Studies on certain aspects of the reproductive biology of mouth-brooding tilapia, *Oreochromis mossambicus* (Peters) from Assam, India

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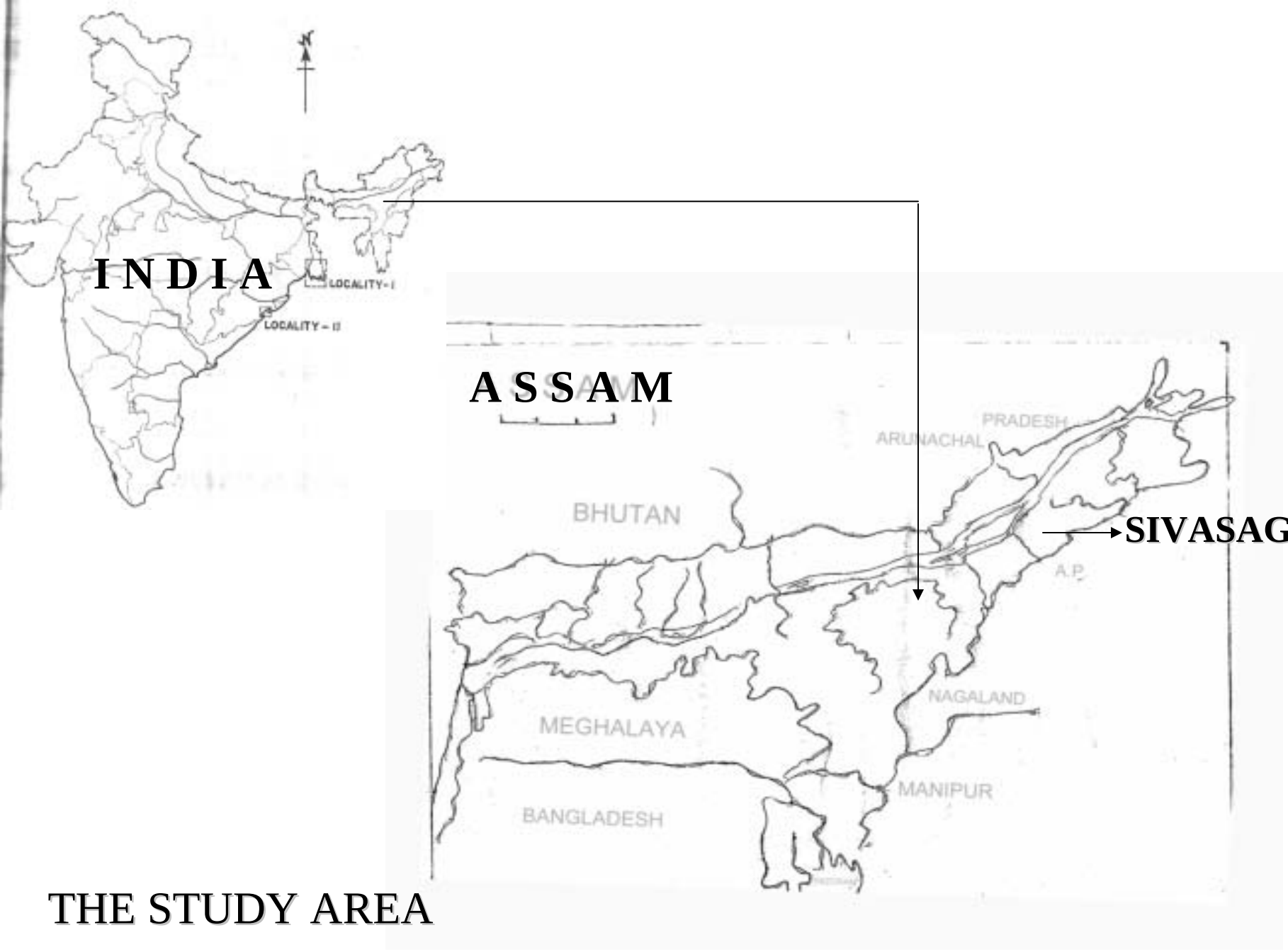

THE STUDY AREA

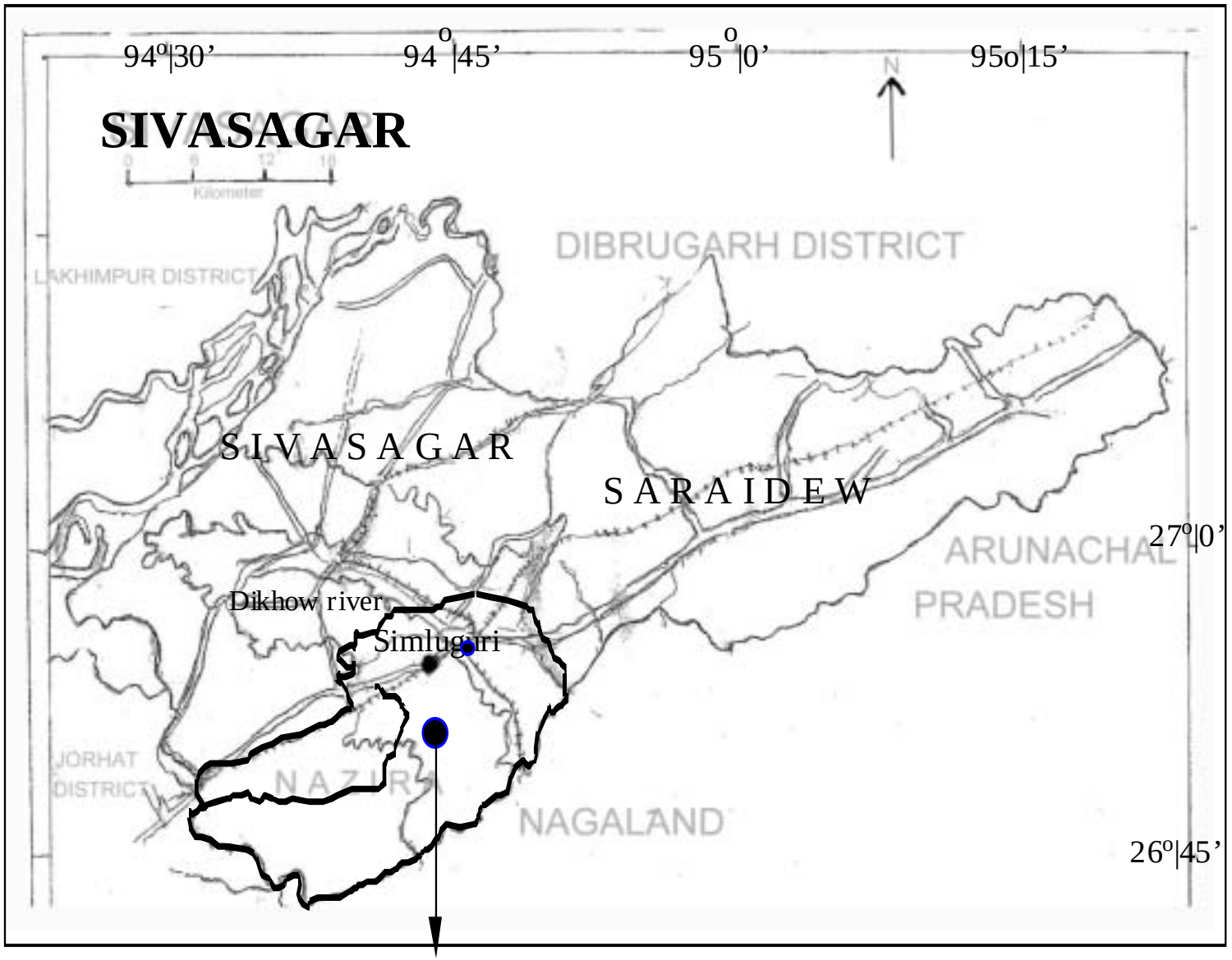

Fig. 1. Location map of the study area

FIG p/3a. *O.mossambicus* Mature male

Caudal fin (light pink)

FIG p/3b. *O.mossambicus* Mature Female

Fig. p / 1. Photograph of the Pond in WINTER

Fig. p / 1. Photograph of the Pond in MONSOON

The experimental pond located at Nazira in Sivasagar District of Upper Assam, India.

Latitude 26°54’36” N and Longitude 94°43’54” E at an altitude of 94 m above msl.

The experiment was carried out for three years (1996 - 1999).

The specimen of *Oreochromis mossambicus* (Peters) for the present study had been collected on a monthly basis from a small perennial domestic pond of Nazira Town of Sivasagar district in Upper Assam. Water of the pond was analyzed on a monthly basis. The pond bottom soils samples and the pond biota including fauna, flora and plankton had been recorded seasonally. The four season had been recorded according to climatic condition of Assam as winter (December – February); pre-monsoon (March – May); Monsoon (June - September) and autumn (October – November).

Morphometric features of the pond

Geographic location	
Latitude	26°54’36”N
Longitude	94°43’54”E
Altitude	94 (approximate) meters above mean sea level.
Type	Old perennial
Shape	Square
Area (m^2)	91.54
Water Colour	Slightly transparent, slightly greenish from pre-monsoon to monsoon and light brown from autumn to winter.
Pond	Muddy bottom mostly covered with decayed organic matter.
Average maximum depth (m)	4.98
Average minimum depth (m)	1.98
Utility	Used for drinking and washing purpose

Methods used :

Seasonal progression of gonads recorded by physical examination of testes and ovary

- Assessment of various maturity stages - modified classification of Kesteven (1960) and Crossland (1977).
- The GSR or Co-efficient of maturity -Hopkins (1979).
- Maturity stage of entire population - length maturity key (Kesteven,1960).
- The attainment of sexual maturity (M_{50}) - (Hopkins and Mann, 1978)

- Spawning periodicity - progressive change in the intra-ovarian ova diameter for a period of three years.
- The absolute and relative fecundity - Bagenal (1967) and Hardisty (1964).
- Histological preparation of gonads - Histological techniques (Patki et.al.,1989 and Lal, 2001).

RESULTS AND DISCUSSION

Table 1. Degree of maturation and the morphology of the gonad in different stages of maturity of *O.mossambicus*

Stage	Degree of maturation	Months of availability	Ova diameter (mm)	Description of the gonads
I	Immature or virgin and resting adult	Throughout the year	0.045 – 0.055	Ovaries very small, thin, thread like pale in colour, occupying a small part of the body cavity. Testes is thin, slender translucent and pale in colour. Both the gonad invisible to the naked eye.
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MORPHOLOGICAL PICTURES

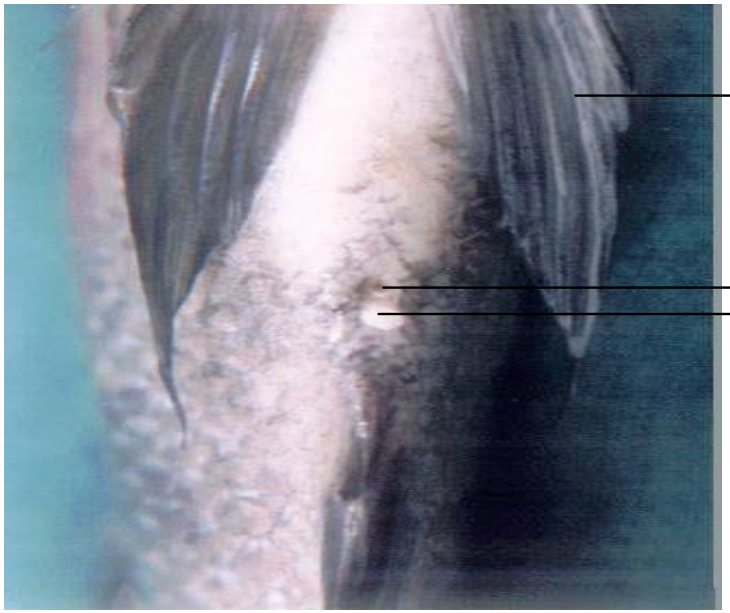


Fig p/4a. Genital papilla of mature male (TL 17 cm).

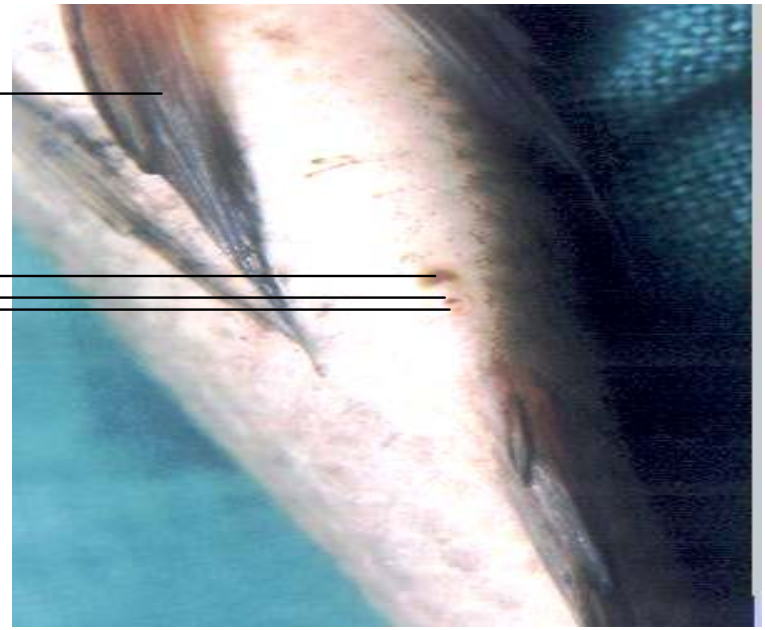


Fig p/4b. Genital Papilla of mature female (TL 21 cm).

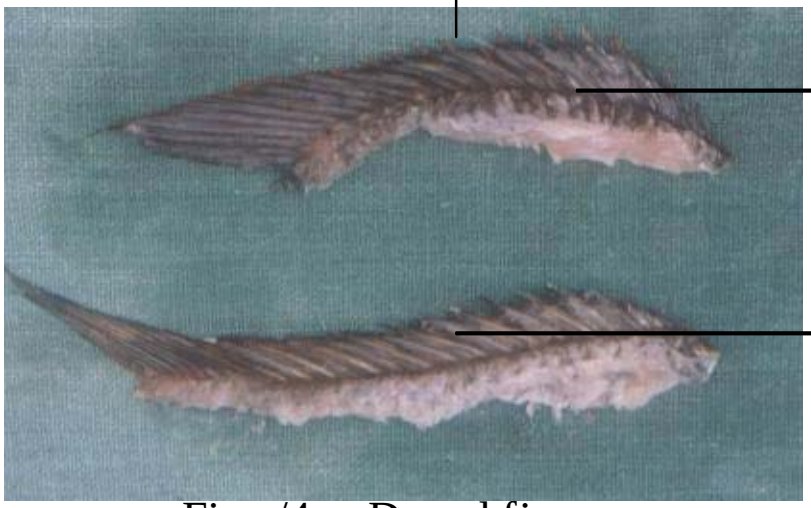


Fig.p/4c. Dorsal fin

Fig.p/4d. Pectoral fin

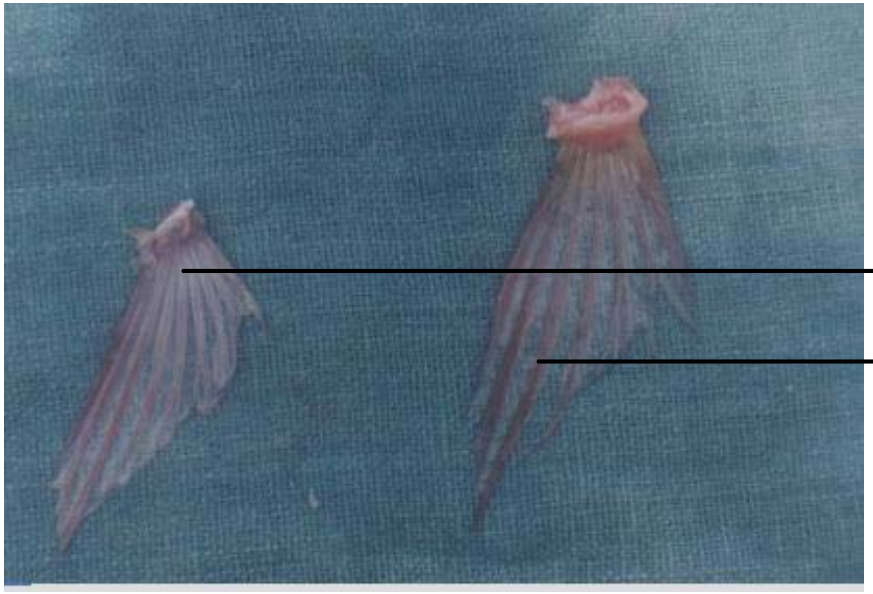


Fig. p/4e. Ventral fin

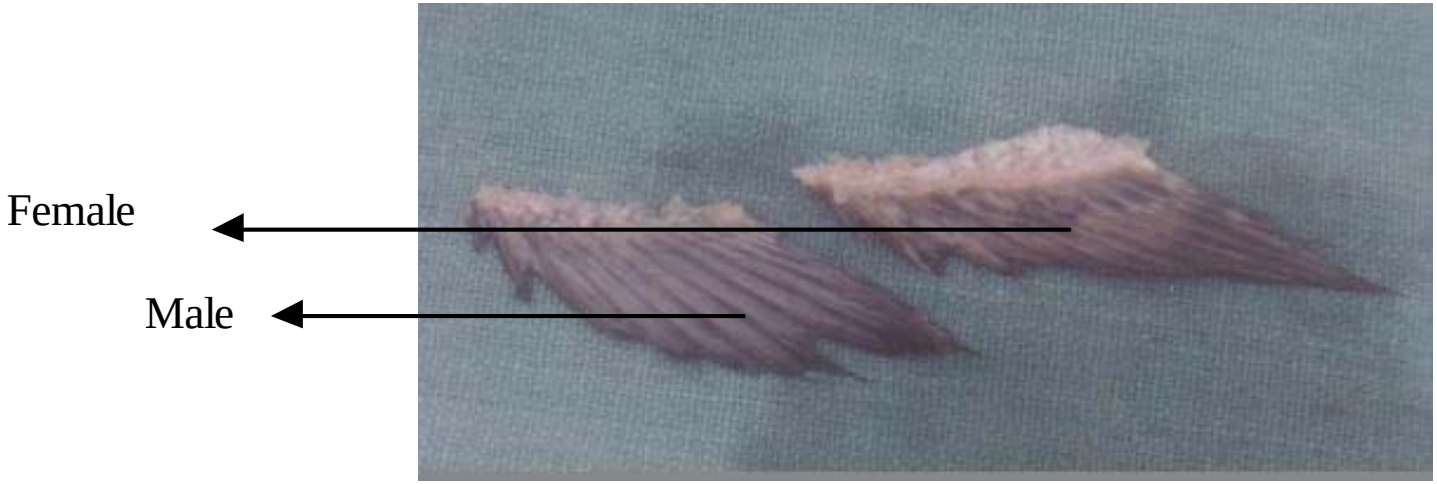


Fig p/4f. Anal fin

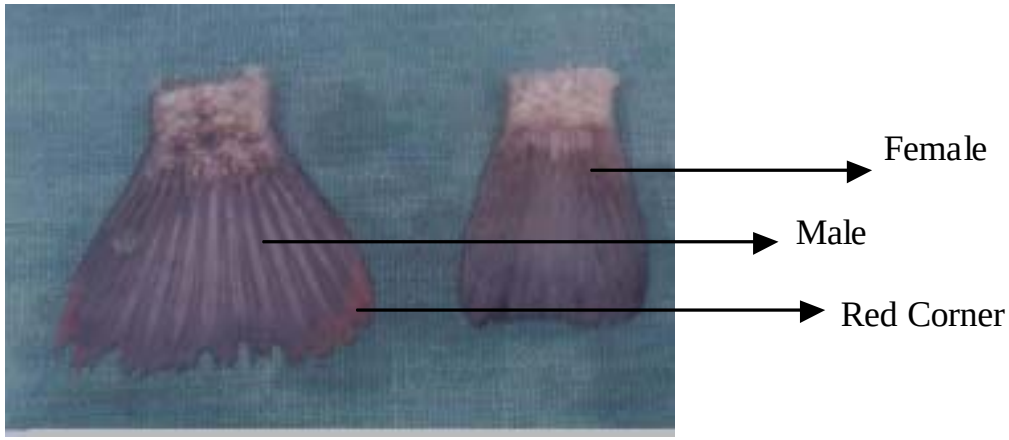


Fig. p/4g. Caudal fin

Fig.p/4h. Caudal fin with red corner & dorsal fin with red margin in the mature male.

Fig.p/10a. Testes Stage I Immature phase

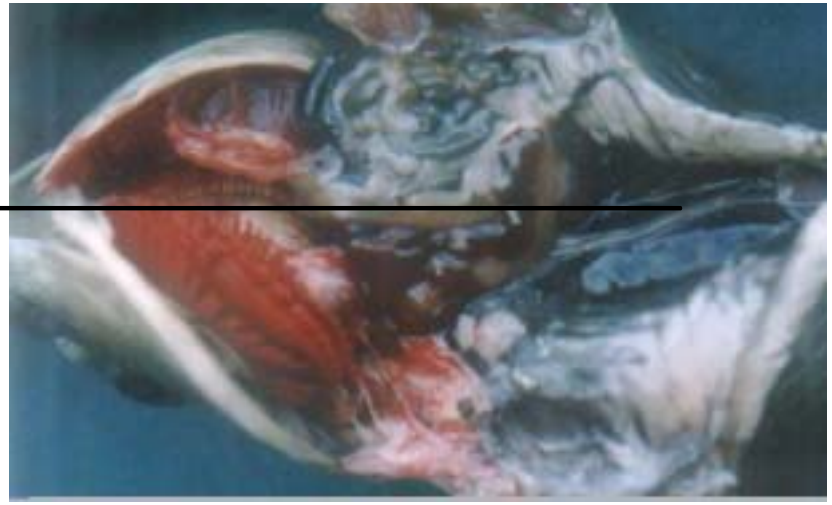


Fig.p/10b. Testes Stage II Early maturing phase

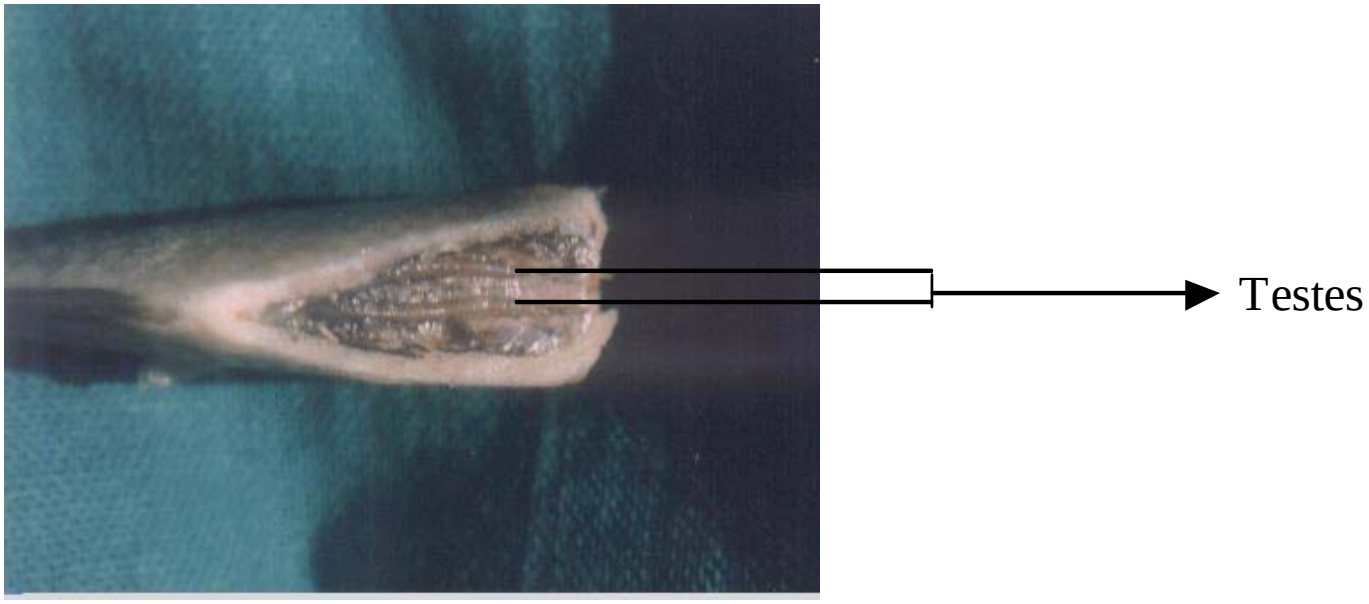


Fig. p/10c. Testes Stage III Advanced / late maturing phase

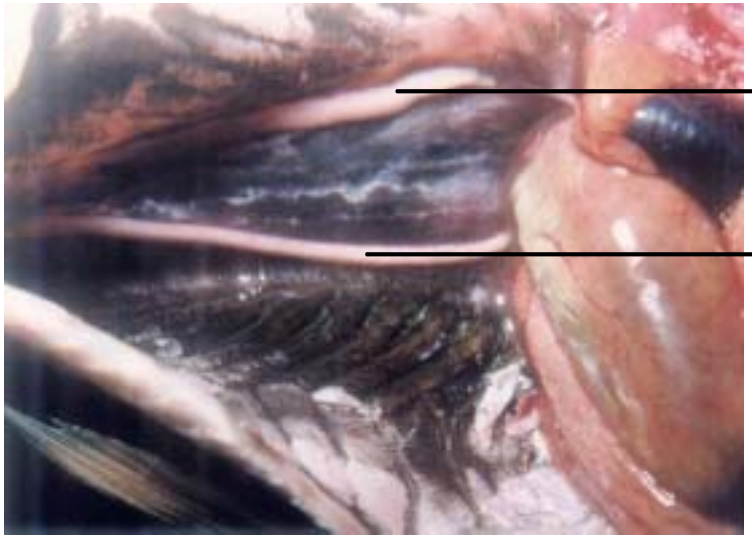


Fig. p/10d. Testes Stage IV pre Spawning phase

Fig. p/10e. Testes Stage V Spawning phase

Fig. p/10f. Testes Stage V Spawning phase

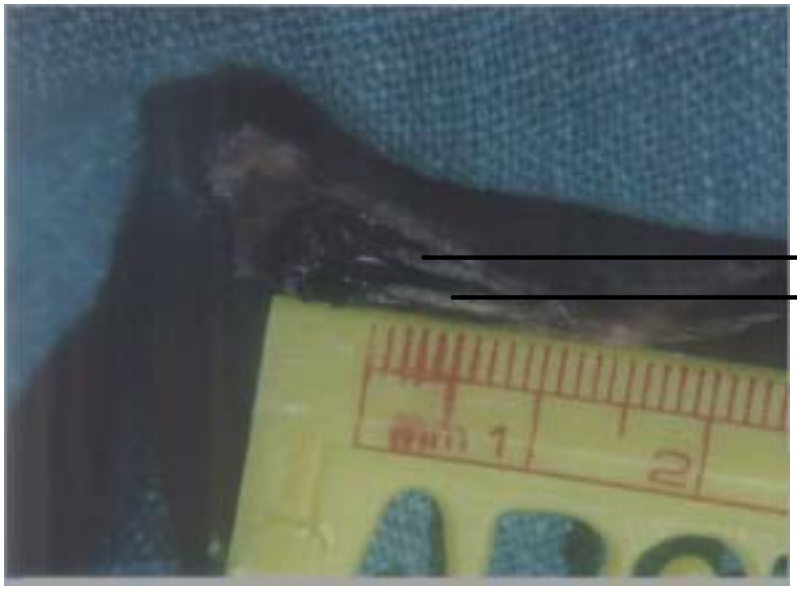


Fig.p/12a. Ovary – Stage II Early maturing phase

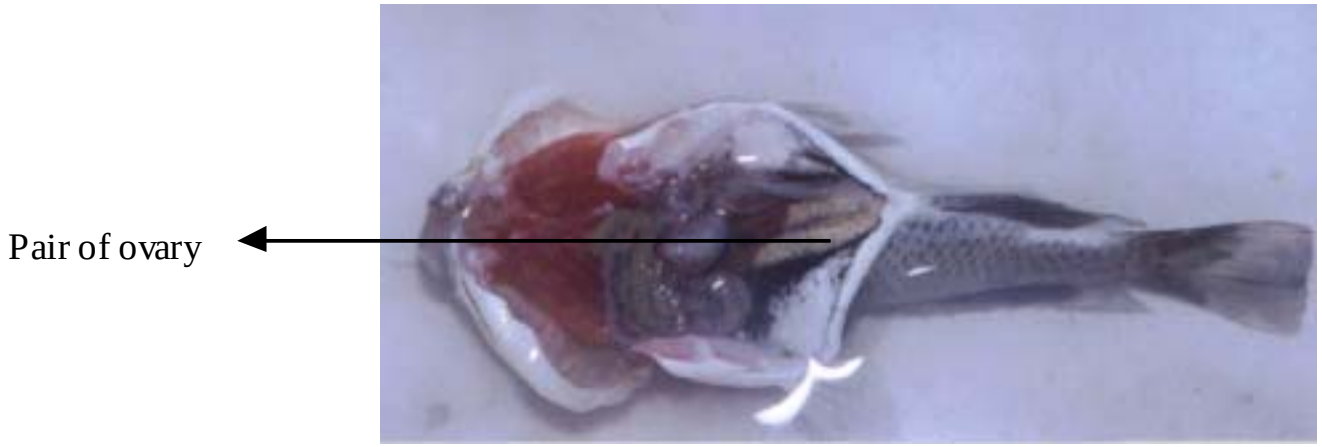


Fig. p/12b. Ovary – Stage III Advanced / Late Maturing Phase

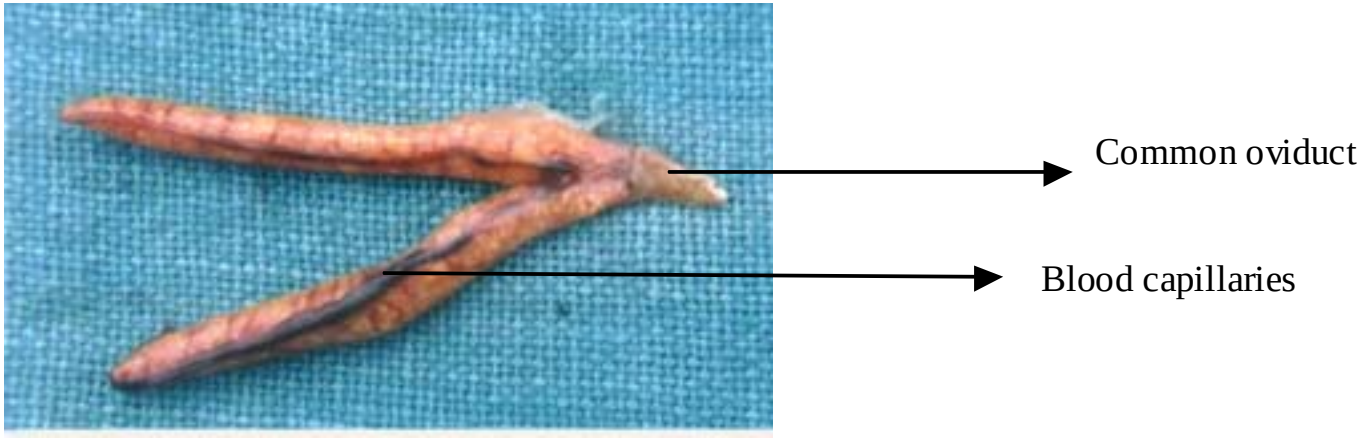


Fig. p/12c. Ovary – Stage IV Pre-spawning Phase

Blood capillary

Ripe eggs visible through the ovarian wall

Fig.p/12d. Ovary – Stage V Spawning Stage,
length 2.9 cm

Mature ovary occupied
most part of the abdominal cavity

Fig.p/12e. Ovary – Stage – V Spawning, Length 3.5 cm.

T E S T E S

Non-zonal, lobules loosely organised anterior middle and posterior parts of testes showed morpho-histological variations in different maturity stages. All the parts showed similar stages of development in a particular maturity stage

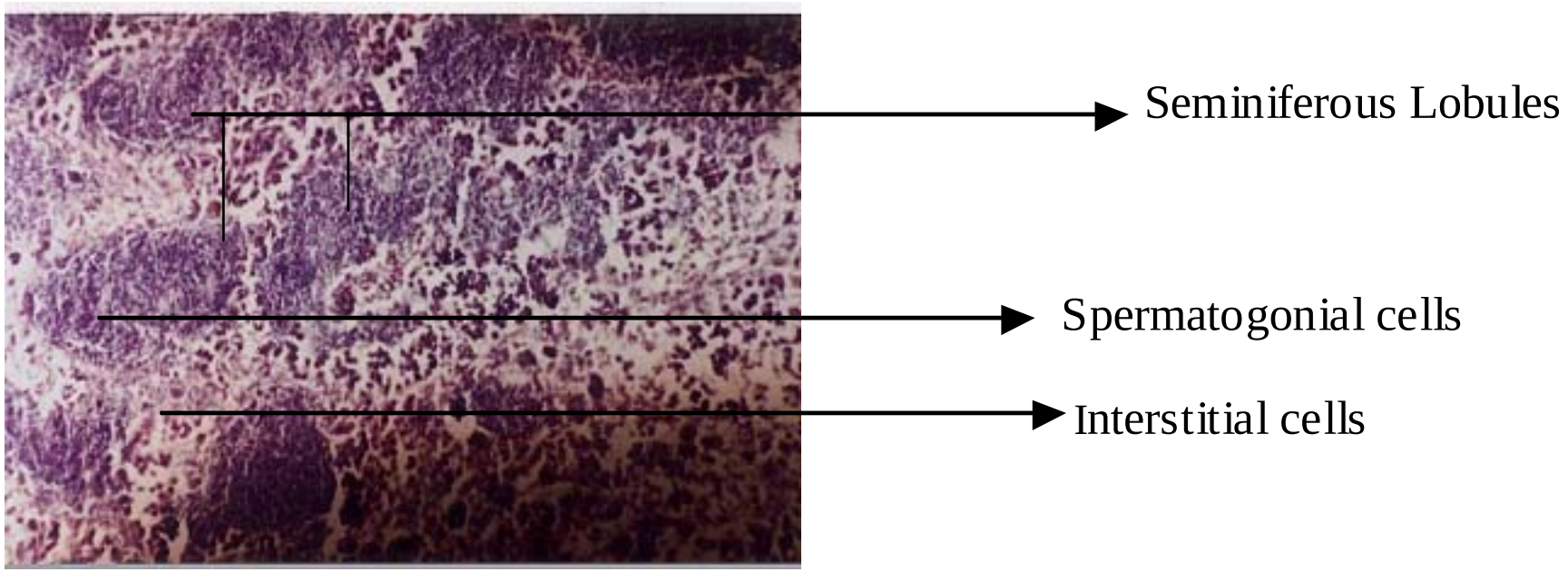


Fig.1a. Photomicrograph of T.S.of Testes.
Stage I immature phase (5x X 10)

IMMATURE : Numerous SPERMATOGONIA observed inside the small SEMINIFEROUS LOBULES. Spermatogonia are large spherical cells with a large round central nucleus with distinct nucleolus.

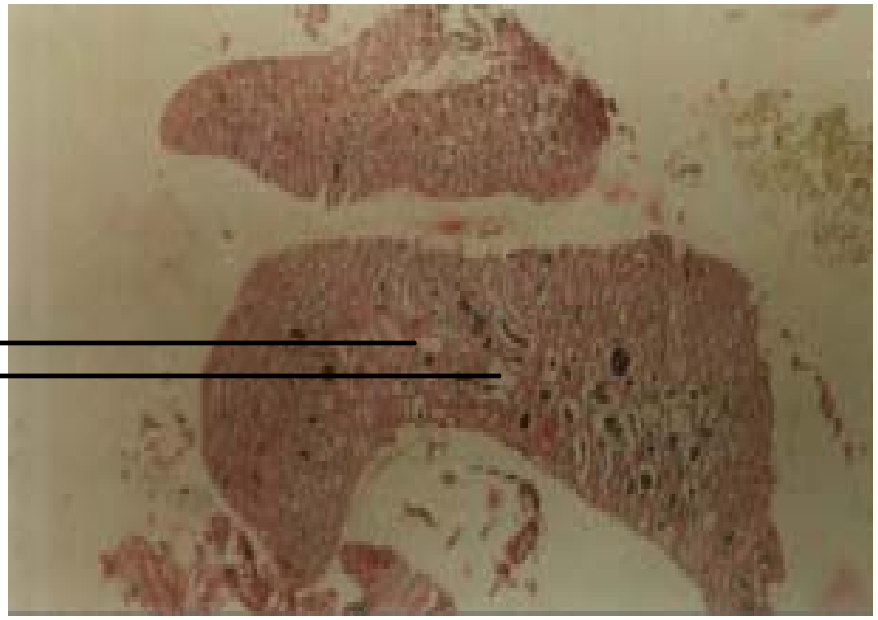


Fig.1b. Photomicrograph of Testis
Resting Stage (5x X 10)

RESTING : Numerous SPERMATOGONIA observed inside the small SEMINIFEROUS LOBULES. Spermatogonia are large spherical cells with a large round central nucleus with distinct nucleolus.

Sperm mother cells

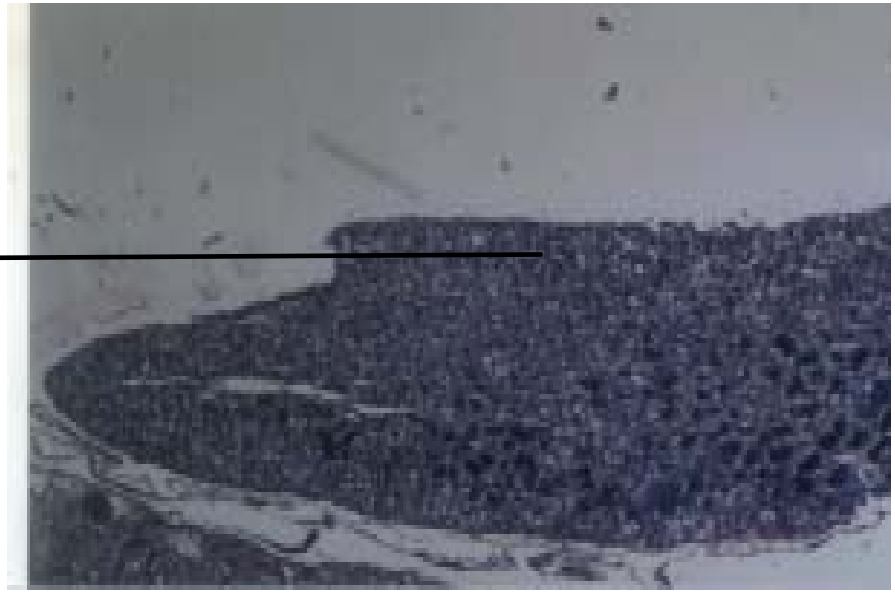


Fig. 1c. Photomicrograph of T.S.of Testes.
Stage II early maturing phase (5x X 10)

EARLY MATURING : SLOW MITOTIC ACTIVITY. Sparmatogonia start dividing and transformed into SPERM MOTHER CELLS.

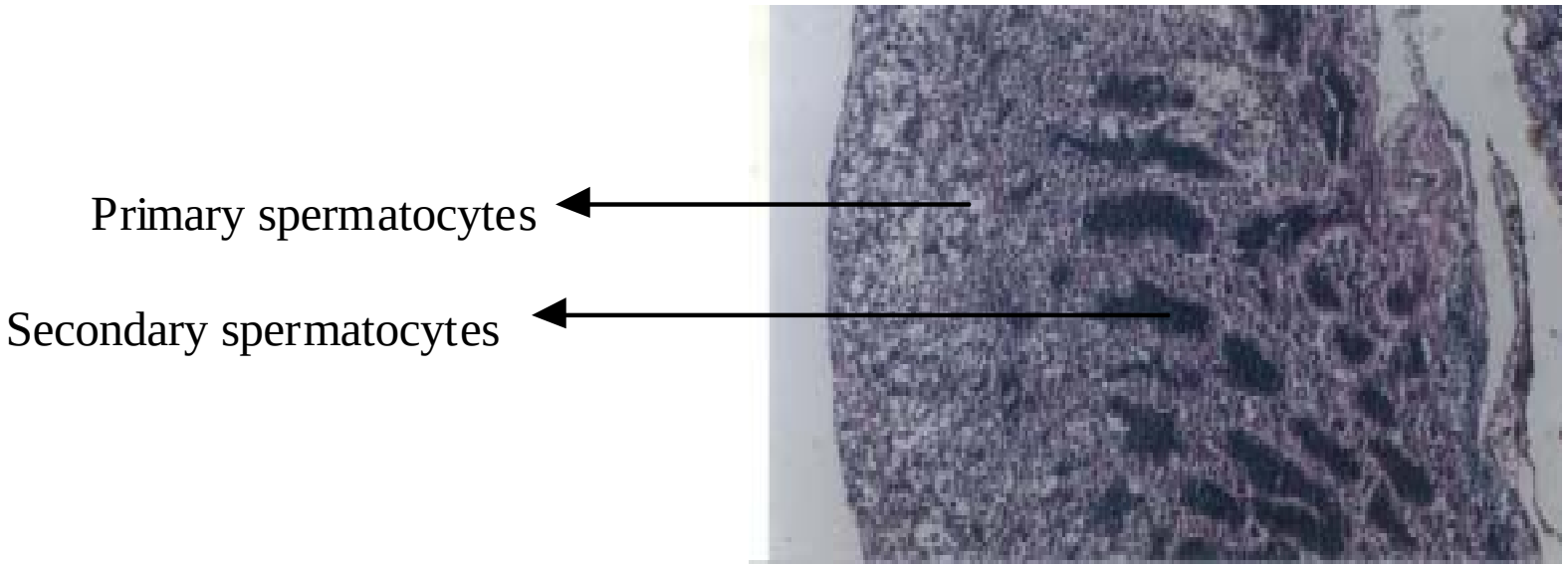


Fig.1d. Photomicrograph of T.S.of Testes
Stage III developing phase (10x X 10 middle part)

<u>DEVELOPING</u> :Intense SPERMATOGENESIS seen during later part of his phase.
SPERMATOGONIA DECRESES IN NUMBER.
Numerous PRIMARY AND SECONDARY SPERMATOCYTES visible.

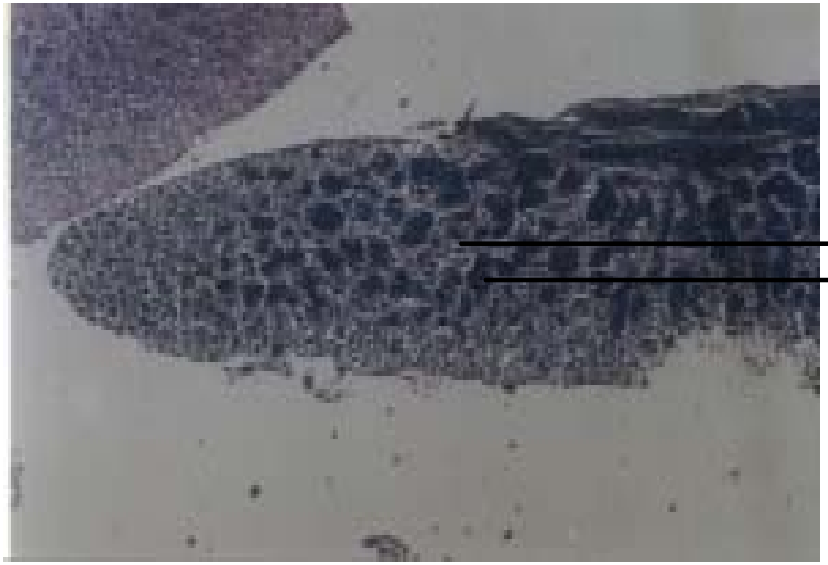


Fig. p/11c.Photomicrograph of T.S.of Testes.
(Stage III developing phase 5x X 10)

DEVELOPING : Primary spermatocytes are smaller than spermatogonia and posses a darkly stained nucleus. They give to secondary spermatocytes, which are still smaller than primary spermatocytes with clump chromatin material.

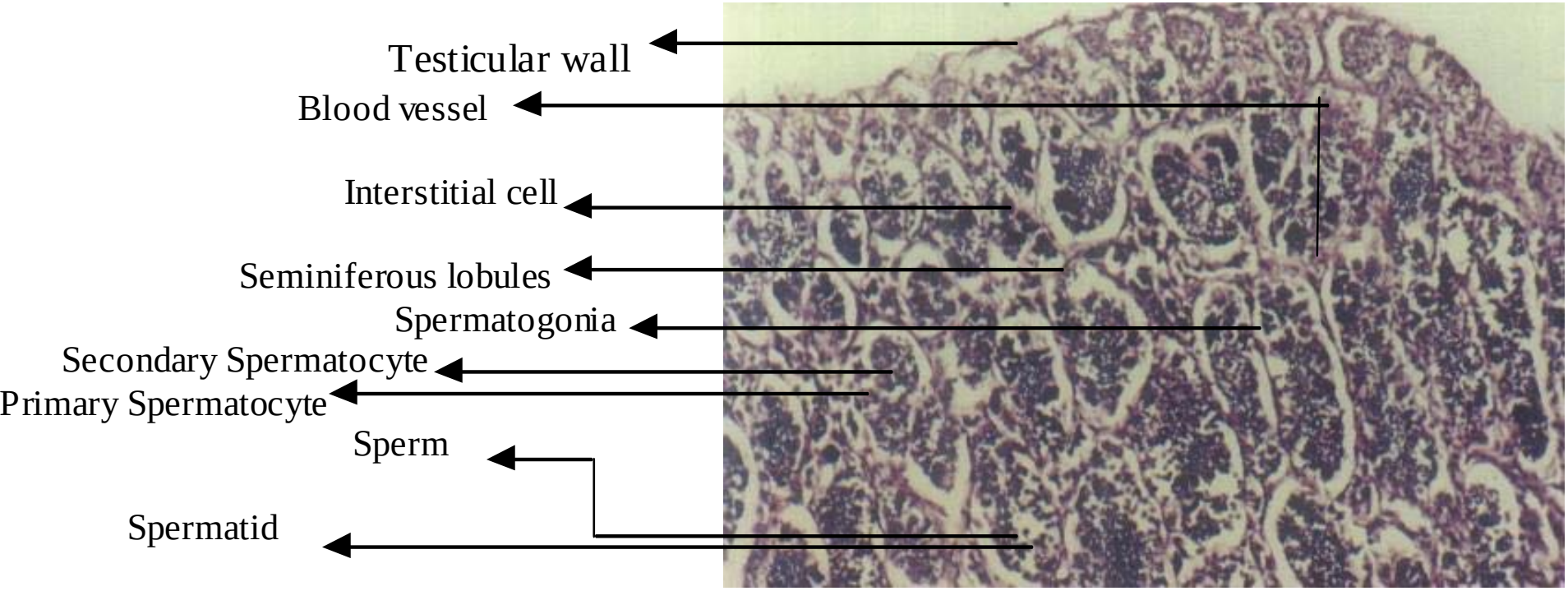


Fig.1e. Photomicrograph T.S. of Testes
Pre spawning phase Stage IV (10x X 10)

PRE-SPAWNING :Blood capillaries become conspecuous, seminiferous lobules are larger in size and full of sperm. Spermatogia are few. All stages of spermatogenesis seen in various lobules

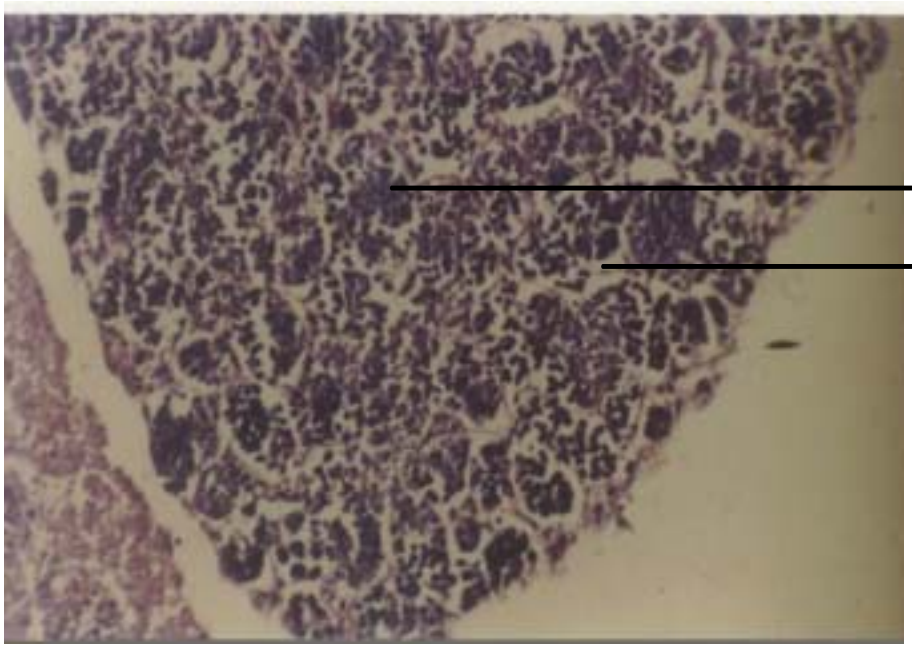


Fig.p/11e. Photomicrograph T.S. of Testes
(Interior part Stage IV pre spawning phase)

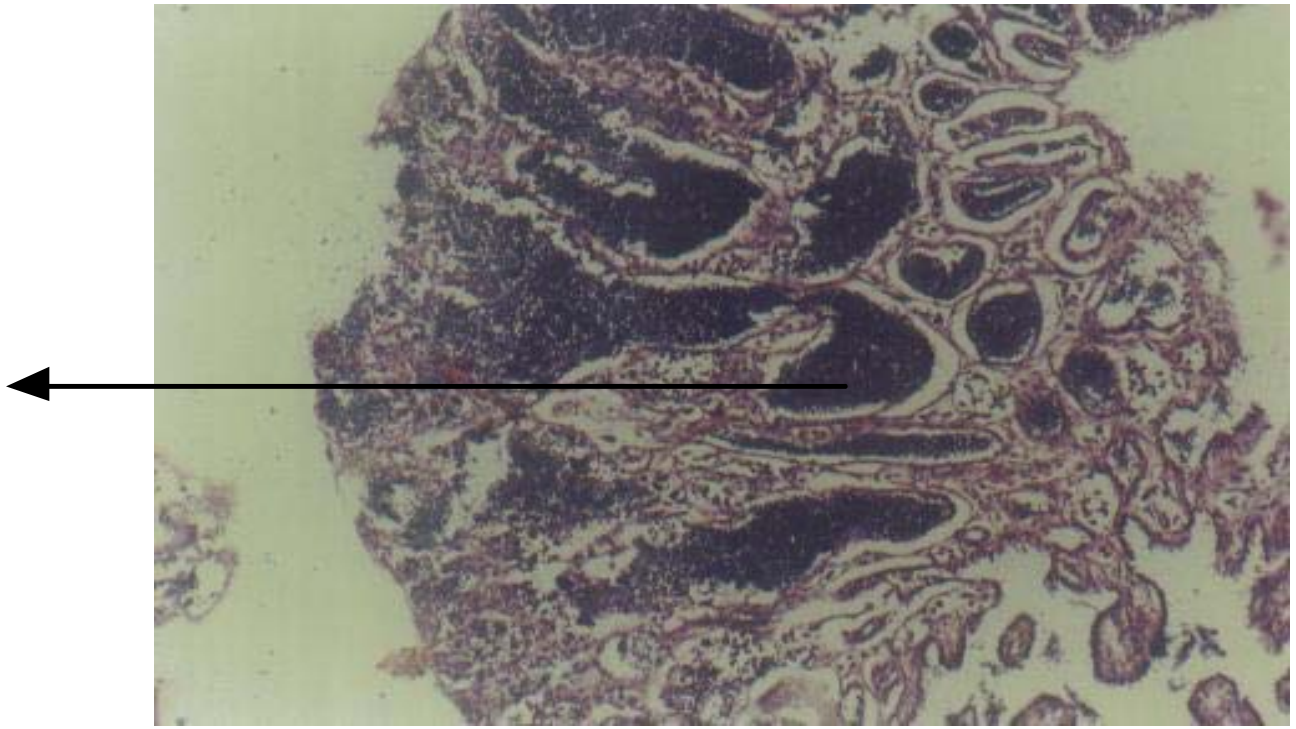


Fig.p/11h. Photomicrograph T.S.of Testes
Stage V spawning phase 10x X 10

<u>SPAWNING</u> :Smaller deeply stained SPERMATIDS with eliptical nucleus and slightly reduced SPERMS seen. Seminiferous lobules became empty because of release of sperms.

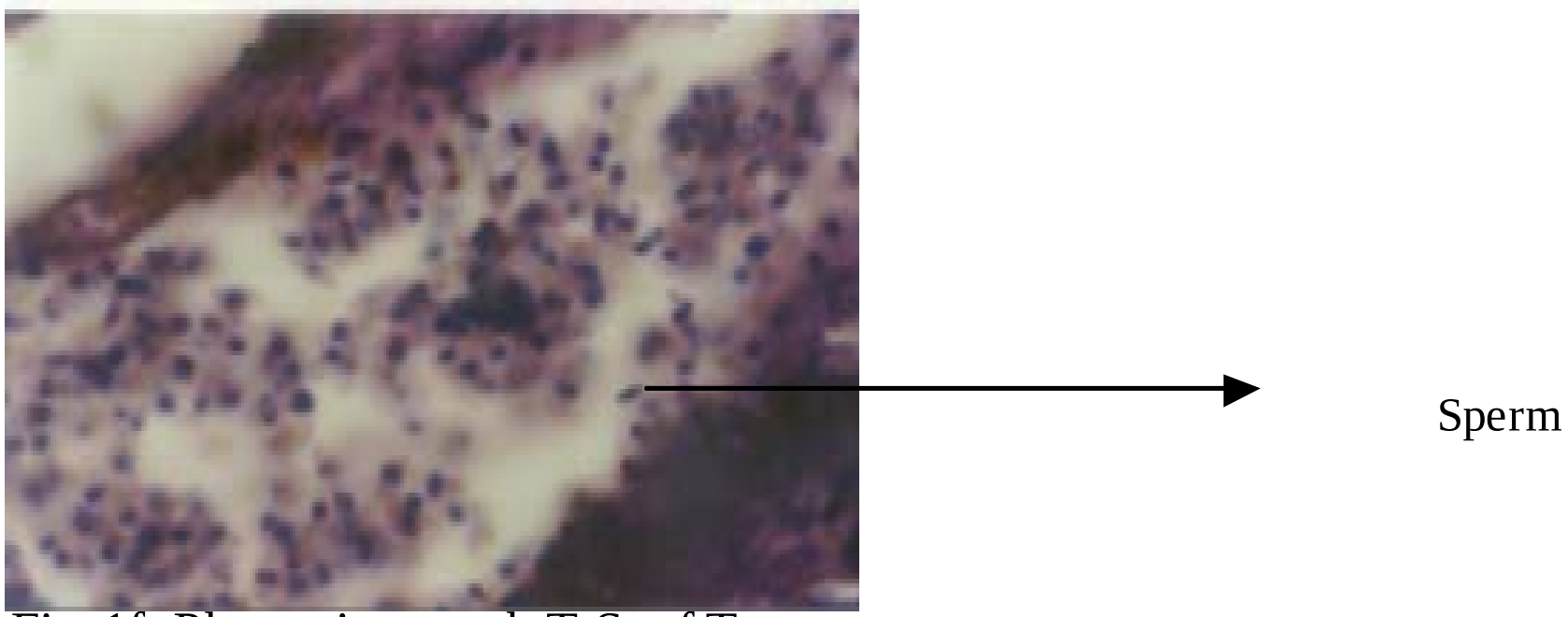


Fig. 1f. Photomicrograph T.S. of Testes
Stage V spawning phase (100x X 10)

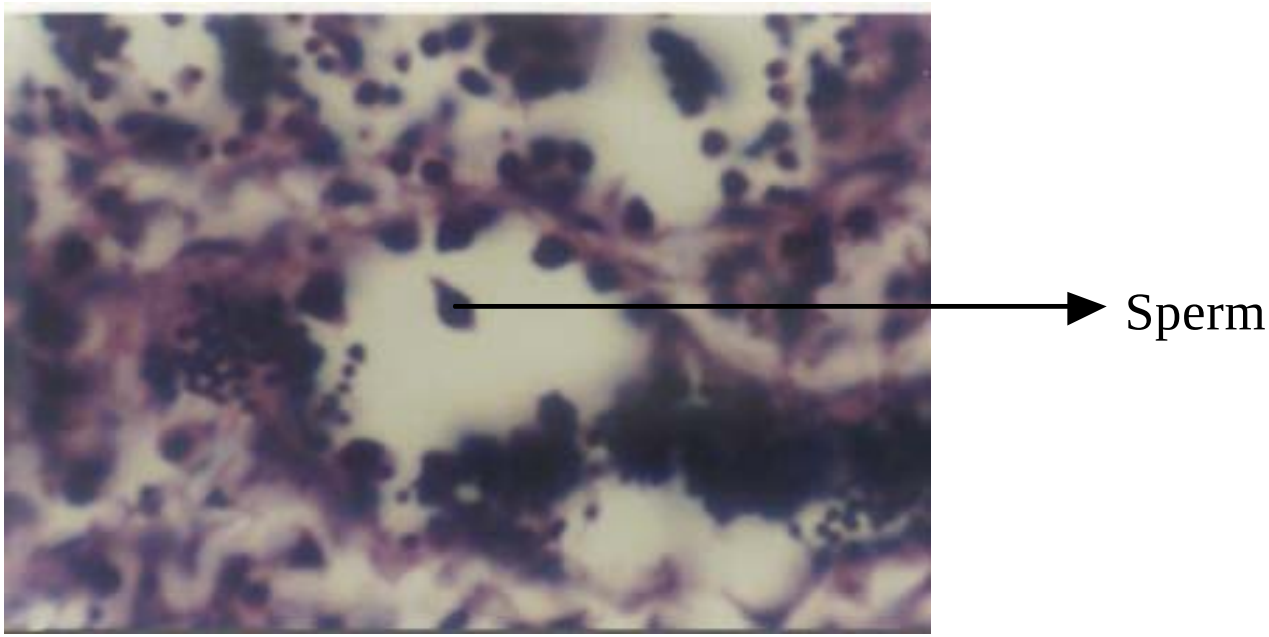


Fig. p/11g. Photomicrograph T.S. of Testes
Stage V spawning phase 10x X 10 (oil)

Collapsing seminiferous tubules
Residual sperm

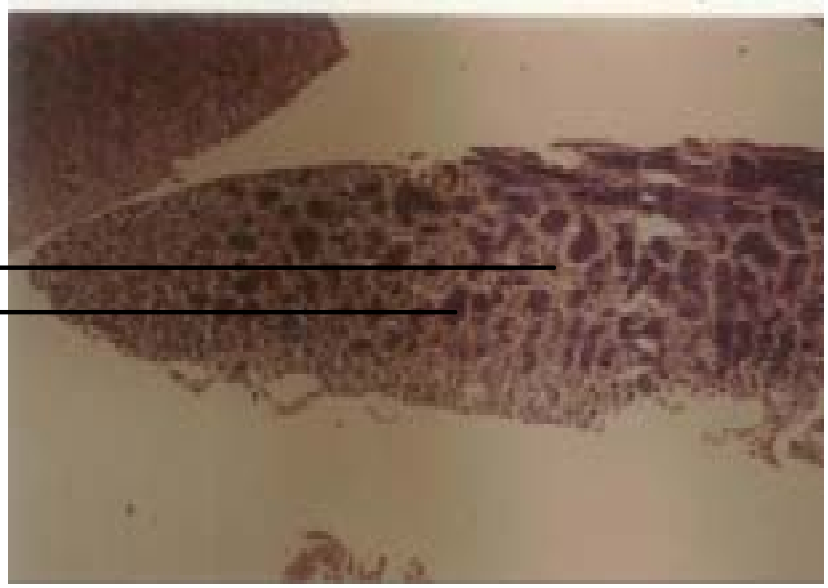


Fig.1g. Photomicrograph of T.S.of Testes (anterior part) Stage VI Spent phase (5x X 10)

SPENT : Empty & collapsing seminiferous lobules are seen, some of which contain residual or unexpelled sperm.

O V A R I E S

* Ovary wall is fairly thick during non-breeding season.

* Becomes thin and highly vascular during the spawning period.

* Ovarian lumen is loosely organised and zonation is not apparent.

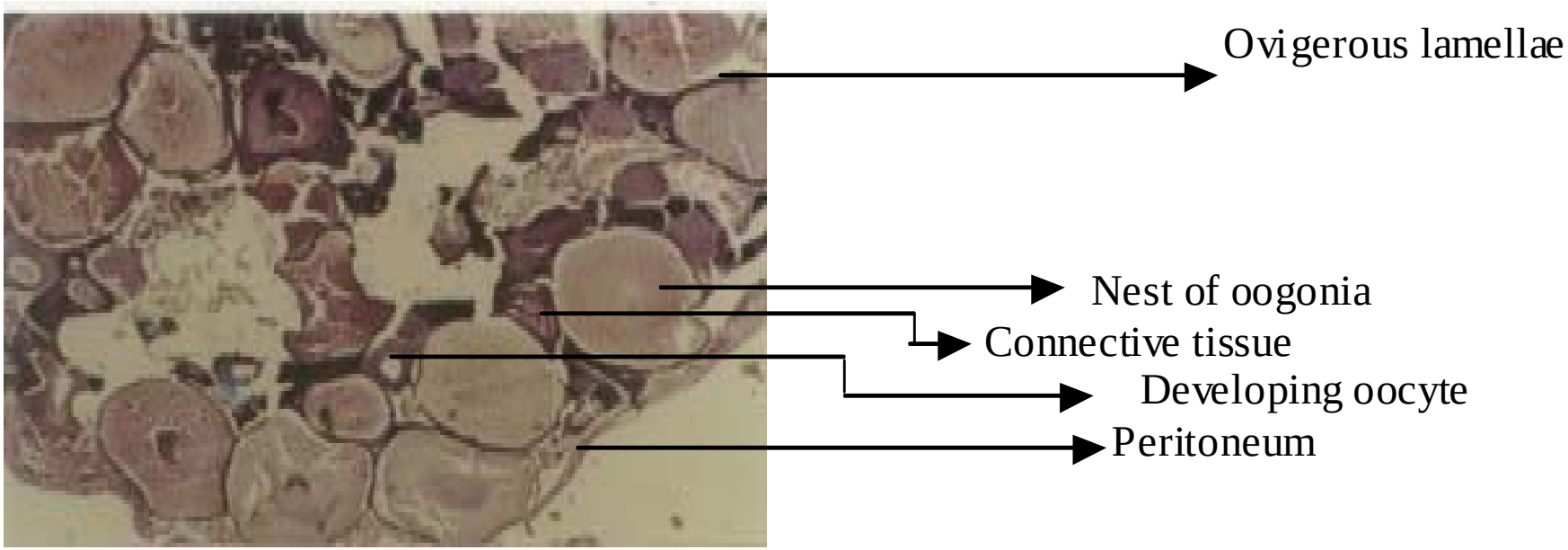


Fig.2a. Photomicrograph T.S. of Ovary
Stage I Immature phase (5x X 10)

<u>IMMATURE</u> : Germ cells, oogonium are found in bunch in the ovigerous lamellae.

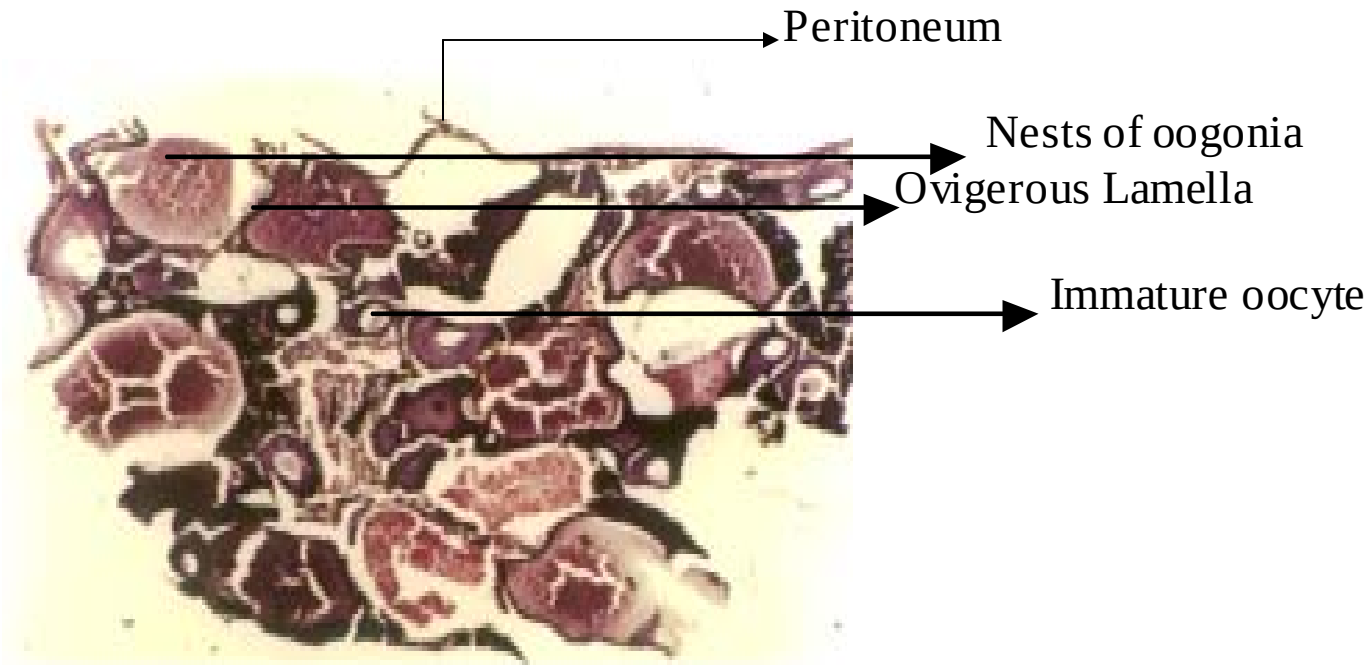
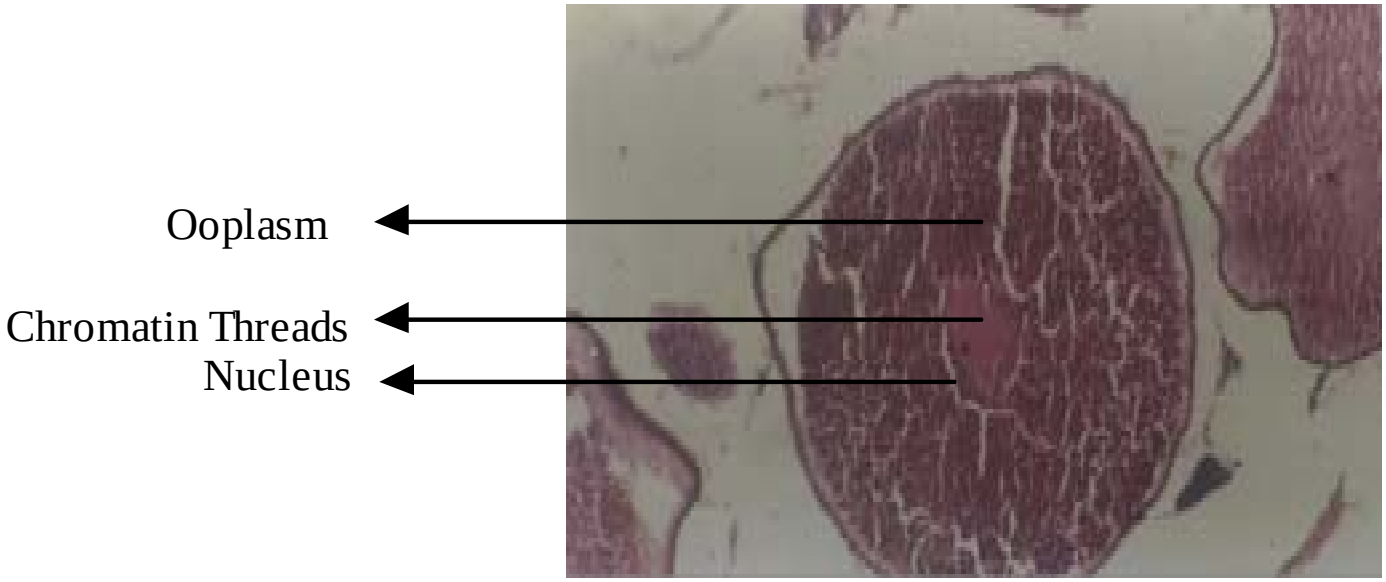


Fig.2b. Photomicrograph T.S. of Ovary
Resting phase (5x X 10)

<u>RESTING</u> : Ovigerous lamellae having nest, of oogonia and immature oocytes Stage I and Stage II are visible



Fig,2c. Photomicrograph T.S. Ovary showing Oogonium (5x X 10)

In the early stage of oogenesis oogonium is a large cell with large nucleus and prominent nucleolus surrounded by narrow rim of ooplasm which ar chromophotic.

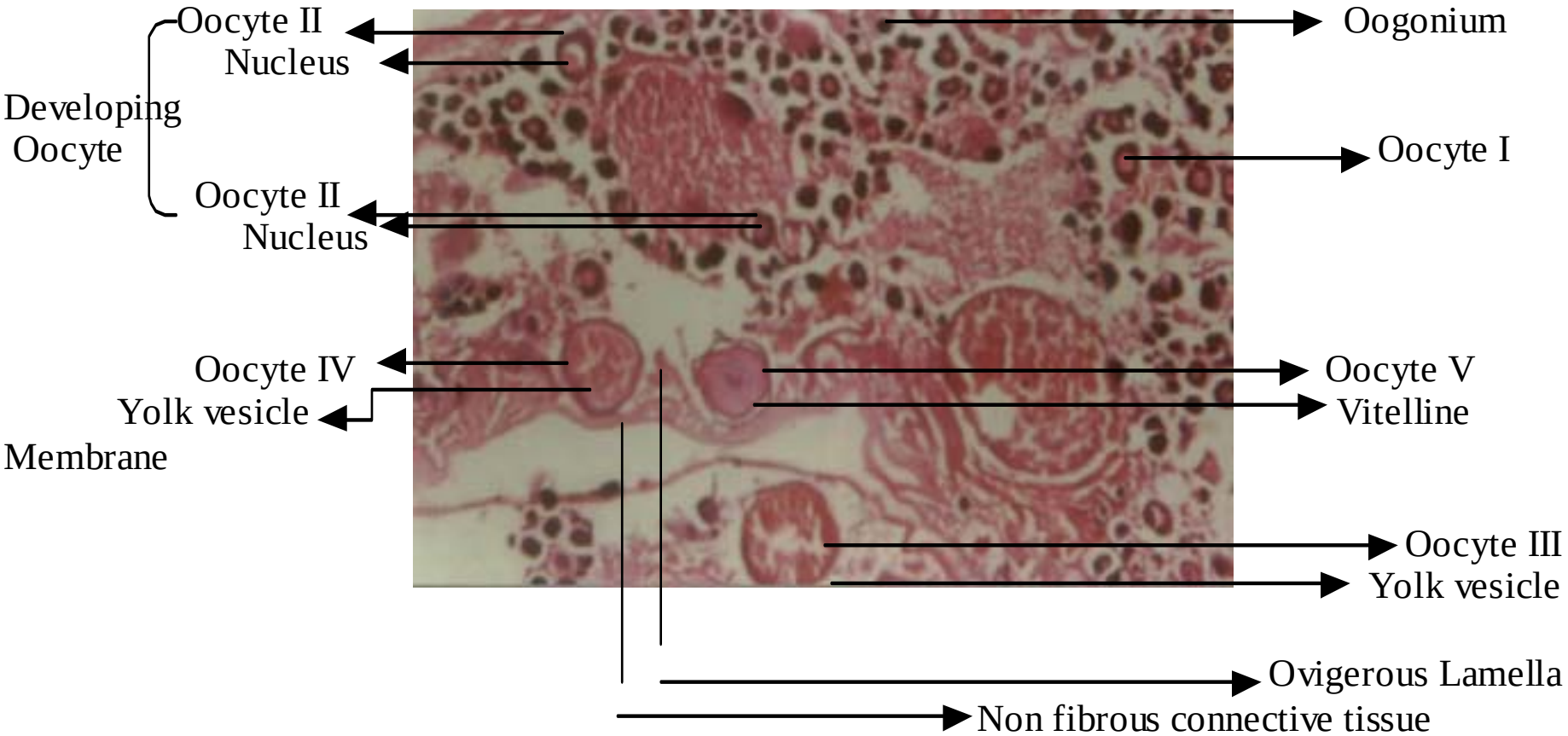


Fig.2d. Photomicrograph T.S. Ovary – Stage II Early Maturing Phase (5x X 10)

<u>EARLY MATURING</u> : Numerous oocytes in different stages of development. Oocytes Stage I to Stage III are in large number. Stage IV and Stage V they are few in number.

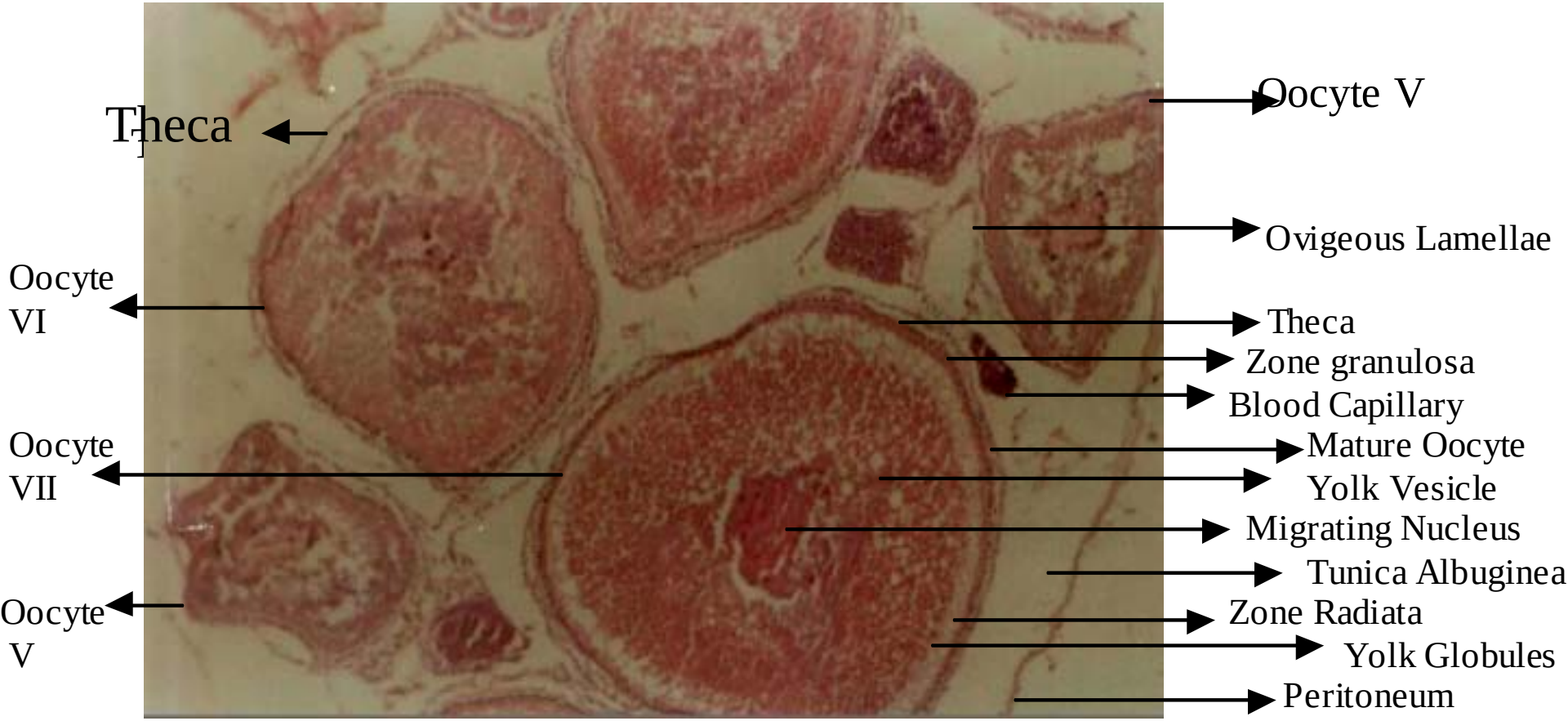


Fig.2e. Photomicrograph T.S. of Ovary
Stage –III developing phase (5x X 10)

DEVELOPING : Vascular supply increased blood capillaries become conspicuous. Immature oocytes reduced. Stage IV and Stage V oocytes large in number. Stage VII may also seen.

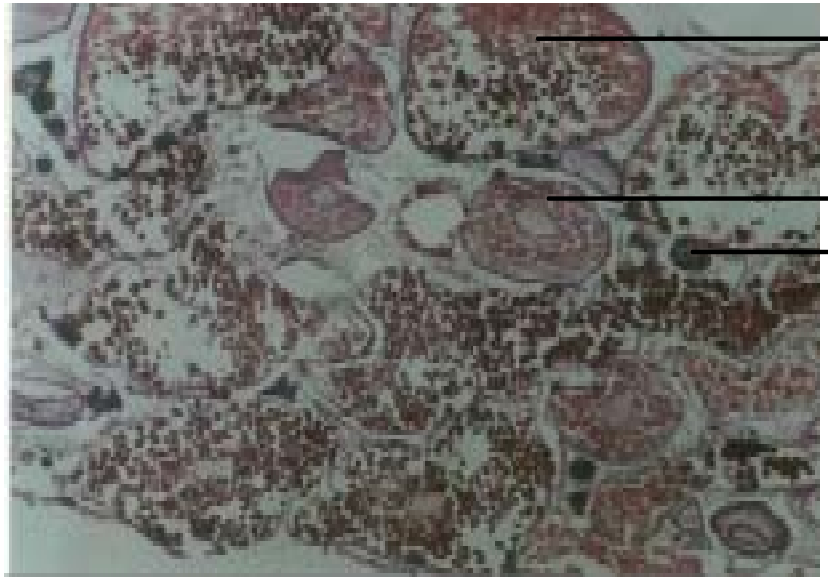


Fig.2f. Photomicrograph T.S. Ovary – Stage IV pre-spawning (5x X 10)

PRE-SPAWNING :Large number of OVA in Stage VII, Ripe eggs and Non-hypertrophic Atresia also observed.

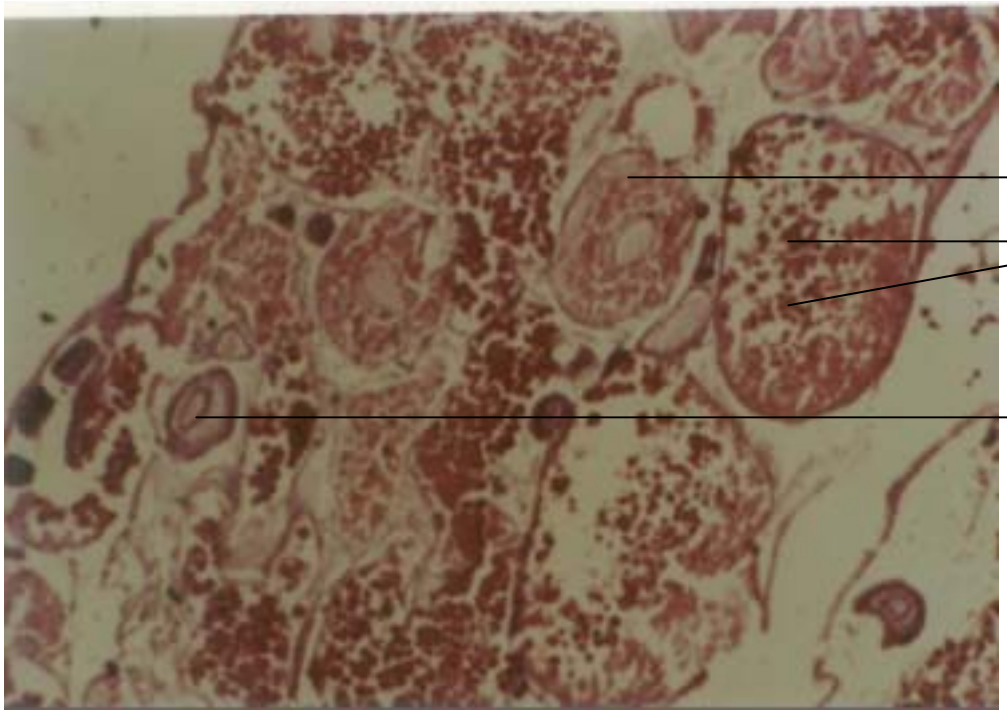


Fig. Photomicrograph T.S Ovary
Stage IV PRE-SPAWNING 5x X 10

Ripe eggs

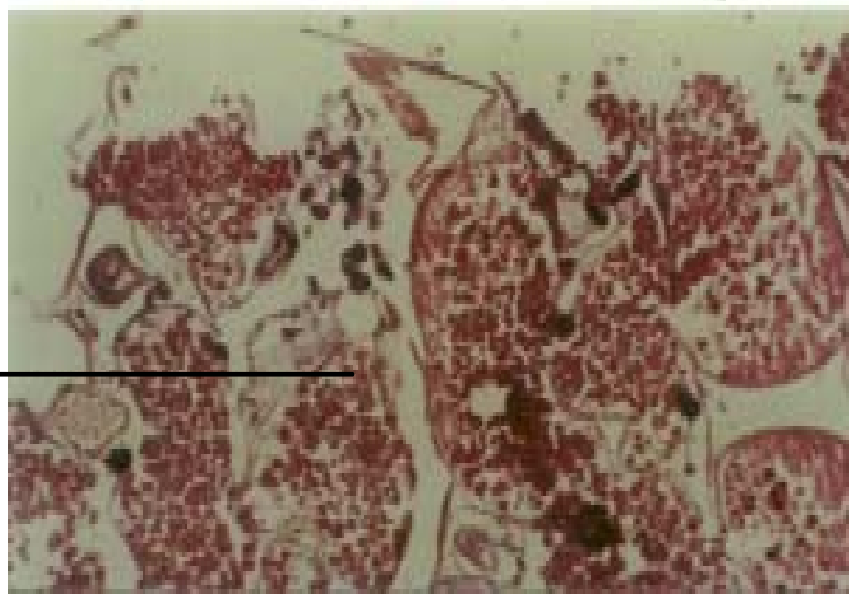
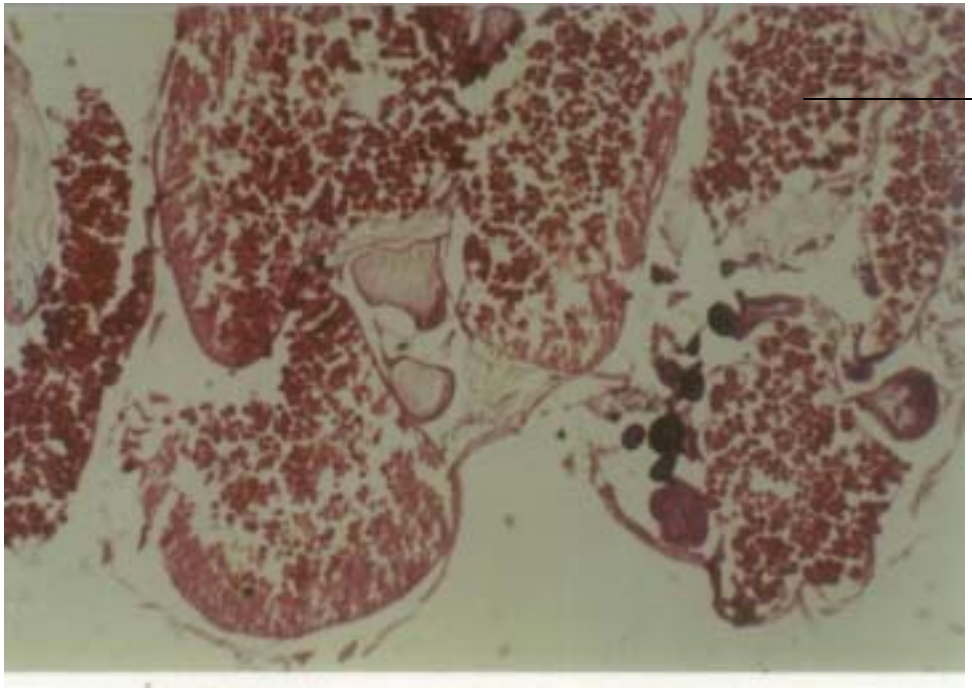


Fig 2g. Photomicrograph T.S. of Ovary
Stage V Spawning phase (5x X 10)

<u>SPAWNING</u> : RIPES OVA come out by rupturing of follicular epithelium



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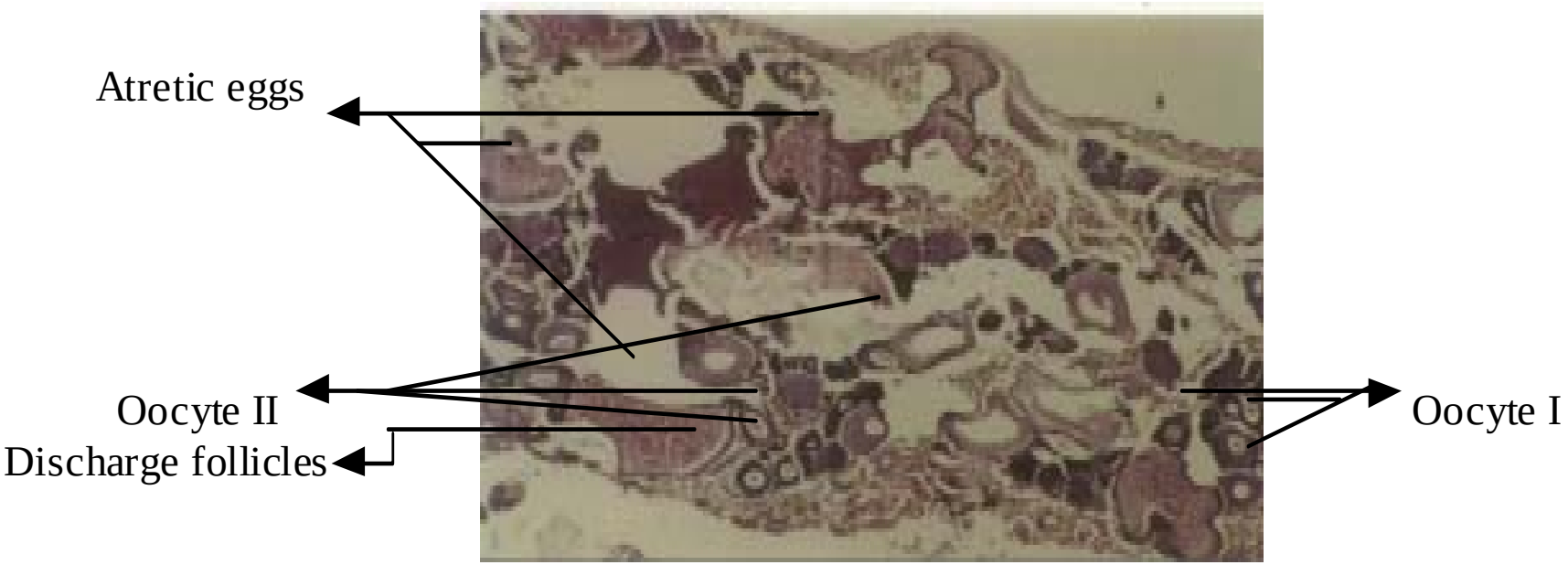

Fig.2h. Photomicrograph T.S. of Ovary, Stage VI Spent phase (5x X 10)

SPENT : ATRETIC AND DISCHARGED FOLLICLES along with STAGE I AND STAGE II OOCYTE

Table 1. Degree of maturation and the morphology of the gonad in different stages of maturity of *O.mossambicus*

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G.S.R and seasonal cycle of maturation

The cycle of maturation and monthly variation of gonadosomatic ratio provide good indication of the extent of development of gonad with respect to the time of year. Gonad staging on a descriptive scale allows a rapid qualitative assessment of the breeding state and gonad weight give a quantitative record of changes in the gonad condition (Crossland, 1977).

Table 2. Average monthly fluctuations in the K, GSR and GSI

Months	K	G.S.R.	G.S.I.
January	1.77	0.28	7.1
February	1.85	0.37	6.5
March	2.05	0.54	5.9
April	1.88	0.48	5.5
May	2.36	0.55	5.7
Jun	2.27	0.51	4.9
July	2.60	0.60	5.2
August	2.55	0.58	4.5
September	2.37	0.59	4.7
October	2.25	0.56	3.9
November	2.09	0.55	4.2
December	1.67	0.18	7.5

G.S.R VARIED FROM 0.18 (DECEMBER) TO 0.60 (JULY).
High correlation between GSR and condition factor (r = 0.88).
Negative correlation between GSR and GSI (r = - 0.84).

High correlation between GSR and condition factor, K (r = 0.88) and occurrence ripe specimen stage IV and stage VI during March to October is the indication of maturity in these months.

Negative correlation between GSR and GSI (r = - 0.84) is the indication that during maturation of the gonad the fish takes less amount of food suggesting that the developing of the gonads are highly effected the feeding habits of the fish during breeding season.

Certain physical parameters of water such as turbidity, water temperature, dissolved oxygen, free carbon-di-oxide and total alkalinity also studied to examine the effect of these abiotic parameters on gonadal maturation.

It was observed that in both aquarium and pond condition the species spawns four times in a year, between March and October, when temperature was favourable spawning activity of the species has not been observed during winter (November to February) when water temperature drops down below 18^{o}C. It has further been noticed that seasonal peaks in the G.S.R. values coincided with the peaks in the percentage of occurrence of matured individuals. The result on GSR indicates that both the males and females mature at the same time of the year, the peak breeding period being June and July.

Seasonal variation of water quality parameters (Physical)

Parameters	Seasons			
	Winter	Pre-monsoon	Monsoon	Autumn
Water Temperature (^{o}C)	17.63	23.13	28.98	21.4
pH	7.75	7.78	8.13	7.83
Turbidity (ppm)	28.48	7.89	4.92	5.29
Conductivity	36.07	33.9	21.35	23.13
TDS	385	334	152	164
Suspended Solids (SS)	108	73	55	63

Seasonal variation of water quality parameters (Chemical)

Parameters	Seasons			
	Winter	Pre-monsoon	Monsoon	Autumn
TH	127.1	116.6	60.0	63.0
DO	1.5	2.6	4.5	3.0
FCO_2	16.01	8.13	7.13	11.31
HCO_2	113.3	122.8	151.8	140.6
TA	114.6	149.6	182.6	179.6
CL	27.07	29.53	54.22	39.88
K	7.4	13.3	37.0	21.8
TP	8.69	6.43	2.95	3.4
NA	26.3	28.0	44.8	30.0
SILICATE	10.83	7.59	6.6	7.1
NO_3N	0.547	0.413	0.320	0.410
NO_2N	0.041	0.065	0.092	0.070
AMMONIA	0.111	0.124	0.180	0.145
AMMONICAL NITROGEN	0.43	0.32	0.25	0.30
SO_4	22.64	22.75	119.36	54.10
MA	24.41	19.13	6.93	17.18
CA	18.8	18.2	8.8	15.1
IRON	3.16	3.03	1.72	2.82
COD	71.67	44.50	34.67	38.70
BOD	14.0	8.63	4.77	8.15

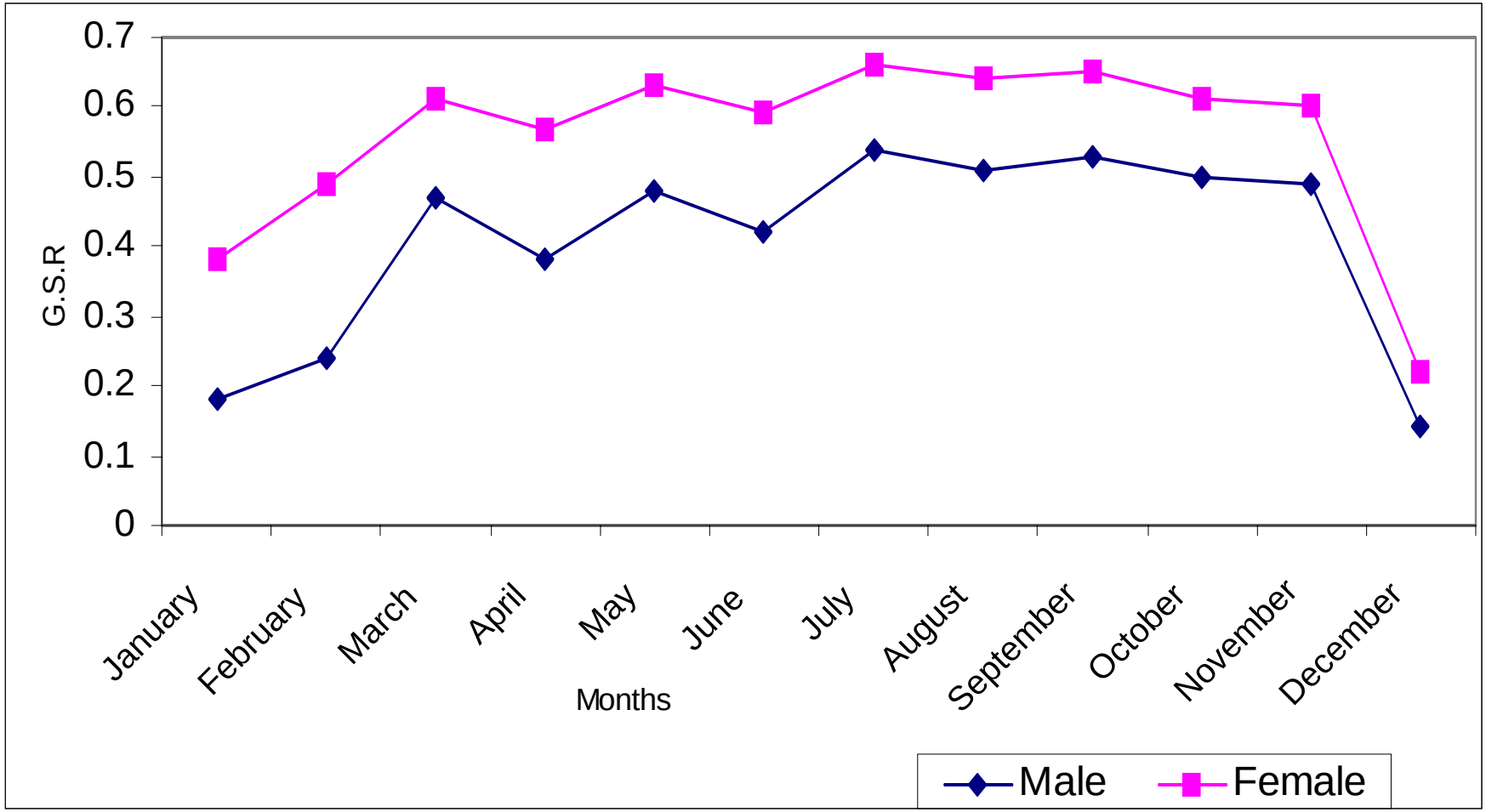

Fig. 3. Monthly Variations of Gonadosomatic ratio (GSR) of Male & Female fish

G.S.R. value showed 4 peaks in March, May, July & September coinciding with the spawning of the species in North-Eastern India.

Ovadiameter and seasonal cycle of maturation

* In the present study it was observed that the ova-diameter increased (0.045 – 1.5 mm) alongwith the progression of the maturity stage (Table.1).

* Measurement of ova-diameter and their frequency polygon distribution at different time of the months of the year was a common method for determination of maturity cycle of the fish (Macer, 1974).

* The progressive change observe in the intra-ovarian diameter for a period not less than a year can give an idea of the spawning periodicity of the fish studies (Biswas, 1993).

* From the percentage occurrence of mature ova in different months (Fig.4) it is inferred that in *O.mossambicus* the mature ova showed 4 peaks suggesting 4 times of spawning in a year.

In other words, the fish was a batch spawner and only gravid ova are released at one time during spawning season.

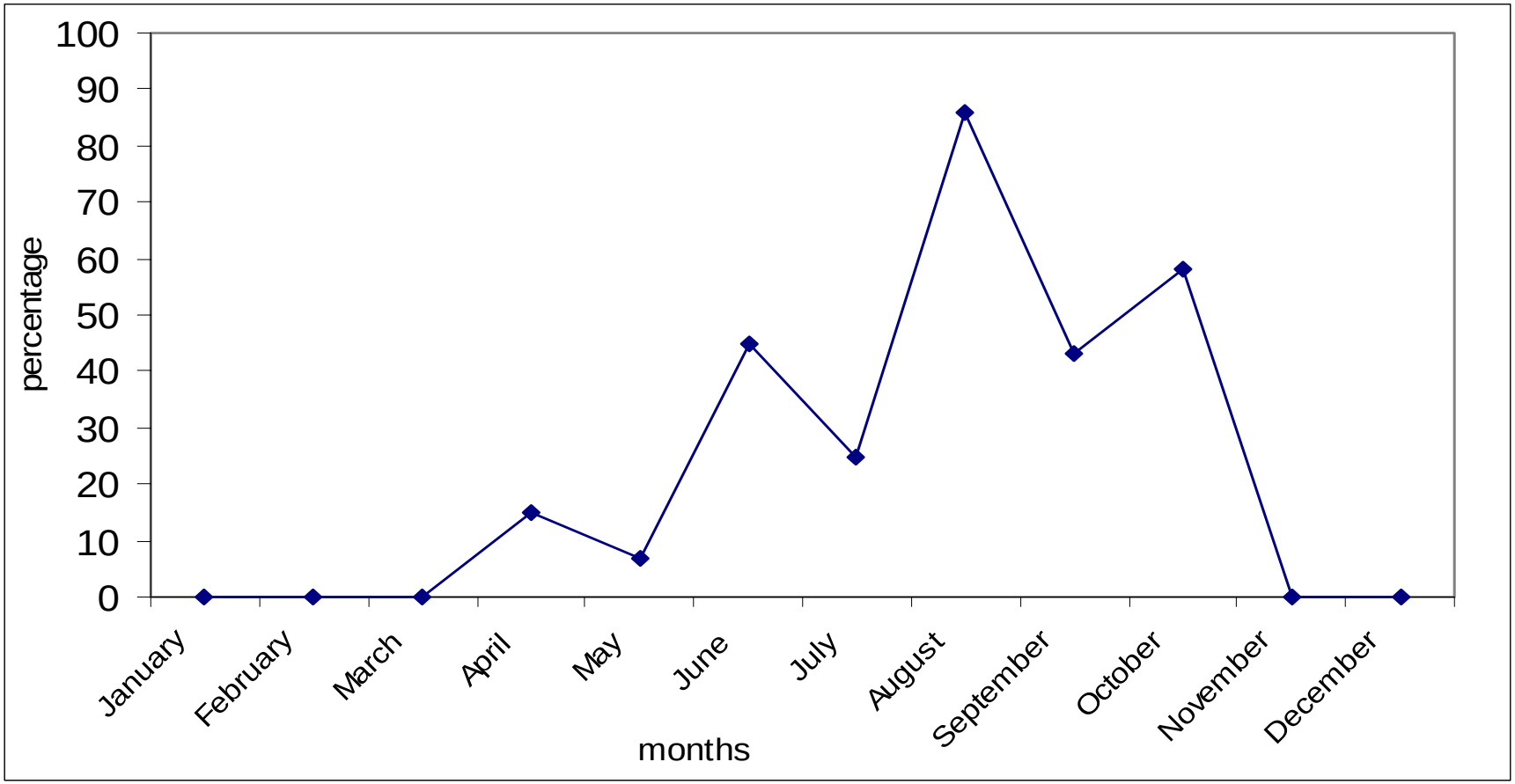

Fig.4. Frequency polygon of mature ova-diameter in different months

Length at first maturity and determination of M_{50}.

- Close relationship between maturity and the length of the fish
- *O.mossambicus* attains maturity length - 6.5 to 180 mm and age 2 and 5 months (Chimitz 1955, Pongsuwana, 1956 and Mironova, 1969)
- In the present study sexual maturity observed in the length group : - female length 5-10 cm, male length 10 - 15 cm.
- 50% male mature -> average length 12.5 cm
- 50 % female mature -> average length 7.5 cm
- Female matures at the shorter length than their male counterparts (Table.3)

Table 3. Percentage of maturity of various length groups

Size groups (cm)	Sex	Immature Stage I	Maturing Stage II & III	Mature Stage IV & V
0-5	Female	100	-	-
	Male	100	-	-
5-10	Female	25	25	50
	Male	75	20	5
10-15	Female	-	25	75
	Male	-	50	50
> 15	Female	-		100
	Male	-		100

Fecundity of Tilapia *(O.mossambicus)* showed wide variation : -

- 1. Hora & Pillay (1962) : The female tilapia lays 75 - 250 eggs at a time.
- 2. Mironova (1969) : Fecundity tilapia ranged between 80 and 1000 eggs.
- 3.Chimitz (1955) & Chang kong Tam (1962): 8 - 9 cm long females incubate 80 eggs. 15 cm long female nursed 800 eggs. A six months old female incubate 180-300 eggs whereas a eighth month old incubate 350-500 eggs.

- 4. De Silva and Chandrasoma (1980): Fecundity of *O.mossambicus* varied between 360-1775 for fish ranging from 20 to 31.9 cm in length and 145 to 538 gm in weight.
- In the present study absolute fecundity 100 - 850 for a size range of 7.6 to 19.9 cm (6.45 gm to 155.73 gm in weight)

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- The low fecund could well be attributed to the parential care (Anon 2000).
- Low fecundity of *O.mossambicus* in these region might be due to prolonged breeding season.
- The logarithmic relationship between fecundity and different body parameters were found to be linear.
- Fecundity and body length was found to the most closely related (r = 0.99).

The relative fecundity ranged between 6-16 (Table.4): -

- Anon (2000): The fecundity of *O.mossambicus* varied from 431 - 1012 eggs / 100 gm body weight.
- Riedel (1965): The fecundity of *O.mossambicus* varied from 660 - 1750 eggs/100 gm body weight.
- In the present study the fecundity varied from 546 - 1550 eggs / 100 gm body weight similar to those recorded elsewhere.

Table 4. Absolute and relative fecundity

Total Length (cm)	Body Weight (gm)	Ovary Weight (gm)	Absolute Fecundity	Relative Fecundity
7.6	6.45	0.028	100	16
9.8	12.92	0.042	150	12
10.2	26.12	0.113	179	7
11.7	28.32	0.327	200	7
12.6	35.57	0.330	250	7
13.5	45.2	0.380	414	9
14.2	50.71	0.486	511	10
15.0	53.82	0.575	569	11
17.3	108.65	0.725	620	6
19.9	155.73	0.835	850	6

Abstract : Certain aspects of reproductive biology

- Morpho-histological studies of gonads revealed the existence of six maturity stages.
- Gonadosomatic index (GSR) indicated that the breeding season extended from March to October.
- Frequency polygon of ova-diameter showed 4 peaks suggesting 4 times of spawning of the fish in this part of the country.
- First sexual maturity observed - 5 to 10 cm length groups in female and 10 to 15 cm in male.

- 50 % of male mature at an average length of 12.5 cm and 50 % of female mature at an 7.5 cm length groups.
- Absolute fecundity 100 to 850, Relative fecundity 6 to 16.
- Positive correlation between GSR and condition factor (r = 0.88). Negative correlation between GSR and GSI (r = - 0.84).
- High correlation was observed between Fecundity and total length (r=0.99), Fecundity and body weight (r=0.85), Fecundity and body weight (r = 0.62).

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