05 |
| 6 | 74.00 ± 3.50 | 13.80 ± 1.50 | 5.80 ± 0.61 | 0.42 ± 0.05 |

The data pertaining to changes in biochemical parameters was subjected to analysis of variance (Anova; two way with interaction) to test significant changes (i) between different tissues at various stages of maturity (ii) between different stages of maturity in various tissues. Since the interaction between the two sources of variation (tissue and stage) was significant at 0.1% level, the main effects were tested against interaction. The results indicated, significant variation of all parameters between tissue at 5% level. But btween stages, only protein, lipid show significant variations at 5% level. (Table 15).

The variation in the composition of blood serum at different stages of maturity, was investigated by a one way analysis of variance applied seperately for each biochemical parameter. (Table 13). The results show significant variation of all parameters ($P < 0.001$), between different maturity stages.

Electrophoretic profile of serum proteins at different stages of maturity of female L. calcarifer are presented in Fig. 22 and data is summarized in Table 16. A total of 24 bands; Rf value ranging from 0.009 0.969 , with varying thickness and staining intensities are detected.
In stage 3 serum, a strong protein band (No. 20) having a low Rf value (0.124) appears in zone I of the gel. This band become diffused in stage 5 and disappear in stage 6. This band stain positively for carbohydrate, lipid and calcium and is not observed in the electrophoretic profile of mature male.

**Table 15 Result of the analysis of variance of biochemical parameters between maturity stages and tissues in female L. calcarifer**

| Parameters | Source of variation | Calculated F. values. | Remarks |
|---|---|---|---|
| Moisture | Between Tissues | 5.85 | $P < 0.05$ |
| | Between Stages | 1.03 | N S |
| | Interation | 70.23 | $P < 0.001$ |
| Protein | Between Tissues | 6.43 | $P < 0.005$ |
| | Between Stages | 3.71 | $P < 0.05$ |
| | Interaction | 135.46 | $P < 0.001$ |
| Lipids | Between Tissues | 9.13 | $P < 0.05$ |
| | Between Stages | 4.68 | $P < 0.05$ |
| | Interaction | 163.71 | $P < 0.001$ |
| Cholestrol | Between Tissues | 6.71 | $P < 0.005$ |
| | Between Stages | 0.641 | N S |
| | Interaction | 32.15 | $P < 0.001$ |

* N S - Not Significant.

Degrees of freedom (df) tissues = 2
stages = 5
interaction = 10

Table. 16 Details of protein fractions, their relative fraction ($R_f$) Values and staining characteristics in the blood serum of female Later calcarifer during different stages of maturity.

| | | | MATURITY STAGES | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | STAGE II | | | | STAGE III | | | | STAGE IV | | | | STAGE V | | | | STAGE VI | | | |
| | Band No. | $R_f$ Values | General Proteins | Glyco Proteins | Lipo Proteins | Calcium Bind-Proteins | General Proteins | Glyco Proteins | Lipo Proteins | Calcium Bind-Proteins | General Proteins | General Proteins | Lipo Proteins | Calcium Bind-Proteins | GGeneral Proteins | Glyco Proteins | Lipo Proteins | Calcium Bind-Proteins | General Proteins | Glyco Proteins | Lipo Proteins | Calcium Bind-Proteins |
| | 1 | 0.969 | XX | - | - | - | XX | - | - | - | XX | - | - | - | XX | - | - | - | XX | - | - | |
| | 2 | 0.926 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | |
| | 3 | 0.890 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | |
| Zone III | 4 | 0.863 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | |
| (6-11cms) | 5 | 0.814 | XXXX | XX | XX | XX | XXXX | XX | XX | XX | XXXX | XX | XX | XX | XXXX | XX | XX | XX | XXXX | XX | XX | XX |
| | 6 | 0.742 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | |
| | 7 | 0.691 | XX | - | - | - | XX | - | - | - | XX | - | - | - | XX | - | - | - | XX | - | - | - |
| | 8 | 0.631 | XXXX | XX | XX | XX | XXXX | XX | XX | XX | XXXX | XX | XX | XX | XXXX | XX | XX | XX | XXXX | XX | XX | XX |
| | 9 | 0.583 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - |
| | 10. | 0.532 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - |
| | 11 | 0.461 | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXX | XXXX |
| | 12 | 0.390 | XXXX | XXXX | - | - | XXXX | XXXX | - | - | XXXX | XXXX | - | - | XXXX | XXXX | - | - | XXXX | XXXX | - | - |
| Zone II | 13 | 0.340 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - |
| (3-6cms) | 14 | 0.310 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - |
| | 15 | 0.292 | XXXX | - | XX | XX | XXXX | - | XX | XX | XXXX | - | XX | XX | XXXX | - | XX | XX | XXXX | - | XX | XX |
| | 16 | 0.278 | XXXX | XX | - | - | XXXX | XX | - | - | XXXX | XX | - | - | XXXX | XX | - | - | XXXX | XX | - | - |
| | 17 | 0.246 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - |
| | 18 | 0.207 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | | - | |
| | 19 | 0.170 | XXXX | - | XX | - | XXXX | - | XX | - | XXXX | - | XX | - | XXXX | - | XX | - | XXXX | - | XX | |
| Zone I | 20 | 0.124 | - | - | - | - | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XX | XX | XX | XX | - | - | - | - |
| (0-3cms) | 21 | 0.078 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - |
| | 22 | 0.039 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - |
| | 23 | 0.018 | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - | X | - | - | - |
| | 24 | 0.009 | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX | XXXX |

X Narrow and Sharp bands

XX Broad and diffuse bands

XXXX Broad and intensely stained bands

- Absence of Bands.

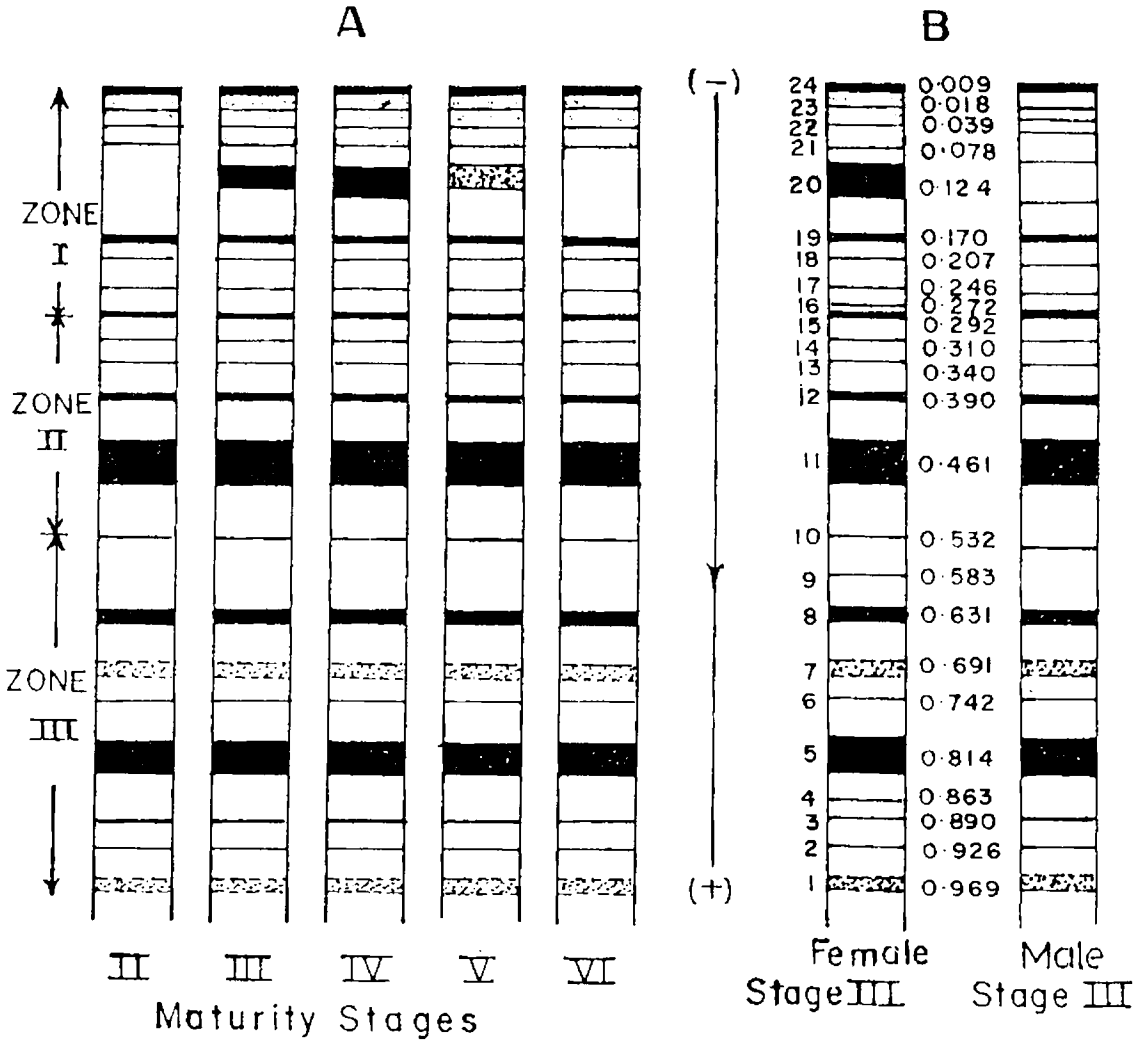



FIG. 22. A. Electrophoretic profile of serum proteins corresponding to different stages of ovarian recrudescence in L. calcarifer.

B. Comparision of serum protein proteins of mature male and female L. calcarifer.

## DISCUSSION

The data presented in the current section reveal the cyclic changes in the levels of certain metabolites in the somatic as well as gonadal tissue with respect to reproductive developments. During the periods of high gonadal activity the resources of metabolites, including reserves in somatic tissue appear to be translocated via circulatory system towards the gonads. These interrelationships, in generalized form, appear to exhibit similarity in both the sexes, however, the magnitude of variations in males is less probably owing to the lack of any phenomenon parallel to vitellogenesis of females.

The recruitment of the oocytes to vitellogenic phase coincides with increasing levels of total serum protein contents as well as appearance of new band in the electrophoretic profile. Staining characteristic of the band suggests this female specific protein to be a complex with lipid as well as carbohydrate moiety and calcium ions; indicating it to be vitellogenin. Vitollogenin found in serum during the period of vitellogenic growth is precursor of much of the egg yolk in teleosts like other non-mammalian vertebrates and is a calcium binding glycolipophosphoprotein (Wallace, 1978). Protein bound phosphate contents vary in the range of 3.04 to 52.0 ug/ml in the serum of L. calcarifer. Considerable variations with respect to this parameter existing among teleosts, has been attributed probably to variability in phosphate contents of vitellogenin ( van Boheman et al., 1981; Craik and Harvey, 1984). Protein bound phosphate is found in large amounts only in the serum of vitellogenic females of oviparous vertebrates, hence has been used as a reliable index of serum vitellogenin levels ( van Bohemen et al., 1981; Craik and Harvey, 1984; Tinsley, 1985; Garg, 1988).

Total serum calcium was found to have correlation with ovarian maturation but not with testicular maturation, and is in agreement with the observations on other teleosts (Oguri and Takada, 1967; Yaron, et al; 1977). The variations in this parameter parallel those in protein bound

phosphorous, probably due to binding of calcium to vitellogenin (Pang, 1973; Whitehead et al; 1980; van Bohemen and Lambert, 1982; Pandey, 1991). Björnsson and Haux (1985) demonstrated that the increase in total serum calcium content is due to the calcium containing vitellogenin while the free calcium level remain unaltered. Estradiol - 17 β , potent to induce hepatic synthesis and secretion of vitellogenin ( Selman and Wallace, 1983) has been found to have hypercalcemic effect (Oguri and Takada, 1967; Aida et al, 1973; Hori et al., 1979) as well as elevates the serum calcitonin levels (Björnsson et al., 1989) Serum calcitonin levels have been found to have definite relationship with ovarian maturation (Fouchereau-peron et al., 1990). It has been argued that the rise in calcitonin level may be to protect calcium pools during the periods of high calcium demand for binding with vitellogenin. (Björnsson, et al, 1989). Functionally the binding of calcium to vitellogenin appear necessary to keep protein in solution (Follett and Redshaw, 1974). Calcium bound vitellogenin may provide vital source of calcium for the embryogeny after its sequestration into oocyte (Bjornsson et al., 1989). Apart from association of calcium with vitellogenin, it has been found to have regulatory function in steroidogenesis in prevoulatory follices ( vanden Kraak, 1991).

Changes in protein bound phosphate and total calcium levels in serum suggest that the vitellogenin appear during stage 3, in the serum and the level increases thereafter. However, the decline in vitellogenin level at stage 5, when most of the oocytes have completed vitellogenic growth, agrees well with the cytological evidence of existence of single clutch of synchronously developing vitellogenic oocytes, to be shed in the single spawning. In multiple spawners e.g. Misgrunus anguillicaudatus serum vitellogenin has been observed to be at high levels, while a group of oocytes has already completed growth; probably to support the succeeding clutch (Teranishi et al., 1981).

Pattern of fluctuations in hepatic protein contents has important physiological significance if viewed in the light of changes in other parameters

associated with vitellogenesis. The decline in total hepatic protein contents during exogenous vitellogenesis may be due to synthesis and secretion of vitellogenin (Plack and Frazer, 1971; van Bohemann et al., 1981; Selman and Wallace, 1983). Vitellogenin synthesizing role of hepatocytes is established, however largely through indirect evidences. Vitellogenin has not been localized cytologically in any teleost or lower vertebrate. Inability to get direct evidence has been attributed to lack of storage of this protein, probably composed from its precursors just prior to secretion (Selman and Wallace, 1983). It seems quite probable that high hepatic protein contents in previtellogenic stages may be due to accumulation of precursor proteins. Plack et al. (1971) opined that the precursor proteins can be stored in liver.

Besides mobilization of proteins in the form of vitellogenin, liver may contribute for other associated ovarian growth processes. Hamazaki et al. (1986) demonstrated that the glycoprotein, used for the formation of inner layer of zona pellucida in Oryzias latipes are derived from liver.

Love (1975) classified fishes into "fat" fishes (those store fat in muscles) and "lean" fishes (those store fat in liver). L. calcarifer with liver lipid content (5-10%) more than muscles (1-3%) may be categorised among "lean" fishes. Changes in lipid contents in various tissues studied depict close relationship with reproductive cycle. Several authors have described close association between lipid metabolism and reproduction in teleosts (Korsgaard and Petersen, 1979; Hori et al., 1979; Wiegend and Peter, 1980; Elizabeth, 1987; Gopalakrishnan, 1991). Fish utilize lipid resources in relation to reproduction primarily for (i) source of energy to sustain as well as meet the demand of higher muscular activity during breeding associated events ii) synthesis of materials for gametogenesis iii) synthesis of steroids.

Pronounced increase in the number of lipid bodies during vitellogenesis, observed cytologically; is reflected in the ovarian total lipid content profile. One of the important most likely source of the extra lipid can be vitellogenin. Hori et al. (1979) in Carassius auratus and Campbell and

Idler (1980) in Salmo gairdneri, found total lipid to constitute 21 to 22% of vitellogenin. Besides, lipoproteins, a distinct fatty acid complement has been found in vitellogenin of S. gairdneri (Leger et al., 1981).

Korsgaard and Petersen (1979) reported that in teleosts ovarian lipid synthesis is minimal as compared to other tissues especially liver. This appear to be true for testis too probably necessitate mobilization of lipid from reserves in somatic tissue indicated by depletion in muscles as well as liver and rising levels in serum with the advancement of gonadal development. The depletion in hepatic lipid contents may be partly contributed for incorporation into vitellogenin. The mobilization declines when the vitellogenesis is near cessation causing slight rise in hepatic lipid levels. Owing to lack of any such phenomenon, males exhibit less pronounced variations in lipid contents (in both somatic as well as gonadal tissues), than females. Idler and Bitners (1960) observed that in Oncorhynchus nerka 8% of muscle lipids is transferred to the ovary as compared to only 0.5% transferred to the testis.

Initiation of steroidogenesis, in the gonads, through mobilization of endogenous cholestrol, to produce pregnanolone, precursor for both androgen and estrogen (Nagahama, 1987) has been suggested to be gonadotropin dependent (Jayashree and Srinivasachar, 1979; Sen and Bhattacharya, 1981; Deb et al. 1985; Petrino et al., 1989).

Testicular cholestrol dynamics in L. calcarifer depict a conspicuous decline during the stages of active spermatogenesis. Parallel to this, ovarian cholestrol level decreases during the gonodotropin dependent phase (vitellogenic phase) of the oocyte growth. This pattern of changes probably reflect consumption of cholestrol to meet high demand of active steroidogenesis at these stages in both the sexes (Deb and Bhattacharya, 1986; Diwan and Krishnan, 1986; Guha and Mikherjee, 1987; Elizabeth, 1987; Gopalakrishnan, 1991). Elevation of cholestrol level in the testes, packed with mature spermatozoa and ovaries with fully grown oocytes probably point out relatively low steroidogenic activity. Subsequent fall in

cholestrol contents in spent gonads reitrate the utilization of the metabolite for steroid synthesis resulting in maturation ovulation/spermiation. These interpretations are supported by the serum cholestrol profile. High level maintained during active stages of gametogenesis may be to cater the high demand of the substrate in the gonads. Deb and Mukherjee (1986) demonstrated relationship between circulatory and ovarian cholestrol levels and suggested the former to be the major source of substrate for ovarian steroidogenesis in Anabas testudineus. Similar relationship has been reported to exist between circulatory and testicular cholestrol levels in Cyprinus carpio (Guha and Mukherjee, 1987). It appear quite probable that the high serum cholestrol content are the consequence of mobilization from somatic tissues reserves especially liver; observed in several teleosts (Chaturvedi et al. 1976; Miller, 1971; Muthukaruppan, 1987; Elizabeth, 1987;Gopalakrishnan 1991).

Water forms the major constituent of the tissues. Its fluctuations in the body tissue, besides influenced by environmental factors, osmotic properties of the cells and other physiological activities are ultimately linked to accumulation or decline of metabolites, is ultimately reflected in the water out of the tissues (Marais and Erasmus, 1977). Accumulation or decline of metabolites is ultimately reflected in the fluctuations in the water contents of the tissue, influenced by the osmotic properties of the cells and other phsiological activities besides environmental factors (Marais and Erasmus, 1977). This probably explain satisfactorily the observed variations in somatic as well as gonad water contents in relation to gametogenesis but for the sharp rise in water contents in the ripe ovary, where there have not been any decline in metabolic component. This rapid uptake of water is probably responsible for drastic rise in oocyte size following completion of vitellogenic growth during maturation (approx. 400 - 500 /um to 700 /um). This phenomenon of gonadal hydration before ovulation has been described in several teleosts. (Kapur and Toor, 1978; Wallace and Selman, 1981; Craik and Harvey, 1984; 1987; Greeley et al.,1986;Selman and Wallace, 1989; Mcpherson et al.;1989) and is more pronounced in marine teleosts which spawn pelagic eggs. Hydration of the oocytes reduces their density to the extent that mature eggs become buoyant in sea water so

as to facilitate dispersal by water currents (Fulton, 1898; Craik and Harvey, 1987).

Mcpherson et al. (1989) demonstrated the influx of potassium ions into the oocytes, largely responsible for osmotic changes effecting hydration in Fundulus heteroclitus. Yolk proteoysis, earlier considered to be the effector for osmotic changes (Greeley et al., 1986b) was found to be an independent event associated with oocyte maturation.

Testicular moisture contents also exhibit sharp rise during the periods of spawning. The significance of gonadal hydration in males is probably to facilitate the free flow of milt and cause intralobular pressure during spermiation. (Billard et al., 1982; Elizabeth, 1987).

# CHAPTER IV
# SUMMARY

## CHAPTER IV

## SUMMARY

Lates calcarifer, though distributed through out the Indian coast, does not contribute much to the capture fishery. The fish has been recoginised to have vast aquaculture potential but availability of seed still remains a bottleneck. The thesis is aimed at providing the necessary information pertaining to various characteristics of natural reproductive cycle. Despite considerable progress achieved in the artificial propagation techniques in several countries of Indo-Pacific, little attempt has been made towards exploring the basic mechanisms underlying various processes associated with the reproduction. Against this background, the current work has been taken up which provide descriptive account of the process of gametogenesis and the biochemical changes associated with.

L. calcarifer displays a complex sexuality, where lower length classes are dominated by males and larger ones by females but for a few exceptions. The majority of the male fish mature after attaining the length in the range of 600 to 650 mm. The length of majority of sex-inverting males was found to be 885 mm. L. calcarifer population is digynous represented by two types of females; primary and secondary. The former develop directly from the immature fish whereas the later category include those derived from males; constituting majority of the female population.

The sex ratio is in the favour of females, which appear to be the artefact in the population under study. Since, in a protandric hermaphrodite, males usually outnumber the females. This deviation probably indicate the selective loss of males from the population. The imbalanced sex ratio may be responsible for the high proportion of gravid fish undergoing involution.

L. calcarifer exhibits seasonally synchronized reproductive cycle. Spawning appear to start after the onset of rains, the season extending from mid October to December. L. calcarifer is a highly fecund fish, the

fecundity estimate ranging from 3.85 to 30.18 million eggs. L. calcarifer does not appear to undergo multiple spawning, as no partially spawned fish was encountered during the study. Moreover, this inference find support from histological and physiological evidences too.

The gonad maturation cycle has been classified into six stages of maturity corresponding to changes in macroscopic as well as microscopic characteristics. The testes are paried strap like organs, packed with seminiferous lobules containing cysts of germ cells and sertoli cells. On the basis of optical as well as ultrastructure studies, seven stages of spermatogenesis has been identified. The primordial germ cells, mitotically dividing cells are found in clusters in the newly differentiating testes only and transform into spermatogonia type A. Spermatogonia type A appear to exist in two forms; type Aa (active cells) and type As (stem cells). The stem cells remain dormant in the lobules and become active to resume the spermatogenic cycle in the recovering spent stage for the next spawning season. Spermatogonia type A are individually surrounded by sertoli cell processes and divide mitotically to give rise to spermatogonia type B, organized in the germinal cysts. The primary spermatocytes formed after several divisions of spermatogonia type B, are destined to undergo meiosis. The first meiotic division yields secondary spermatocytes which in turn produce spermatid after second meiotic division. Spermatid transform into spermatozoa, thrrough spermiogenesis, a process of concurrently occurres events viz. condensation of nuclear chromatin; development of axoneme and migration close to articular fossa in the nucleus; relocation of mitochondria and extrusion of excess cytoplasm. The spermiogenesis of L. calcarifer appears to belong to type B class, due to lack of nuclear notation. Mature spermatozoa has morphological features of a primitive type spermatozoa.

The ovaries derived from post-spawned testes are paired, cylindrical organs consisting of ovigerous lamellae the site for oocyte development. The newly formed ovary has clusters of mitotically dividing primordial germ cells, which give rise to oogonia. The dynamics of cytological events during the oogenesis, from oogonia to egg, parallel those found in other

teleosts, in general. The primary growth phase is characterized by chromatin nucleolus, early perinucleolus and late perinucleolus stages. During this period, oocytes grow in size and display high nucleocytoplasmic interaction. The nucleolar material are extruded into the cytoplasm in the form of nuage, considered to be involved with biogenesis of mitochondria and ribosomes. The nuage material was found to be major component of Balbiani's vitelline body. During primary growth phase, oocyte accumulates organelles, required for further growth of oocyte. A group of oocytes is recruited into secondary growth phase characterized by appearance of cortical alveoli, lipid droplets and vitelline envelope. Pinocytotic activity, probably related to the sequesteration of exogenous yolk precursor proteins. During vitellogenic stage, the yolk material is incorporated into yolk spheres. The fully grown oocyte (at the end of vitellogenic growth) is tightly packed with mature yolk spheres. Concurrently vitelline envelope grows in width and become architecturely complex; differentiated into external and internal layers. Close to ovulation, the oocytes undergo maturation and are referred as egg. The processes associated with maturation involve resumption of meiosis indicated by break down of germinal vesicle; lipid droplets coelsc to form single oil globule, compaction of vitelline envelope. The oocytes grow significantly during this period, probably due to rapid uptake of water.

The cyclic nature of reproductive recrudescence is reflected in the biochemical composition of both somatic as well as gonad tissues. In general, the reproductive development occurs at the cost of metabolic constituents translocated from the somatic sources like liver and muscle. The transportion appear to be carried out through blood circulation. The fluctuations in biochemical composition are more pronounced in females than males, due to larger investments required to accomplish yolk accumulation in the oocytes. The yolk precursor protein or vitellogenin appear to be synthesized in the hepatocytes. Vitellogenin is a clacium binding glycolpophosphoprotein which appear as a band in the electrophoretic profile of serum proteins at stage 3 (Rf = 0.124), becomes diffused at stage 5 and finally disappear at stage 6. The sequestration of vitellogenin is evidenced by pinocytotic activity near the oocyte surface at ultrastructural

level. Vitellogenin level in serum, indicated by protein bound phosphate and total calcium contents, appear to be maximum at stage 4 thereafter start decreasing, indicating the development of single clutch of oocytes to be shed simultaneously, as mentioned earlier.

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