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 under study 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 mangrove 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 abdomina1 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 distinguishes 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

<table>
<thead>
<tr>
<th>Stage</th>
<th>Macroscopic characteristics</th>
<th>Histological characteristics</th>
<th>Spermatogenic response</th>
<th>Quantity of spermatogenesis</th>
<th>Standard gonadal weight (Gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 0</td>
<td>The testes indistinguishable from the viscera.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stage 1</td>
<td>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.</td>
<td>The undifferentiated stroma cells with large vacuoles form major part of the lobe primordial germ cells with highly basophilic nuclei present in clusters.</td>
<td>0.00</td>
<td>0.00</td>
<td>1.8217</td>
</tr>
<tr>
<td>Stage 2</td>
<td>The testes are opaque, light pale with deep longitudinal furrows.</td>
<td>Can be classified into developing virgin and recovering spent. Lobules contain mainly spermatogonia 'type A' along the periphery, with a few cysts of spermatogonia 'type B' and primary spermatocytes.</td>
<td>0.60</td>
<td>0.00</td>
<td>4.6903</td>
</tr>
<tr>
<td>Stage 3</td>
<td>The testes thicken and are wedge-shaped in cross section. The spermatozoa squeeze out from the cut testes.</td>
<td>Most of the interstitial areas are occupied by the lobules, contain mainly the cysts of spermatocytes and spermatids with spermatozoa in the lumina and sperm ducts ducts some spermatogonia are still present.</td>
<td>1.48</td>
<td>0.32</td>
<td>6.8814</td>
</tr>
<tr>
<td>Stage 4</td>
<td>The testes are thick with rounded lateral margins and prominent ventral lobes.</td>
<td>Lobules are characterized by thin walls and lumina are packed with spermatozoa. Cysts of spermatocytes and spermatids still present but no active spermatogonia.</td>
<td>2.96</td>
<td>2.14</td>
<td>10.7391</td>
</tr>
<tr>
<td>Stage 5</td>
<td>The ventral lobes are enlarged considerably. Pressure on the belly causes milk to extrude from urogenital aperture.</td>
<td>Lobules and ducts are packed with spermatozoa.</td>
<td>2.80</td>
<td>3.00</td>
<td>15.1014</td>
</tr>
<tr>
<td>Stage 6</td>
<td>The testes appear collapsed and appear like a strap.</td>
<td>The lobule walls are wrinkled and start thickening; lumina still have residual spermatozoa.</td>
<td>0.12</td>
<td>1.11</td>
<td>6.8838</td>
</tr>
</tbody>
</table>
Table 3. Salient features of ovaries of *L. calcarifer* corresponding to various stages of maturity.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Macroscopic characteristics</th>
<th>Histological characteristics</th>
<th>Largest oocyte diameter (μm)</th>
<th>Standard gonad weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1 (newly formed)</td>
<td>The ovaries are deep reddish in colour, recently derived from testes after sex inversion.</td>
<td>Ovigerous lamellae are packed with chromatin nucleolus and early perinucleolus stage oocytes with a few at late perinucleolus stage.</td>
<td>110</td>
<td>78.1941</td>
</tr>
<tr>
<td>Stage 2 (developing virgin/ recovering spent)</td>
<td>The ovaries are thick walled, translucent, with pinkishred hue.</td>
<td>Chromatin nucleolus stage oocytes present but perinucleolus stage oocytes dominate.</td>
<td>140</td>
<td>82.9456</td>
</tr>
<tr>
<td>Stage 3 (maturing)</td>
<td>The ovaries increase in size, translucent and cream in colour</td>
<td>Perinucleolus stage oocytes are present; oocytes entering gonadotropin dependent phase (cortical alveoli stage) predominate the population.</td>
<td>160 - 270</td>
<td>168.1938</td>
</tr>
<tr>
<td>Stage 4 (mature)</td>
<td>The ovaries are markedly larger in size, with thinner wall and yellow hue start appearing</td>
<td>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.</td>
<td>260 - 500</td>
<td>406.7025</td>
</tr>
<tr>
<td>Stage 5 (ripe)</td>
<td>The ovaries are deep yellow in colour, with thin walls, distended and occupying most of the body cavity</td>
<td>The oocytes undergo final maturation, migration of germinal vesicle and break down, coalescence of oil droplets.</td>
<td>450 - 700</td>
<td>628.793</td>
</tr>
<tr>
<td>Stage 6 (spent)</td>
<td>The ovaries are flaccid, reduced in size, with purple yellow colour. The wall becomes tough and wrinkled.</td>
<td>Post ovulatory follicles; previtellogenic oocytes dominate along with a few residual yolky oocytes.</td>
<td>140</td>
<td>127.9911</td>
</tr>
<tr>
<td>Stage 5A. (involuting)</td>
<td>The ovaries are deep red in colour, progressively decrease in size and have watery consistency.</td>
<td>Vitellogenic oocytes at various stages of atresia, often surrounded by blood vessels.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
FIG. 5. Representative oocyte size-frequency profiles corresponding 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 maturational stage 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 protandric 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 discernible 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
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 occurring in January (Fig. 12).

SEX RATIO

The sex ratio was found to be

\[ \frac{0.83}{1} \text{ in favour of females} \left( \chi^2 = 4.6864 \right) \]

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

FIG. 9. Variation of the frequencies with length of mature male L. calcarifer.
FIG. 11. Percentage of male L. calcarifer examined that were changing sex.
FIG. 12. Percentage of transitional male *L. calcariifer* 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 spermatzoa 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 progression through maturity stages 3 and 4, characterized by active spermatogenesis, to the ripe (stage 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 dominance 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. 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 (G_s,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 maintains 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 2 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 occur.

**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, presumably 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 artificial 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 hermaphroditism. Patnaik and Jena (1976) encountered the smallest mature female of length 700 mm in India. Wongsomuk 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 occurrence 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 hermaphrodite (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
Davis (1982) fitted Gompertz 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 synchronised 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 occurrence 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 Queensland, 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 southeasterly east 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. Dunstan (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 extended nature of the breeding season.

The observed fecundity 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 up to $46 \times 10^6$ (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, 1984b; Greeley et al., 1987). As defined for L. niloticus (Ogutu-Chiango, 1983) the actual reproductive potential of L. calcarifer will depend upon the number of eggs that are fertilized and hatch, the environmental conditions to which the offspring are exposed and the growth rate of the offspring. 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 reveals a dissimilar situation where sex-ratio favours females, yet importance of high fecundity deserves recognition as only a small proportion 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 agreement 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 occurrence in fish ovary during prespawning, spawning and post-spawning periods and has been studied extensively in numerous 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 mobilise and relocate large energy investments rather than turning it into waste, if conditions conducive for spawning do not occur (Ham and Leesan, 1961). Besides, it ensures that young ones hatch only when conditions are favourable for their survival (Wooton 1982; Lam, 1983; Guaraya, 1986).

In a protandric hermaphrodite, 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 pheromone 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. 2, 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 Queensland 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. Mangrove 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 predominant 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; Bruslé, 1987; Bruslé-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, separated 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 proliferation (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 1a). 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 1b). 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 pinocytic vesicles near the plasmalemma. Zone between myoid cells and basal lamina consists of connective tissue containing collagen 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 usually present in these areas, are of irregular shape, distributed single or in clusters (Plate 1c). 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 1d).
PLATE I

a. Light micrograph showing organization of testis with thick tunica albuginea (TA), sperm duct (SD) on dorsal side (D). Seminiferous lobules (SI) surrounded by lobular walls (Iw) 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.
<table>
<thead>
<tr>
<th>Topography</th>
<th>Primordial germ cells</th>
<th>Spermatagonia type A</th>
<th>Spermatagonia type B</th>
<th>Primary spermatocytes</th>
<th>Secondary spermatocytes</th>
<th>Early spermatid</th>
<th>Late spermatid</th>
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<tr>
<td>Association</td>
<td>clusters</td>
<td>form layers around lobules</td>
<td>isolated in cysts</td>
<td>in cysts</td>
<td>in cysts</td>
<td>in cysts</td>
<td>in cysts</td>
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<tr>
<td>with sertoli</td>
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<tr>
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<td>ovoid</td>
<td>ovoid</td>
<td>ovoid</td>
<td>ovoid</td>
<td>ovoid</td>
<td>spherical</td>
</tr>
<tr>
<td>Length (µm)</td>
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<td>11.5-13.8</td>
<td>11.5-13.8</td>
<td>8.5-10.2</td>
<td>6.8-8.5</td>
<td>5.3-6.4</td>
<td>4.0-4.6</td>
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<tr>
<td>Width (µm)</td>
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<td>10.0-12.8</td>
<td>10.0-12.8</td>
<td>7.8-9.0</td>
<td>6.3-7.6</td>
<td>4.5-5.5</td>
<td>2.9-3.4 (diameter)</td>
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<tr>
<td>Nuclear shape</td>
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<td>near spherical</td>
<td>near spherical</td>
<td>ovoid</td>
<td>near spherical</td>
<td>spherical</td>
<td>kidney shaped</td>
</tr>
<tr>
<td>Length (µm)</td>
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<td>7.1-7.8</td>
<td>7.1-7.8</td>
<td>5.2-6.8</td>
<td>3.8-4.7</td>
<td>3.2-3.8</td>
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<td>Width (µm)</td>
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<td>6.3-7.0</td>
<td>6.3-7.0</td>
<td>3.4-4.8</td>
<td>3.4-4.1</td>
<td>2.6-3.1</td>
<td>1.1-1.2 (diameter)</td>
</tr>
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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, separated from each other by strands of sertoli cell cytoplasm. Lobular configuration of the testis is still not fully established (Plate IIc,d; IIIa). GAa are more or less oval cells with regular 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, mitochondria, 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 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) separated 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 are also visible (arrow). Sperm heads (Sd) in the adjoining cyst indicate completion of spermiogenesis. X 4,000.
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 mitochondrion. 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 abundant 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 IIIId cysts containing GB are present while neighbouring cysts have already completed spermiogenesis.
a. Electron micrograph showing part of a germinal cyst containing spermatogonia type B. Nuclei (N) surrounded by cytoplasm containing mitochondria (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.
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, mitochondria, intermitochondrial cement is observed till pachytene stage. Intercellular bridges are visible. Occasionally bundles of microtubules can be found close to nuclear envelope. First meiotic division of primary spermatocytes give rise to secondary spermatocytes.

5. Secondary spermatocytes (SSC)

SSC are much smaller cells with very scant cytoplasm surrounding an intensely staining basophilic nucleus (Plate Vd). Cytoplasm 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 within 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). Lumen contains 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 spermatids (St). The lumen of lobule contains spermatozoa (SZ). X 100.
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 golgi 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.
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 membrane. 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 disintegration 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 homogeneous chromatin. Articular fossa (af) has scalloped boarder. Fibres are also visible in the granular chromatin (arrow 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), horizontal 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.
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. Occasionally, 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 Ville). Plasma membrane make a loop separating 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 phagocytised by sertoli cells. This makes the plasma membrane to closely adhere to the nucleus in the mature spermatozoon (Plate VIIlc).

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 VIIb). Mid piece is constituted by ring of eight to ten mitochondria. Tail consists of a central axoneme encovered by plasma membrane sheath. The axoneme is characterized by typical 9 + 2 pattern of microtubules. The nine peripheral microtubule doublets are attached individually to outer plasma membrane as well as two central microtubules 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 (arrow) are also visible. X 33,000.

b. Electron micrograph showing spermatids at the end of sperm development. Plasma membrane (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 longitudinal 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 separate mitochondria form flagellum (F). Axoneme (A) is also visible. X 26,000.
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 membrane (P). X 96,000.

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

c. Light micrograph showing lobules (L) packed with spermatozoa (SZ) in a running ripe testis. Blood vessel (BV) is also visible. X 100.
Mature spermatozoa are released from germinal cyst into the lumen of lobule. Initially, the spermatozoa are seen as clumps with their tails held together (Plate IXb). Gradually, individual spermatozoa separate 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 spermatozoa 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. calcifer 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 /um 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 Xla). Various stages of development up to the formation of egg are described below.

Primordial germ cells

Primordial germ cells are small polygonal cells, occurring in clusters in the newly developing ovary (Plate Xlla) and characterized by large strongly basophilic nucleus. These cells proliferate mitotically (Plate Xllb).
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.
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 XII

a. Light micrograph of newly differentiated ovary showing abundant clusters of primordial germ cells (arrow) along with early previtelligenic 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 than that in (a). Note less abundance of primordial germ cell clusters (arrow). The oocytes are predominantly at perinucleolar stages. X 140.

d. Electron micrograph of an oogonium showing nucleus (N) with a distinct nuclear envelope (arrowheads). Cytoplasm has polar distribution of cell organelles containing mitochondria (mt) with associated ciment (ci) and nuage (nu). Pe follicle cell (PFC) with dense nucleus (N) is also visible. X 13,200.
Gradually, with the advancement of growth, primordial germ cells decline in abundance (Plate XIIc).

Oogonia (approximately 10 \( \mu \text{m} \))

Oogonia are oval or nearly spherical cells with regular outline (Plate XIIIa). Usually occur 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 than oogonia. Nucleus can be oval or elongated with dense clumps of chromatin.

Primary growth Phase:

Chromatin nucleolus oocytes (10-25 \( \mu \text{m} \) 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, XIIa,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 previtelligenic (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.
Cytoplasm is strongly basophilic and electron dense especially in early stages (Plate XIIIa), tightly packed with ribosomes. Electron-density reduces with the increase in cell size during growth. Circumferential to nuclear envelope is a low density area of cytoplasm. Sense aggregates of nuage material are also seen scattered along the nuclear envelope (Plate Xlib). The Balbiani's vitelline body originates, 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 Xlic). Lightly staining patches, containing mostly mitochondria are confined to perinuclear ooplasm at this stage (Plate Xlild).

The oocytes are contained within the definite follicles, with monolayer of a few follicle cells extending their processes between oolemma and basal lamina (Plate Xlild).

Cytoplasm is less electron dense than 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 size, 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 Xlb; 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 (arrow). 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 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 depicting 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.
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). Occasionally, 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 Balbiani'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 preceding stage, are scattered throughout 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 than in the previous stage. The association between adjacent oocytes show detachment at certain points.

Late Perinucleolar stage (70 - 120 μm).

The oocytes at this stage are round, with a large spherical nucleus with a diameter ranging from 35 to 45 μm (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 richly endowed with cell organelles distributed throughout from perinuclear to cortical area.

Oocyte begin to elaborate microvillar processes towards the overlying follicle cells (Plate Xvlb), 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 μm)**

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

Cortical alveoli are the small vesicles, observed throughout the peripheral ooplasm (Plate XVIc). At ultrastructure 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 XVC).

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 scattered 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 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 precursor) 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.
material deposited between the oocyte microvillar processes of width 4.8 μm. The vitelline envelope attains bilaminar appearance with external (ZE = 2.2 μm) and internal (Z1 = 2.6 μm) layers (Plate XVIIa). Microvillar processes continue to lengthen as the vitelline envelope thickens. 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 elaborate through the perforations in the vitelline envelope.

Vitellogenesis (260 - 500 μm)

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 μm) 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 XVIIa). Vitelline envelope thickness (Plate XVIIa, 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 μm) 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 microscopy 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 an oocyte after completion of vitellogenic growth, compactly filled with yolk spheres (YS). X 140.
Bilaminar, vitelline envelope increase in width (7.8 μm; ZE = 2.6 μm; ZI = 5.2 μm) 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 tilled 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 XVIIIb).

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 border 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 μm).

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 (arrow) 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.
Yolk spheres are compactly packed, resulting in peripheral displacement of ooplasm to a thin rim. Oocyte size increases considerably. The vitelline envelope 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 spawning. 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 I) 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). Germinial 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 degenerating 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.
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 membrane 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, rather 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 previtelligenic oocyte (PVO). X 5750.
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.
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). Loftus 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 reproductively 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 (Nagahama, 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 (Nagahama, 1983). The boundaries of germinal cysts are delimited by plasmalemma of Sertoli cells.

In principle, the sequence of events up to 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. Bruslé' and Bruslé (1978) in _Liza auratus_; Billard (1984) in _Poecilia 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 predominantly 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, Ictalurus punctatus and Perca flavescens. 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 GAd, 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 discrepancies 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 mitochondria. 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 tsawatscha_ 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 appears 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 phenomena have 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 heterogeneity 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. Occasional electron lucent areas observed, are rare among teleosts and have not been assigned any functional significance but arise accidentally 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 accommodate 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 microtubular pattern; ensheathed by plasma membrane. The plasma membrane does not form any lateral ridges or fins as observed in O. maculosus (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; Bruslé-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 ultrastructural level, spermatogonia and oogonia exhibit resemblance to a large extent, indicating the possibility of the cells to be bipotential (Reinboth, 1982). Recently, Shibata and Hamaguchi (1988) demonstrated that biopotentiality is maintained upto several mitotic generations of spermatogonia type B (GB) in Uryzas jalipes. 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; Nagahama 1983; Guraya, 1986; Selman and Wallace, 1989).

Oogonia, found throughout 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 (Garda, 1964; Lehri, 1968, Baumeister, 1973, Monace et al., 1978; Selman and Wallace, 1989). Following this arrest, a period of extensive oocyte growth commences concomitant to folliculogenesis.

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 fragments 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 granulofibrillar 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 reflection 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 occur 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 (Bruslé, 1980) M. cephalus (Gopalakrishnan, 1991) and of many other teleosts (Guraya, 1986), was conspicuously lacking. Nuclear envelope remains more or less regular except occasional formation of contact with nucleoli or bulging due to the presence of lipid bodies adjacent to it.

Balbiani's vitelline body is a predominant 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 organelles. (Clerot, 1976; Hogan, 1978; Bruslé, 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 (Upadhya, 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 alveoli 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 phenomenon 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 multivascular 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 *Oryxias 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 ultrastructure 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, 1983; 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. Steroid 17α, 20β dihydroxyprogesterone is considered to be the principle physiological maturation inducer in teleost oocytes (Fostier et al., 1973, Jalabert and Finet, 1986; Nagahama and Adachi, 1983; 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 agrees well with the previous reports (Kowtal, 1976; Roy et al., 1977; Moore, 1982). Concomitant 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 occurs 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 I). 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 running 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 mordax_ (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, 1983). Sequence of events through which the oocytes are degenerated and resorbed is similar to that described in other teleosts (Saigapur, 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 lipofuscin (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 phenomenon 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 up to 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 up to 2.31 percent (stage 6). Cholesterol 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). Cholesterol 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*.

<table>
<thead>
<tr>
<th>Maturity stages</th>
<th>Moisture contents (%)</th>
<th>Total proteins (%)</th>
<th>Total lipids (%)</th>
<th>Total cholesterol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68.31 ± 3.5</td>
<td>20.67 ± 2.5</td>
<td>3.21 ± 0.5</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>68.47 ± 2.8</td>
<td>22.43 ± 2.0</td>
<td>3.40 ± 0.3</td>
<td>0.43 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>69.63 ± 3.7</td>
<td>20.30 ± 2.3</td>
<td>3.14 ± 0.4</td>
<td>0.41 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>71.47 ± 2.9</td>
<td>19.18 ± 2.8</td>
<td>2.86 ± 0.5</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>73.16 ± 4.0</td>
<td>18.67 ± 2.0</td>
<td>2.82 ± 0.3</td>
<td>0.35 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>74.68 ± 3.1</td>
<td>20.13 ± 1.9</td>
<td>2.31 ± 0.2</td>
<td>0.31 ± 0.05</td>
</tr>
</tbody>
</table>
Table 7 Variation in biochemical parameters (Mean ± SD) in liver tissue at different stages of maturity of male *L. calcarifer*

<table>
<thead>
<tr>
<th>Maturity stages</th>
<th>Moisture contents (%)</th>
<th>Total proteins (%)</th>
<th>Total lipids (%)</th>
<th>Total cholesterol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>61.42 ± 2.8</td>
<td>14.30 ± 1.3</td>
<td>8.60 ± 0.2</td>
<td>2.12 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>62.67 ± 3.1</td>
<td>15.36 ± 1.4</td>
<td>10.23 ± 0.16</td>
<td>1.86 ± 0.01</td>
</tr>
<tr>
<td>3</td>
<td>64.18 ± 4.0</td>
<td>14.14 ± 1.2</td>
<td>9.10 ± 0.12</td>
<td>1.72 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>67.61 ± 4.1</td>
<td>13.36 ± 1.3</td>
<td>8.36 ± 0.2</td>
<td>1.56 ± 0.02</td>
</tr>
<tr>
<td>5</td>
<td>68.15 ± 4.2</td>
<td>13.15 ± 1.6</td>
<td>8.17 ± 1.8</td>
<td>1.51 ± 0.01</td>
</tr>
<tr>
<td>6</td>
<td>68.76 ± 4.1</td>
<td>14.27 ± 1.5</td>
<td>7.84 ± 1.3</td>
<td>1.70 ± 0.01</td>
</tr>
</tbody>
</table>
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 cholesterol 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 do 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_.

<table>
<thead>
<tr>
<th>Maturity</th>
<th>Total protein (g/100ml)</th>
<th>Total lipid (g/100ml)</th>
<th>Total cholesterol (g/100ml)</th>
<th>Protein bound phosphorous (μg/ml)</th>
<th>Total calcium (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.14 ± 0.41</td>
<td>0.85 ± 0.05</td>
<td>0.30 ± 0.02</td>
<td>4.8 ± 0.6</td>
<td>78.15 ± 2.8</td>
</tr>
<tr>
<td>2</td>
<td>7.46 ± 0.61</td>
<td>0.90 ± 0.08</td>
<td>0.34 ± 0.01</td>
<td>4.78 ± 0.8</td>
<td>78.20 ± 3.5</td>
</tr>
<tr>
<td>3</td>
<td>8.24 ± 0.33</td>
<td>1.09 ± 0.07</td>
<td>0.46 ± 0.03</td>
<td>4.77 ± 0.6</td>
<td>77.58 ± 4.7</td>
</tr>
<tr>
<td>4</td>
<td>9.83 ± 0.63</td>
<td>1.24 ± 0.04</td>
<td>0.51 ± 0.02</td>
<td>4.81 ± 0.7</td>
<td>78.18 ± 6.2</td>
</tr>
<tr>
<td>5</td>
<td>9.06 ± 0.80</td>
<td>1.07 ± 0.06</td>
<td>0.39 ± 0.03</td>
<td>3.78 ± 0.03</td>
<td>78.21 ± 5.1</td>
</tr>
<tr>
<td>6</td>
<td>7.42 ± 0.71</td>
<td>0.91 ± 0.02</td>
<td>0.28 ± 0.01</td>
<td>4.80 ± 0.5</td>
<td>78.16 ± 3.9</td>
</tr>
</tbody>
</table>

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*

<table>
<thead>
<tr>
<th>Maturity stages</th>
<th>Moisture contents (%)</th>
<th>Total proteins (%)</th>
<th>Total lipids (%)</th>
<th>Total cholesterol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76.37 ± 2.80</td>
<td>13.41 ± 1.40</td>
<td>1.43 ± 0.41</td>
<td>0.40 ± 0.06</td>
</tr>
<tr>
<td>2</td>
<td>74.21 ± 3.19</td>
<td>14.84 ± 1.20</td>
<td>1.72 ± 0.83</td>
<td>0.51 ± 0.08</td>
</tr>
<tr>
<td>3</td>
<td>73.30 ± 4.20</td>
<td>17.68 ± 1.40</td>
<td>2.48 ± 0.61</td>
<td>0.38 ± 0.07</td>
</tr>
<tr>
<td>4</td>
<td>72.81 ± 3.80</td>
<td>21.68 ± 1.14</td>
<td>3.61 ± 0.73</td>
<td>0.26 ± 0.04</td>
</tr>
<tr>
<td>5</td>
<td>77.83 ± 4.10</td>
<td>22.36 ± 1.20</td>
<td>3.57 ± 0.54</td>
<td>0.25 ± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>79.54 ± 3.70</td>
<td>13.86 ± 1.50</td>
<td>1.23 ± 0.38</td>
<td>0.19 ± 0.06</td>
</tr>
</tbody>
</table>
Table 10 Result of the analysis of variance of biochemical parameters between tissues and maturity stages in male *L. calcarifer*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Source of variation</th>
<th>Calculated F values</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>Between tissues</td>
<td>109.183</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Between stages</td>
<td>14.625</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>8.228</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Protein</td>
<td>Between tissues</td>
<td>5.418</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Between stages</td>
<td>1.068</td>
<td>NS*</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>16.802</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Lipid</td>
<td>Between tissues</td>
<td>21.461</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td></td>
<td>Between stages</td>
<td>1.348</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>63.721</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Cholestrol</td>
<td>Between tissues</td>
<td>168.921</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Between stages</td>
<td>1.760</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>14.191</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* 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 separately 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). Cholesterol 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.

Cholesterol 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 cholesterol 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*.

<table>
<thead>
<tr>
<th>Maturity</th>
<th>Moisture contents (%)</th>
<th>Total proteins (%)</th>
<th>Total lipids (%)</th>
<th>Total cholesterol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>66.25 ± 2.5</td>
<td>21.6 ± 2.5</td>
<td>3.50 ± 0.3</td>
<td>0.38 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>66.63 ± 2.5</td>
<td>24.8 ± 2.3</td>
<td>4.20 ± 0.34</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>69.5 ± 1.5</td>
<td>19.1 ± 2.5</td>
<td>3.30 ± 0.3</td>
<td>0.38 ± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>74.0 ± 2.5</td>
<td>17.5 ± 2.0</td>
<td>2.60 ± 0.2</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>5</td>
<td>76.5 ± 1.5</td>
<td>16.7 ± 1.5</td>
<td>2.76 ± 0.06</td>
<td>0.33 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>77.0 ± 2.0</td>
<td>18.4 ± 2.5</td>
<td>2.25 ± 0.3</td>
<td>0.30 ± 0.01</td>
</tr>
</tbody>
</table>
Table. 12 Variations in biochemical parameters (Mean ± S.D) in liver tissue at different stages of maturity of female *L. calcarifer*

<table>
<thead>
<tr>
<th>Maturity Stages</th>
<th>Moisture contents (%)</th>
<th>Total proteins (%)</th>
<th>Total lipids (%)</th>
<th>Total cholestrol (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64.61 ± 2.7</td>
<td>16.4 ± 1.2</td>
<td>8.8 ± 0.11</td>
<td>2.41 ± 0.001</td>
</tr>
<tr>
<td>2</td>
<td>65.31 ± 3.0</td>
<td>18.6 ± 1.2</td>
<td>10.10 ± 0.09</td>
<td>2.68 ± 0.02</td>
</tr>
<tr>
<td>3</td>
<td>69.64 ± 4.1</td>
<td>14.4 ± 1.3</td>
<td>8.2 ± 0.008</td>
<td>2.20 ± 0.01</td>
</tr>
<tr>
<td>4</td>
<td>72.40 ± 4.00</td>
<td>11.8 ± 1.1</td>
<td>6.8 ± 0.12</td>
<td>1.80 ± 0.01</td>
</tr>
<tr>
<td>5</td>
<td>72.00 ± 4.1</td>
<td>13.4 ± 0.8</td>
<td>5.9 ± 0.11</td>
<td>2.10 ± 0.02</td>
</tr>
<tr>
<td>6</td>
<td>71.76 ± 4.1</td>
<td>14.2 ± 1.2</td>
<td>5.1 ± 0.10</td>
<td>2.00 ± 0.03</td>
</tr>
</tbody>
</table>
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 to 1.71 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 cholesterol 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 parameter 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*.

<table>
<thead>
<tr>
<th>Maturity</th>
<th>Total protein (g/100ml)</th>
<th>Total lipids (g/100ml)</th>
<th>Total cholesterol (g/100ml)</th>
<th>Protein Bound phosphorous (μg/ml)</th>
<th>Total calcium (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>9.24±0.30</td>
<td>0.98±0.035</td>
<td>0.39±0.035</td>
<td>5.2±0.8</td>
<td>74.00±4.6</td>
</tr>
<tr>
<td>2</td>
<td>6.79±0.41</td>
<td>0.36±0.05</td>
<td>0.30±0.05</td>
<td>8.6±1.2</td>
<td>92.00±5.3</td>
</tr>
<tr>
<td>3</td>
<td>9.85±0.75</td>
<td>1.60±0.04</td>
<td>0.52±0.04</td>
<td>32.4±3.6</td>
<td>161 ±7.8</td>
</tr>
<tr>
<td>4</td>
<td>11.01±0.9</td>
<td>1.71±0.04</td>
<td>0.56±0.04</td>
<td>23.3±7.6</td>
<td>218 ±6.7</td>
</tr>
<tr>
<td>5</td>
<td>9.25±0.9</td>
<td>1.56±0.09</td>
<td>0.43±0.05</td>
<td>28.8±2.1</td>
<td>148 ±4.9</td>
</tr>
<tr>
<td>6</td>
<td>7.18±0.75</td>
<td>1.12±0.02</td>
<td>0.25±0.03</td>
<td>6.7±1.6</td>
<td>107 ±4.2</td>
</tr>
</tbody>
</table>

**Calculated F. Value**

<table>
<thead>
<tr>
<th>F. Value</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>48.68</td>
<td>240.61</td>
<td>80.67</td>
<td>170.63</td>
<td>83.14</td>
</tr>
</tbody>
</table>

**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*.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Source of variation</th>
<th>Calculated F. values</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>Between Tissues</td>
<td>5.85</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Between Stages</td>
<td>1.03</td>
<td>N S</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>70.23</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Protein</td>
<td>Between Tissues</td>
<td>6.43</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>Between Stages</td>
<td>3.71</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>135.46</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Lipids</td>
<td>Between Tissues</td>
<td>9.13</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Between Stages</td>
<td>4.68</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>163.71</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>Cholestrol</td>
<td>Between Tissues</td>
<td>6.71</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td></td>
<td>Between Stages</td>
<td>0.641</td>
<td>N S</td>
</tr>
<tr>
<td></td>
<td>Interaction</td>
<td>32.15</td>
<td>P &lt; 0.001</td>
</tr>
</tbody>
</table>

* N S - Not Significant.

Degrees of freedom (df)  
- tissues = 2  
- stages = 5  
- interaction = 10
Table 1: Details of protein fractions, their relative fraction (Rf) values and staining characteristics in the blood serum of female L. terrestris during different stages of maturity.

<table>
<thead>
<tr>
<th>Stage</th>
<th>MATURITY STAGES</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STAGE II</td>
</tr>
<tr>
<td></td>
<td>Band Ab.</td>
</tr>
<tr>
<td>Zone I</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone II</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Zone III</td>
<td></td>
</tr>
<tr>
<td>Zone IV</td>
<td></td>
</tr>
<tr>
<td>Zone V</td>
<td></td>
</tr>
<tr>
<td>Zone VI</td>
<td></td>
</tr>
</tbody>
</table>

X Narrow and Sharp bands
XX Broad and diffuse bands
XXX 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. Comparison of serum protein profiles 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. Vitellogenin 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 glyco phosphoprotein (Wallace, 1978). Protein bound phosphate contents vary in the range of 3.04 to 52.0 μg/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 Bohemen 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 - 17β, 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 (Rouchereau-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 embryogenesis after its sequestration into oocyte (Björnsson et al., 1989). Apart from association of calcium with vitellogenin, it has been found to have regulatory function in steroidogenesis in prevoulsatory follicles (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. Misgurunus 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 cholesterol, to produce pregnanolone, precursor for both androgen and estrogen (Nagahama, 1987) has been suggested to be gonadotropin dependent (Jayashree and Srinivasaschar, 1979; Sen and Bhattacharya, 1981; Deb et al. 1985; Petrino et al., 1989).

Testicular cholesterol dynamics in *L. calcarifer* depict a conspicuous decline during the stages of active spermatogenesis. Parallel to this, ovarian cholesterol level decreases during the gonadotropin dependent phase (vitellogenic phase) of the oocyte growth. This pattern of changes probably reflect consumption of cholesterol 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 cholesterol level in the testes, packed with mature spermatozoa and ovaries with fully grown oocytes probably point out relatively low steroidogenic activity. Subsequent fall in
cholesterol contents in spent gonads reiterate the utilization of the metabolite for steroid synthesis resulting in maturation ovulation/spermiation. These interpretations are supported by the serum cholesterol 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 cholesterol 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 cholesterol levels in *Cyprinus carpio* (Guha and Mukherjee, 1987). It appear quite probable that the high serum cholesterol 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 physiological 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 μm to 700 μm). 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).