STUDIES ON CERTAIN ASPECTS OF THE REPRODUCTIVE BIOLOGY OF MOUTH-BROODING TILAPIA, *Oreochromis mossambicus* (Peters) FROM ASSAM, INDIA

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Abstract

Certain aspects of the reproductive biology of *O. mossambicus* (Peters) from a domestic pond of Assam have been described. Morpho-histological studies of the gonad revealed the existence of six maturity stages in *O. mossambicus* while gonadosomatic index (GSR) indicated that the breeding season extended from March to October. The frequency four polygon of ova diameter showed peaks suggesting four times of spawning of the species in this part of the country. Appearance of first sexual maturity was observed in 5 – 10cm length groups in female and 10 – 15cm in male specimen. Fifty per cent of male mature by the time they attained an average length of 12.5cm and this occurring at 7.5cm in females. Absolute fecundity ranged from 100 to 850 while the relative fecundity varied between 6 and 16. There was positive correlation (r = 0.88) between GSR and Condition factor (K) and negative correlation (r=- 0.84) between GSR and gonadosomatic index (GSI). Fecundity was highly correlated with total length (r = 0.99), body weight (r = 0.85) and ovary weight (r=0.62) of the fish.

Introduction

Oreochromis mossambicus was first introduced to India in 1952. Its culture in Indian water was later discouraged because it was reported to be a serious threat to native carps. Nevertheless, tilapia has been privately reared in certain pockets of North-East India.

The reproductive biology of tilapia have been widely investigated from different parts of the globe (Arnoult, 1954; Chervinski, 1961a and 1961b; Riedel, 1965; El-zarka *et al.*, 1970a; Siddiqui, 1977; Hodgkiss and Mann, 1978; Huner, 1980; Arthington and Milton, 1987; Rawat and Sheikher, 1991; Msiska and Costa-Pierce, 1999). However, there are conflicting views on the onset of maturity and reproductive cycle of tilapia. Furthermore, there has been no report on the maturity and spawning of tilapia from the sub-tropical Himalayan region. Therefore, an attempt has been made to study certain aspects of the reproductive biology of mouthbrooder tilapia (*Oreochromis mossambicus*) from a stagnant water body in Upper Assam.

Materials and methods

The experimental pond was located at Nazira in Sivasagar District of Upper Assam (Lat. 26°54′36″N and Log. 94°43′54″E at an altitude of 94 m above msl). For studying the various biological parameters the fish reared both in aquarium and nylon hapa in the pond were used. The experiment was carried out for three years (1996-99).

Seasonal progression of gonads was recorded by physical examination of testes and ovary. Assessment of various maturity stages was based on the modified classification of Kesteven (1960) and Crossland (1977). The GSR or co-efficient of maturity were calculated following Hopkins (1979). Length-maturity key as proposed by Kesteven (1960) was used to ascertain the maturity stage of the entire population. The attainment of sexual maturity (M_{50}) was calculated by using graphic method (Hodgkiss and Mann, 1978). Spawning periodicity was determined by observing the progressive changes in the intra-ovarian ova diameter for a period of 3 years. The absolute and relative fecundity were estimated following Bagenal (1967) and Hardisty (1964), respectively. The histological preparation of micro slides of gonads and processing were done by adopting standard histological techniques (Patki *et al.*, 1989; Lal, 2001).

Results and discussion

Morpho-histology of the gonad

Morphological description of ovary and testis at different stages of maturity and other matter are given in Table 1 which reveals that mature specimens are available between March and October.

Testes

The testes were non-zonal, lobules were loosely organized and the zonation was not apparent. The different parts of the testis i.e., anterior, middle and posterior parts showed morphohistological variations in different maturity stages. However, all the three parts of the testis showed similar stages of development in a particular maturity stage. In both immature (Fig. 1a) and resting phase (Fig. 1b), numerous spermatogonia were observed inside the small seminiferous lobules. The spermatogonia were large, spherical cells containing a large round, central nucleus with distinct nucleolus. Gradually slow mitotic activity was seen in early maturing phase and the spermatogonia started dividing and transformed into sperm mother cells (Fig. 1c). Intense spermatogenesis was seen during the latter part of this phase (developing). Spermatogonia decreased in number and numerous primary and secondary spermatocytes were visible (Fig.1d). The primary spermatocytes were smaller than spermatogonia and possessed a darkly stained nucleus. They gave rise to the secondary spermatocytes, which were still smaller than primary spermactocytes with clump chromatin material. In pre-spawning phase, blood capillaries became conspicuous, the seminiferous lobules were larger in size and full of sperm. Spermatogonia were few and all stages of spermatogenesis can be seen in various lobules (Fig. 1e). The smaller deeply stained spermatids with eliptical nucleus and slightly reduced sperms were seen in this stage. In spawning phase, the semiferous lobules became empty because of release of sperms (Fig. 1f).

At the end of spermatogenesis the seminiferous lobules were packed with sperm masses. In spent phase, the empty and collapsing seminiferous lobules were seen, some of which contain residual or unexpelled sperm (Fig. 1g).

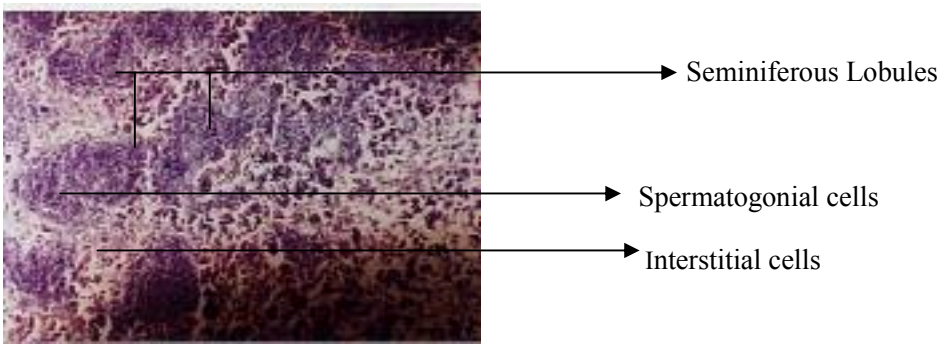


Fig.1a. Photomicrograph of T.S. of Testes.
Stage I immature phase (5x X 10)

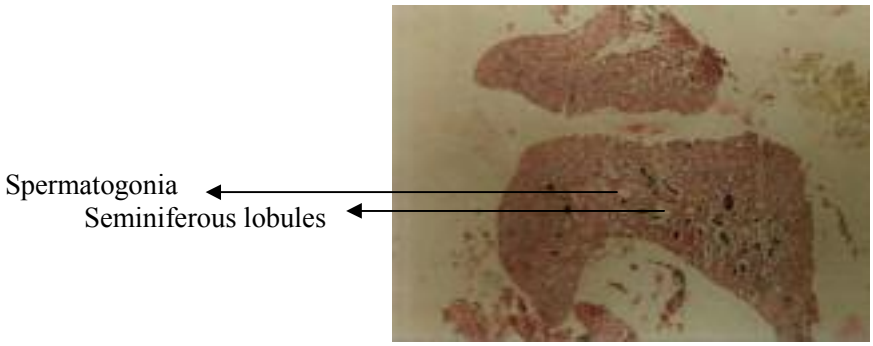


Fig.1b. Photomicrograph of Testis
Resting Stage (5x X 10)

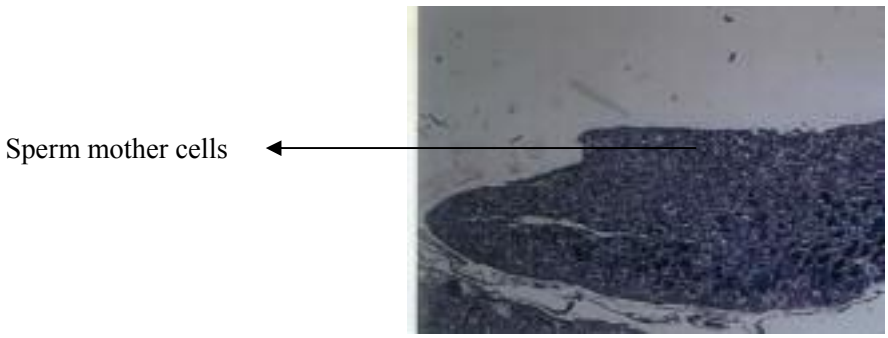


Fig. 1c. Photomicrograph of T.S. of Testes.
Stage II early maturing phase (5x X 10)

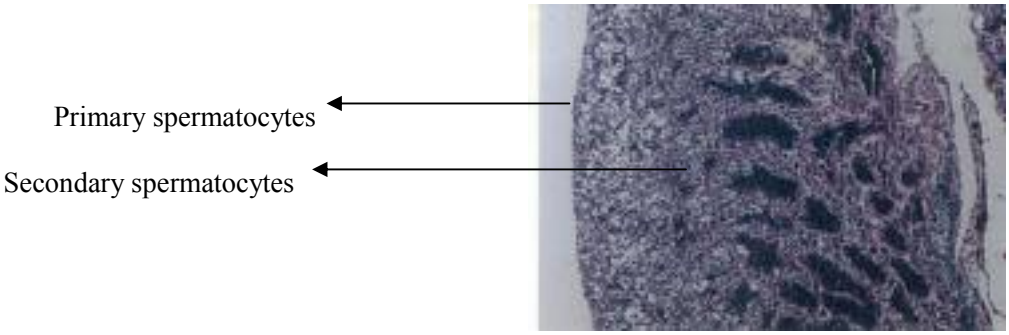


Fig.1d. Photomicrograph of T.S. of Testes
Stage III developing phase (10x X 10 middle part)

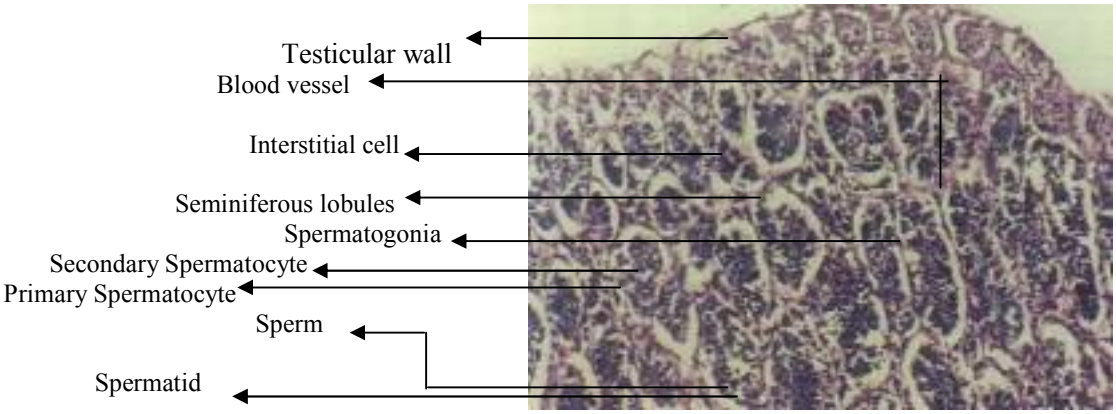


Fig.1e. Photomicrograph of T.S. of Testes
Pre spawning phase Stage IV (10x X 10)

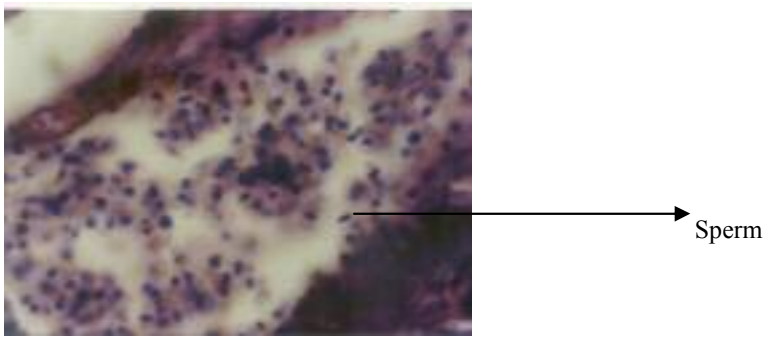


Fig. 1f. Photomicrograph T.S. of Testes
Stage V spawning phase (100x X 10)

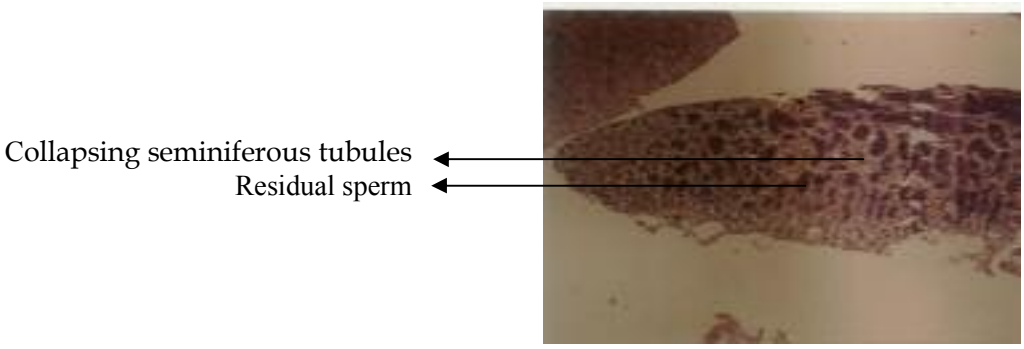


Fig. 1g. Photomicrograph of T.S.of Testes (anterior part)
Stage VI Spent phase (5x X 10)

Ovaries

The wall of the ovary was fairly thick during the non-breeding season but became thin and highly vascular during the spawning period. The ovarian lumen was loosely organized and the zonation was not apparent. In the ovigerous lamellae the germ cells, oogonium were found in bunch in the immature phase of the ovary (Fig. 2a). In resting phase, the ovary showed, ovigerous lamellae, having nests of oogonia, and immature oocytes in the stage I and II were visible under microscope (Fig. 2b). In the earlier stages of oogenesis, oogonium was a large cell with large nucleus and prominent nucleolus surrounded by narrow rim of ooplasm which were chromophobic (Fig. 2c). In early maturing phase, the ovary contains numerous oocytes in different stages of development (Fig. 2d). Oocytes in Stage I to III were large in number and Stage IV and V were few in number. Descriptions of each stage of oocyte are as follows; a) Oocyte I, this was spherical in shape with strongly basophilic cytoplasm, central nucleus, having 2 or 3 nucleoli; b) Oocyte II, there was further increase in the size of the oocyte and basophilia of the cytoplasm. Nucleus enlarged in size and the number of nucleoli increased; c) Oocyte III, there was further increase in the size of the oocyte, and was distinguished by the appearance of a thin layer of follicular cells around the cytoplasm. A large number of small, clear vacuoles called the yolk vesicles, appeared in the periphery of the ooplasm. The vesicles appeared red in eosin dye; d) Oocyte IV, as the oocyte grows further, the yolk vesicles increased in number and fill the entire ooplasm. e) Oocyte V; a vitelline membrane or zona radiata was also clearly visible, between the ooplasm and the follicular layer or the zona granulosa; f) Oocyte VI, this was characterized by the appearance of yolk in the form of minute granules in the extravascular ooplasm. The yolk granules fused to form larger globules. A thin layer of fibroblasts (theca) was also distinguishable outside the follicular layer; g) Oocyte VII, there was heavy deposition of yolk globules. Migratory nucleus was seen towards the periphery. Some yolk vesicles were pushed towards the periphery of the egg and form cortical alveoli. In developing phase, vascular supply increased and the blood capillaries became conspicuous. Also immature oocytes were reduced in number while stage IV and stage V oocytes were present in large number. A few stage VI oocytes may also be seen (Fig. 2e). In pre-spawning phase, a large number of ova in stage VII and ripe eggs were seen in the ovary. Some atretic follicles were also observed in this stage (Fig. 2f). In spawning phase, ripe ova come out by rupturing of follicular epithelium (Fig. 2g). In spent phase, the ovary showed atretic and discharged follicles, along with stage I and II oocyte (Fig. 2h).

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.
II	Early maturing	March to September	0.056 -0.090	Ovaries become slightly larger and increase in weight and volume with minute opaque whitish eggs occupied about half of the body cavity. Testes become enlarge, flat, increase in weight and volume, and creamy white in colour. Both the gonad are readily seen without any aid.
III	Developing	March to October	0.091-0.85	Ovaries distended occupied, about 2/3 of abdominal cavity with large pale yellow eggs. Testes enlarge, increase in weight and volume, light pinkish and thicker in size and look more vascular. Blood capillaries become conspicuous.
IV	Developed / pre spawning	March to October	0.86 – 0.99	Ovary becomes more enlarged occupying almost entire body cavity, with large number of big, turgid, spherical, translucent, deep yellow ripe ova. Testes become soft turgid pinkish red and increase in weight and volume. Blood capillaries prominent. Roe to milt run with slight pressure.
V	Spawning	April to October	1.0 – 1.5	Ovary walls become thin almost transparent. Riped eggs are visible through the ovarian wall and some riped eggs are present in the oviduct. Testes become flabby, thin and dull white in colour.
VI	Spent	April to late October	0.052 - 0.17	Gonad shrunken having loose walls. Ovaries are flaccid, shrinked and sac like, reduced in volume. Ovary contains ripped unspawned darkened eggs and a large number of small ova. Testes become flabby, thin and dull white in colour.

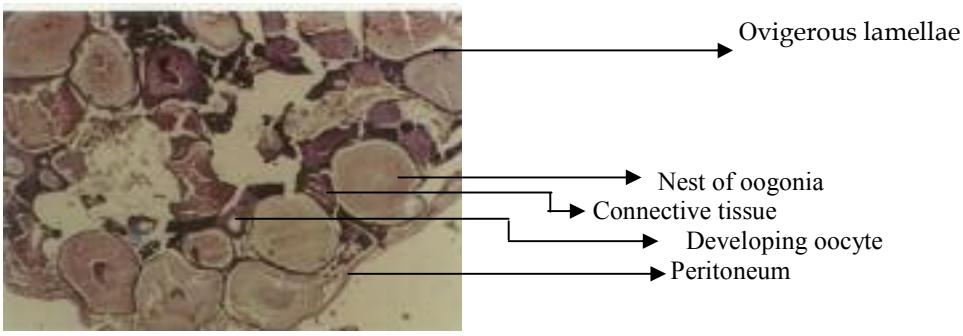


Fig. 2a. Photomicrograph of T.S. of Ovary
Stage I Immature phase (5x X 10)

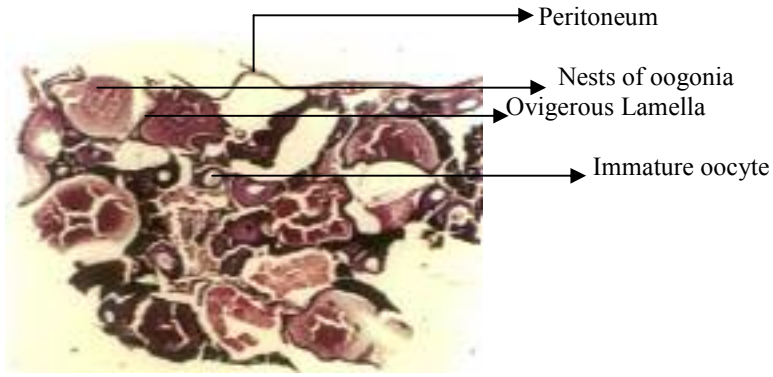
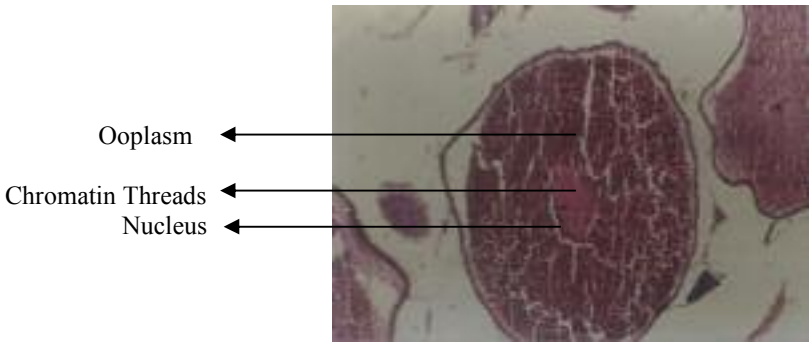


Fig. 2b. Photomicrograph of T.S. of Ovary
Resting phase (5x X 10)



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

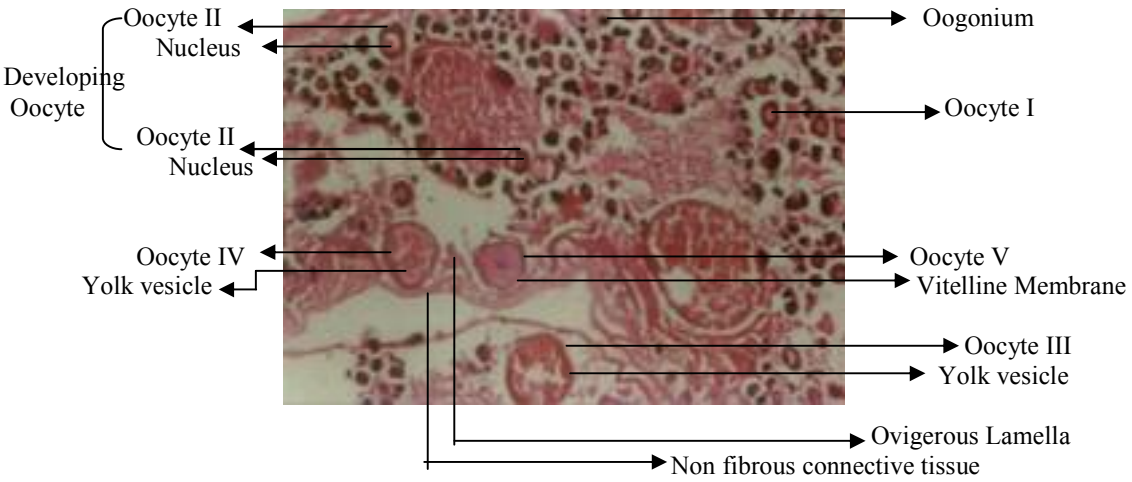


Fig. 2d. Photomicrograph of T.S. Ovary – Stage II Early Maturing Phase (5x X 10)

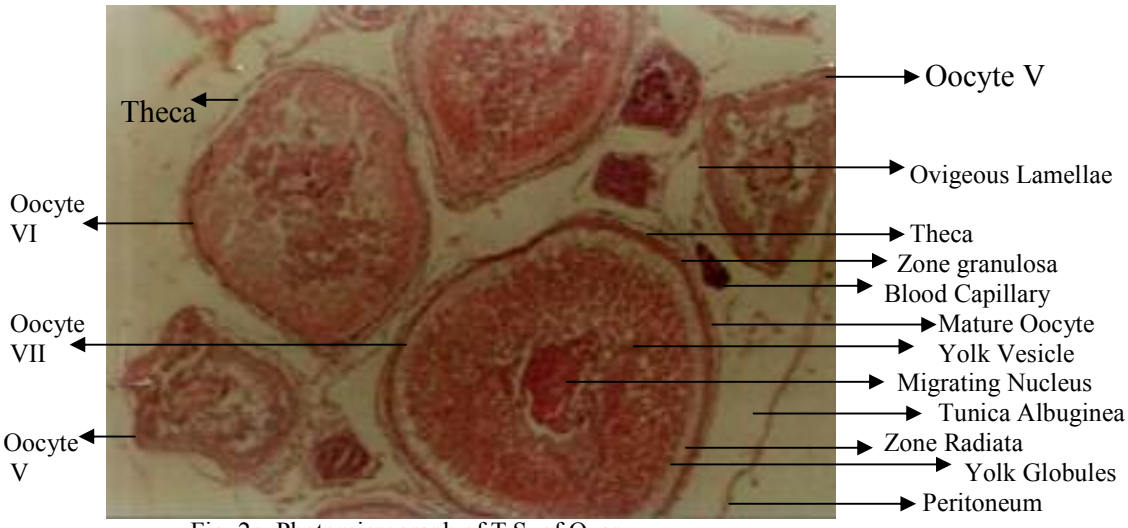


Fig. 2e. Photomicrograph of T.S. of Ovary
Stage –III developing phase (5x X 10)

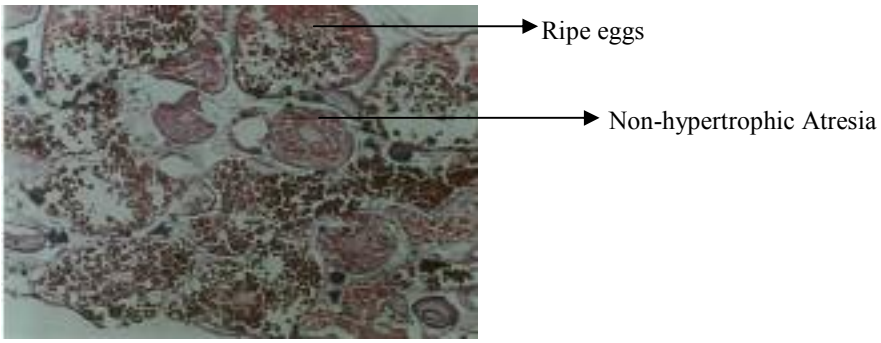


Fig. 2f. Photomicrograph of T.S. Ovary –
Stage IV pre-spawning (5x X 10)

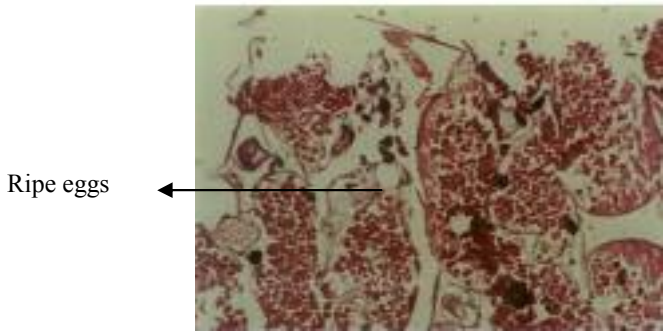


Fig 2g. Photomicrograph of T.S. of Ovary Stage V Spawning phase (5x X 10)

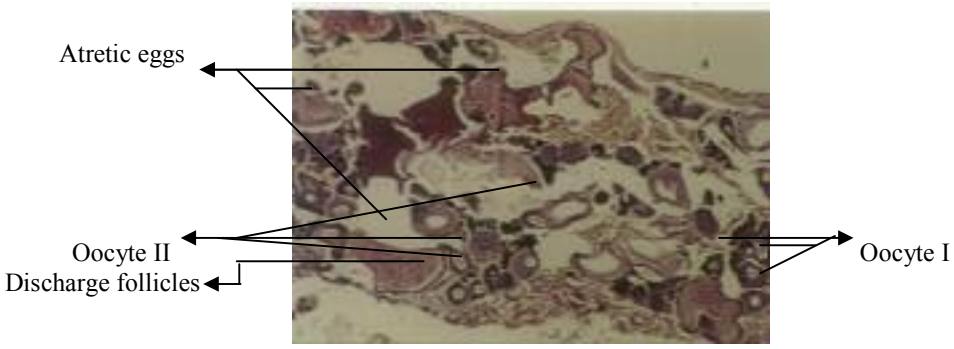


Fig. 2h. Photomicrograph of T.S. of Ovary, Stage VI Spent phase (5x X 10)

Gonadosomatic ratio (GSR) and seasonal cycle of maturation

The cycle of maturation and monthly variation of gonadosomatic ratio provides 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 gives a quantitative record of changes in the gonad condition (Crossland, 1977). In the present study the GSR varied from 0.22 (December) to 0.66 (July). The GSR value showed four peaks in March, May, July and September coinciding with the spawning period of the species in North-Eastern India (Fig. 3). De Silva and Chandrasoma (1980) also observed four peaks with a range of 0.35 to 1.4 in the GSR of *O. mossambicus.* Monthly variations in the GSR have been compared with the K-values and GSI (Table 2) and certain physical parameters of water such as turbidity, water temperature, dissolved oxygen, free carbon dioxide and total alkalinity to examine the effect of these abiotic parameters on gonadal maturation. High correlation was observed between GSR and Condition factor (r = 0.88). The occurrence of ripe specimen stage IV to VI during March to October and high GSR and K-values during these months are indications of the sexual maturity. However, negative correlation was found between GSR and GSI (r = - 0.84) indicating that during maturation of the gonad the fish takes less amount of food. The negative correlation between GSR and GSI suggests that developments of gonads highly affect the feeding habits of the

fish during breeding season. It was observed that water temperature and plankton density were highly correlated with the GSR value. It was observed that in both aquarium and pond conditions the species spawns four times in a year, between March and October, when temperature was favourable (Hatikakoty, 2002) 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 observed that seasonal peaks in the GSR 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.

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

Month	K	GSR	GSI
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

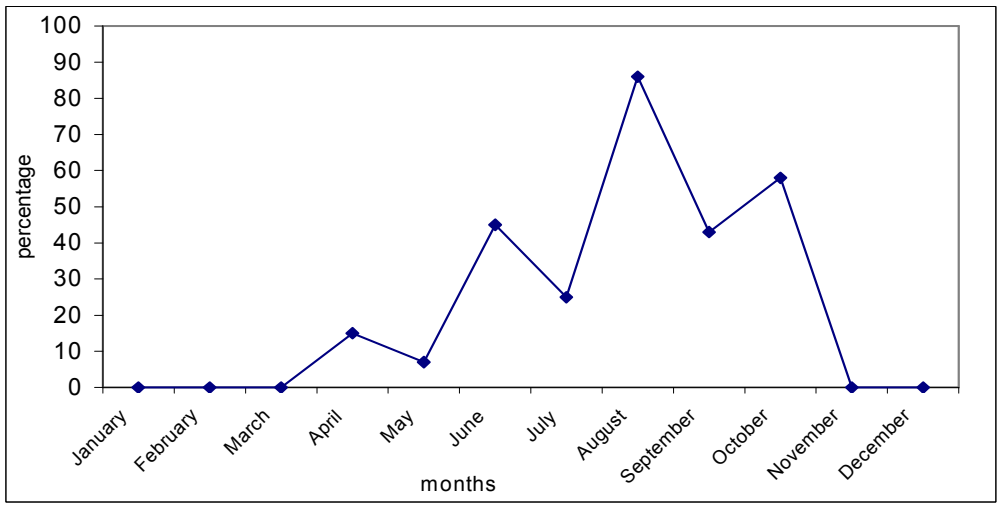


Fig. 3. Monthly Variations of Gonadosomatic ratio (GSR) of Male & Female fish

Ova-diameter and seasonal cycle of maturation

In the present study it was observed that the ova-diameter increased from 0.045 to 1.500mm along with the progression of the maturity stage (Table 1). Measurement of ova-diameter and their frequency polygon distribution at different times of the months in a year was a common method in determining the maturity cycle of the fish (Macer, 1974). The progressive change observed 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 four peaks suggesting that they spawn four times in a year. In other words, the fish was a batch spawner and only gravid ova are released at one time during spawning season.

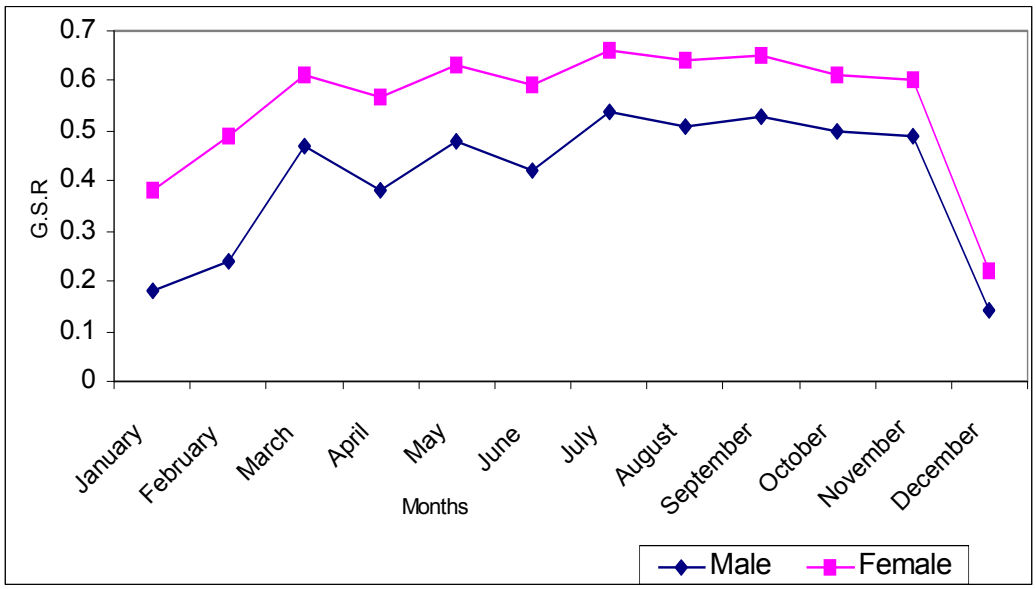


Fig.4. Frequency polygon of mature ova-diameter in different months.

Length at first maturity and determination of M_{50}

There was a close relationship between maturity and the length of the fish. It appears from the works of other investigators (Chimitz, 1955; Pongsuwana, 1956; Mironova, 1969) that *O. mossambicus* attains maturity from 65 to 180mm in length which is about two to five months of age. In this study, appearance of sexual maturity was observed at 5 – 10cm length groups in female and 10 – 15cm in male fish. Fifty per cent of male matures at the average length of 12.5cm and that of female at 7.5cm (Table 3) indicating that females mature at smaller size than their male counterparts.

Fecundity

Fecundity in *O. mossambicus* is very variable as reported by various investigators. According to Hora and Pillay (1962), the female tilapia lays 75-250 eggs at a time. Mironova (1969) reported that the fecundity of tilapia ranged from 80 to 1000 eggs per female. Females of 8 – 9cm long incubate 80 eggs and those 15cm long nursed 800 eggs. A six-month old female was found incubating 180-300 eggs and eight-month old, 350-500 eggs (Chimitz, 1955; Chang Kong Tam, 1962). De Silva and Chandrasoma (1980) found that the fecundity of *O. mossambicus* varied from 360 to 1,775 eggs per female for fish with length that ranges from 20 to 31.9cm and weight ranging from 145 to 538g In this study, the absolute fecundity of the species varied from 100 to 850 eggs per female for a size range of 7.6 to 19.9cm (6.45 to 155.73g) and the relative fecundity ranged from 6 to 16 (Table 4). It was reported that fecundity of *O. mossambicus* varied from 431 to 1,012 eggs/100g body weight (Anon., 2000) whereas Riedel (1965) observed that the fecundity of the species ranged from 660 to 1,754 eggs/100g body weight. In the present study it was observed that fecundity ranged from 546 to 1,550 eggs/100g body weight indicating that the fecundity recorded in the present investigation was similar to those recorded elsewhere. The low

fecundity could well be attributed to the parental care (Anon., 2000). Furthermore, the low fecundity of *O. mossambicus* also might be due to prolonged breeding season.

The logarithmic relationship between fecundity and different body parameters were found to be linear. Among the various parameters, fecundity and body length was found to be the most closely correlated (r = 0.99).

Table 3. Percentage of maturity in various length groups of *O. mossambicus*.

Size groups (cm)	Sex	Immature (Stage I)	Maturing (Stages II &III)	Mature (Stages 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

Table 4. Absolute and relative fecundity.

Total Length (cm)	Body Weight (g)	Ovary Weight (g)	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	45.20	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

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