

Reproductive Biology and Histological Study of Red Lionfish Pterois Volitans from Cuddalore, South East Coast of India

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Abstract

Reproductive study of a fish is an important for fishery resources and conservation management. Their provide information on the basis for early life history and oriented studies. In the present investigation clearly showed that the assessment of size at maturity, fecundity, spawning behaviour and capacity of reproductive in *Pterois volitans*. The graphical representation shows there is the intimate relationship between the length of the ovary and its relative weight. On the graphical representation, in the month of August every year the high values of gonad weight and fecundity were concluded. The gonad weight (GW) can be expressed as $F=9387.9GW+34026$ with an r^2 value of 0.5723. It was recorded for significantly different. The GSI values of female *P. volitans* have also shown similar increasing trend associated with histological changes. A GSI value increase with corresponding histological changes was also noticed in lion fish *P. volitans*.

Keywords: *P. volitans*; Reproductive biology; Histological; Gonadosomatic index; Aquaculture scale

Introduction

The success of any fish species is ultimately determined by the ability of its members to reproduce successfully in a fluctuating environment and thereby to maintain the viable population. Information on the reproductive biology of the candidate species is very much essential for the development of aquaculture industry. The dynamic metabolic activity of a reproduction in most of the fishes and it involves sequential changes in the germ cells. The pattern of these changes in the gonads is typical for each species [1,2]. To understand the physiology of reproduction, the study of the seasonal developmental changes of gonads through both macroscopic and microscopic observations is necessary. In case of hermaphroditic fishes, macroscopic observation may not provide the correct information of the germ cell development during gonadal maturation and has its own limitations [3,4]. Hence, microscopic observation is considered as an important method to get detailed information on the reproductive mechanism of such a fishes. Histological observation can provide information on the internal structural changes in the germ cells. The Pterois, including *P. miles* and *P. volitans*, are gonochoristic with males and females, exhibiting minor sexual dimorphism during spawning [4]. The two genders are morphologically identical and thus cannot be distinguished visually. Males typically grow larger than females with the largest male lionfish recorded as 476 mm total length [5]. The red lionfish are external fertilizers that produce a pelagic egg mass following a courtship and mating process that is not well documented. Like many reef fishes, red lionfish larvae are planktonic. After a few weeks in the plankton stage, larvae settle onto reefs as juveniles. In order to proceed with the artificial means of reproduction and to produce good quality eggs, it is necessary to have basic information on reproductive biology of the species. Information on the reproductive biology of Pterois sp completely is not available. Therefore, the present study has been taken up on the reproductive biology of *P. volitans* from Cuddalore coast.

Materials and Methods

The present study was carried out at the Cuddalore coast, Tamilnadu from January, 2012 to December, 2012. Adult fishes of *P. volitans* were collected by using gill-nett, trawlers, hooks and seafood

markets of selective landing centres. Further, haphazardly selected ovaries were preserved in 5% formalin and processed according to standard histological methods [6] to investigate pathologies associated. Transverse sections were cut from the same region in the centred of each ovary. The light microscope (LM) was used for the examination. All measurements are in micrometres. The fish nomenclature was followed by Fish Base [7]. The size at 50% maturity was then obtained by substituting $P=0.5$ in the equation.

Gonado-Somatic Index (G.S.I)

Gonado-somatic index is an indirect method for estimating the spawning season of a species. It was calculated by following the method of [8,9] using the following formula

$$GSI = \frac{\text{Weight of the gonad}}{\text{Weight of the fish}} \times 100$$

Mostly the average G.S.I values were computed by dividing the total values of each month by the number of fish examined in the month.

Gonado-somatic and fecundity relationship: Fish body weight and weight of gonad gives the gonado-somatic Index (G.S.I.). Meanwhile the development and growth of gonad simultaneously take the place in the fish. It the fish grows; the G.S.I. will high.

The term "fecundity" can be expressed as the number of eggs laid by an individual of a species during the spawning in a single season by the species. In order to assess the population stock of any species, the accurate estimation of the fecundity is essential. This will be useful

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to understand that whether fish has attained the maturity and able to produce the eggs in the spawning period. Present observation was made to study the growth of fish and gonadal development along with egg laying capacity (fecundity) and when the fish attains its first maturity.

Estimation of fecundity

For the estimation of fecundity, gravimetric method was applied. Fresh gonads were removed from the fish within a few hours of capture and their reproductive stage was recorded using macroscopic observation. Gonads obtained from recreational fishers could usually be weighed in gm. Two or three transverse cuts were then made through each gonad to ensure proper medium. Gonado-Somatic Index was observed high in fixation.

Fresh samples

Length (cm) and weight (gm) of each fish was measured. Total length (TL) is measured to the tip of the mouth to the end of the tail. Developments of matured oocytes were measured with the help of Oculometer. Gonado-Somatic Index and fecundity have been calculated during the study.

Fecundity (F) = Total wt of the ovary / Wt of sub sample X No. of mature eggs (ova) in sub-sample

Collection method and preservation

Live fishes collected by using fishing vessels were measured accurately to nearest millimetre (mm) for total length, standard length and total weight (gm). Each fish was dissected to remove the gonads. The dissected tissues were covered with aluminium foil, and packed in labelled 4x5 cm polythene bags. The polythene bags were preserved at -20 °C until the landing of the vessel. After reaching the shore, all the samples were loaded in an ice box and transferred to the laboratory. The ovaries preserved in polythene bags were taken and their weights were recorded up to milligram (mg) level by using electronic balance (Sartorius) for the determination of Gonado-somatic Index (GSI). The GSI for each fish was calculated using the formula of [8,9]. The range and average values of GSI were calculated for each maturity stage. The 'pondreal index' or 'condition factor,' K for each fish was calculated using the formula suggested by [10]. The range and average values of 'K' were determined for each maturity stage. Sex and stage of maturation were determined microscopically.

The gonads were assigned to three different maturity stages as suggested by [11]. The process of oogenesis was studied by utilizing histological preparations of ovaries from females belonging to different gonad maturity stages as recommended by [12] and adopted by [13]. After dissecting the ovary from fresh fish, the ovary was cut into pieces for easy penetration of fixative. The ovary pieces from fresh specimens were fixed in Bouin's fixative and embedded with molten wax (58 °C melting point). The sections (5 mm thick) were stained with Delafield's haematoxylin and counter stained with 1% aqueous eosin.

Oocyte diameter measurements were taken from ovaries belonging to various developmental stages and oocyte size-frequency profiles were constructed to trace the development of ova from immature stage to ripe condition [13-16]. Fecundity estimates were based on sub sampling of unbiased samples of ovaries from gravid fish collected during the peak spawning period as recommended by [17]. The relationship between the fecundity (F) and total length (L), fecundity and total body weight (W) as well as fecundity and total gonad weight of the fish were determined using regression equations.

Results

The reproductive system of females of *P. volitans* includes a pair of ovaries, continued into an oviduct and ends in genital pore. The ovaries are paired egg sacs located behind the stomach and duodenum, below the swim bladder and just above the intestine and connected to it by mesenteries. Each ovary consists of a hollow sac. The right and left lobes are usually unequal in size. Right ovarian lobes are relatively larger than the left; both of them join posteriorly and descend as an oviduct to open in the genital pore immediately behind the anus. The urinary bladder is closely bound to the posterior face of the common oviduct. Supporting mesenteries continue forward from the anterior end of each gonad as ligaments that join a complex of ligaments and mesenteries at the anterior end of swim bladder.

P. volitans ovary is the cyst ovarian type in which matured eggs will be released into the ovarian cavity during the ovulation; the ova will pass through oviduct on their way to go out at the genital pore. The genital pore is seen as a smaller pore behind the anus which would be bigger and pinkish during spawning season. The wall of the gonad is covered externally with a peritoneal layer. The tunica albuginea has an intermixture of longitudinal, oblique and circular muscle fibres.

Morphological classification of the ovary

Stage I: The ovary in the immature stage I is relatively small, translucent and white pinkish in colour (Figure 1).

Stage II: Mature resting female/maturing female stage II of *P. volitans* is defined as an ovarian stage II that had undergone extensive vitellogenesis and recovered into resting state. The ovary is larger than the previous stage and white brownish in colour (Figure 2).

Stage III: Stage III (ripe) is defined as the ovarian stage III in which active vitellogenesis takes place in preparation for spawning in the mature active female/ripe female. The ovary occupies 2/3rd of the body cavity and is yellowish in colour. Oocytes are in stages 1, 2, 3 and 4 with stage 3 ovary dominating during early development of this stage (Figure 3).

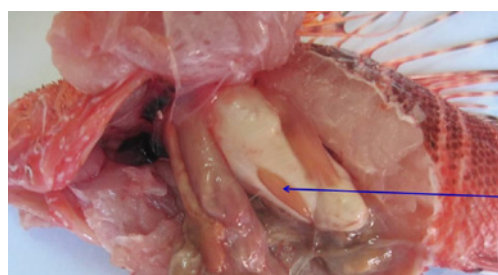


Figure 1: Ovary in the immature stage I *P. volitans*.



Figure 2: Ovary in the mature stage II *P. volitans*.

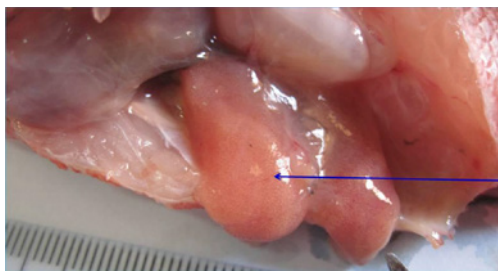


Figure 3: Ovary in the mature stage III *P. volitans*.

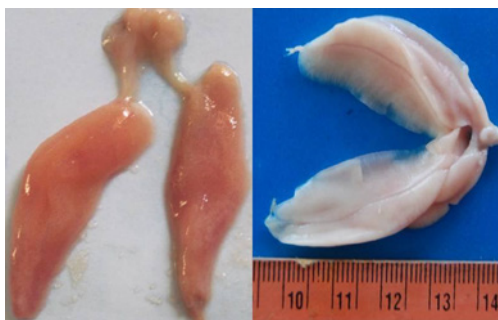


Figure 4: Male testes and female ovary.

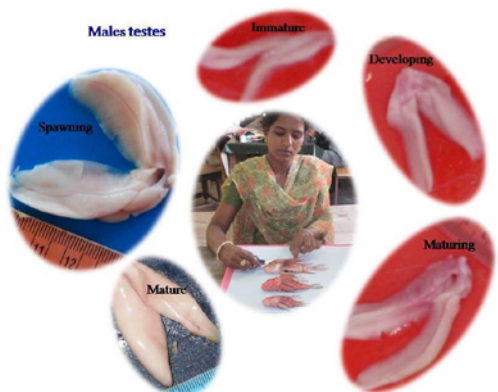


Figure 5: Testes development stages.

Condition factor (K): The condition factor (K) of *P. volitans* was in the range of 1.15-1.61. Highest condition factor (1.61) was observed in stage III of gonadal maturation.

Gonado-somatic index (GSI): In the present study, GSI values of *P. volitans* showed correlation with the maturation of gonad. The immature ovaries in the maturity stage I showed a GSI value of 0.062, the value was 0.234 for the maturing ovaries in the stage II and in the ripe stage ovaries of the maturity stage III, the value was 3.064 (Figure 4).

Males

Immature: Testes are appeared in thread-like structure and present within a transparent membrane (Figures 5 and 6).

Developing: Testes developed uniformly as ribbon-like structure. Surface of testes appears smooth and uniformly textured (Figures 5 and 7).

Mature: Testes Oozes are larger in size and highly convoluted;

sperm cannot be extruded. Body wall incision causes gonads to be expelled from opening (Figure 8).

Spawning: Testes milky sperm freely or extrude sperm when compressed (Figure 9).

Spent: Testes are large, but flaccid, watery, and bloodshot.

Females-Histological study of the ovary (Based on the procure of oocyte)

Stage I-Immature ovary: In this stage, the diameter of oocyte ranged between 17 and 50 μm . The cytoplasm becomes strongly basophilic. A thin follicular layer is surrounded the oocyte at this stage. The ovary contains stage 1 and stage 2 oocytes (Figures 10

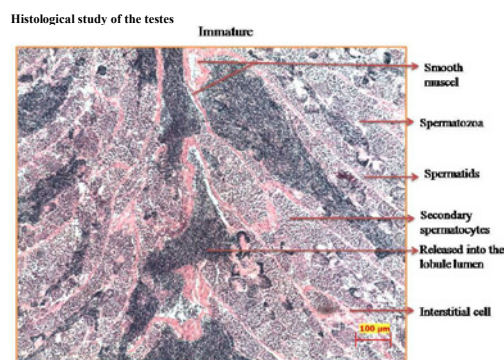


Figure 6: Cross section of testes at immature stage.

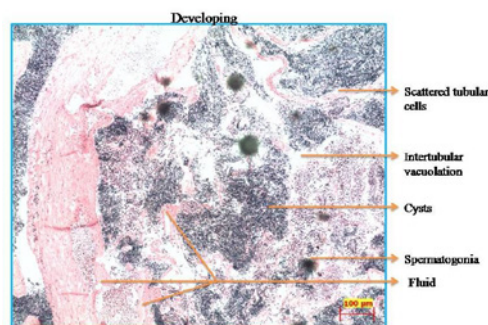


Figure 7: Cross section of testes at developing stage.

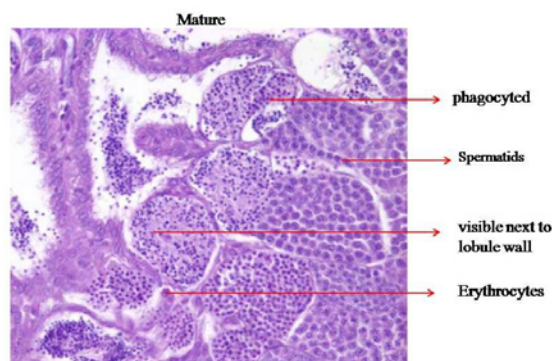


Figure 8: Cross section of testes at mature stage.

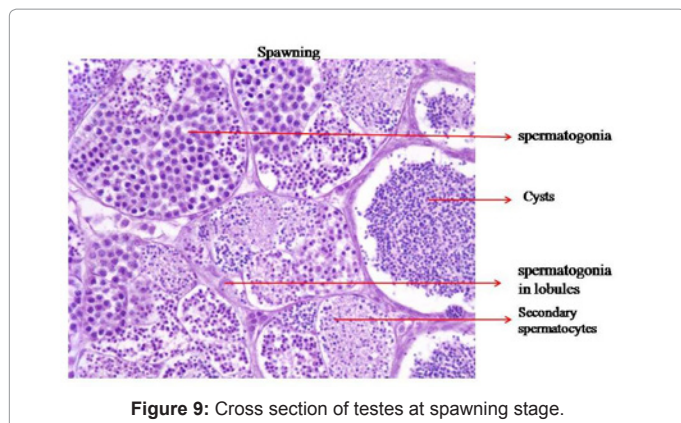


Figure 9: Cross section of testes at spawning stage.

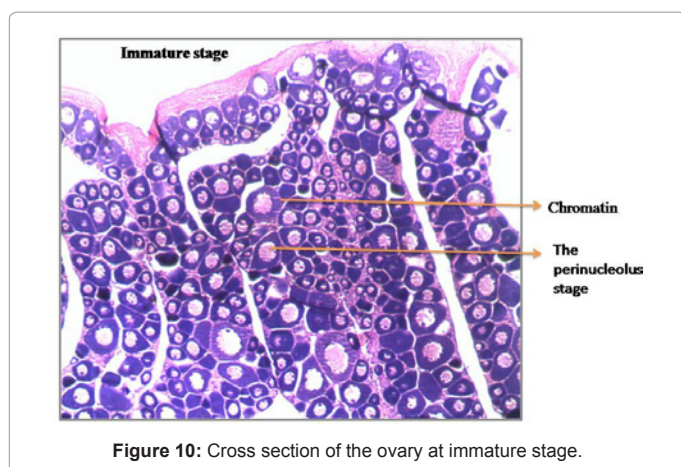


Figure 10: Cross section of the ovary at immature stage.

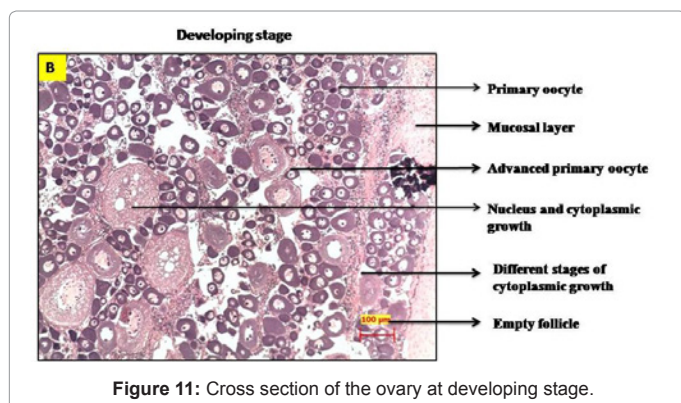


Figure 11: Cross section of the ovary at developing stage.

and 11). Chromatin nucleolus stage oocytes are more abundant than perinucleolar stage oocytes. Primary stage oogonial cells observed in this stage are embedded in the ovigerous tissue and usually found along the periphery of the ovarian lamellae.

Stage II-Developing ovary: At early stage: ovaries are tapered, formed in two distinct, transparent lobes with well-developed blood vessels. No or few individual ova are present. At later stage: developing lobes are filling up to half of the body cavity, with distinctly visible opaque, orange eggs (Figure 11).

Stage III-Mature ovary: The size of oocyte diameter in this stage was from 80 to 520 μm . The oocyte expands generally and regains its rotundity. The nuclei are also increased in relation to its size. The

ovary is containing early and late vitellogenic oocytes. The stage 4 oocytes are abundant in the ovary (Figure 12).

Stage IV-Spawning ovary: Ovaries are larger in size and filled the body cavity. Most of the eggs are the transparent (hydrated) though some opaque eggs may remain. Eggs are extruded from the body under slight pressure or are loose in the ovary and easily separated from each other (Figure 13).

Stage V-Spent ovary: Ovaries are larger, but flaccid, watery, and

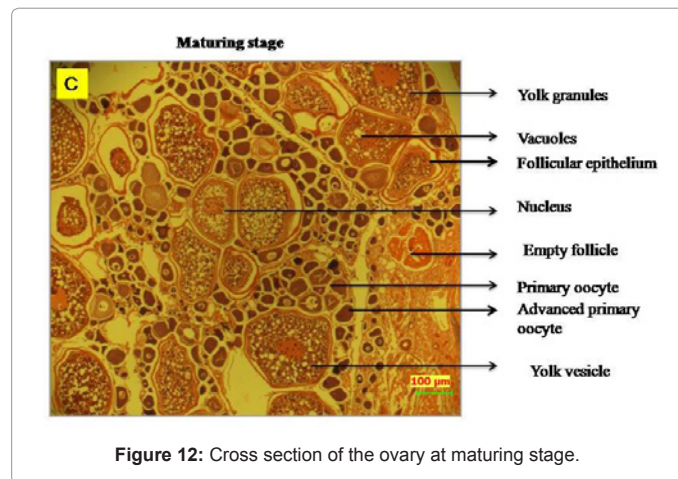


Figure 12: Cross section of the ovary at maturing stage.

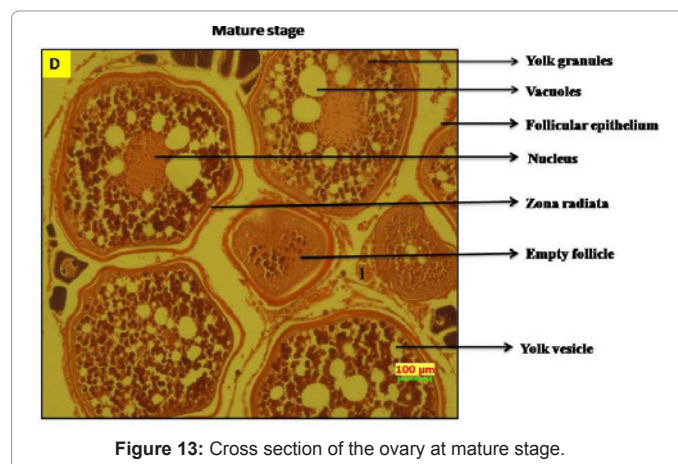


Figure 13: Cross section of the ovary at mature stage.

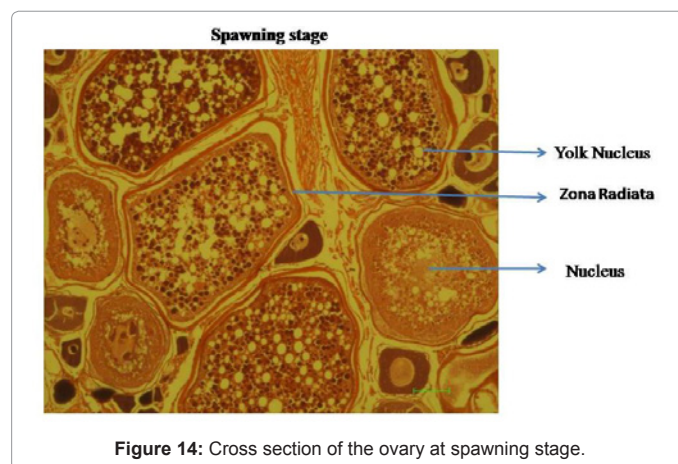


Figure 14: Cross section of the ovary at spawning stage.

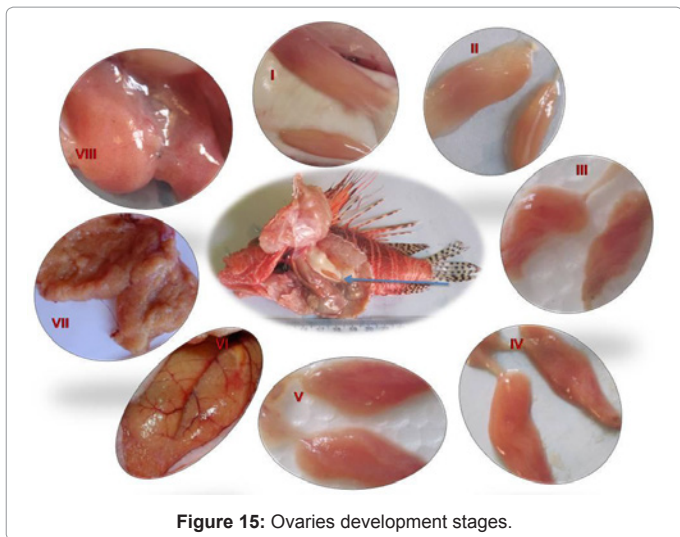


Figure 15: Ovaries development stages.

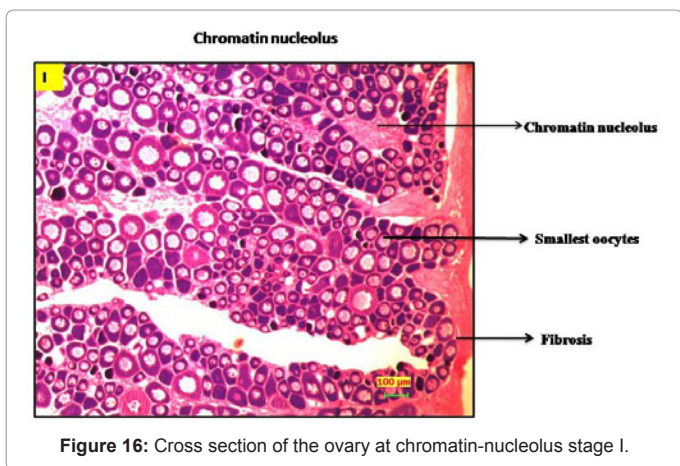


Figure 16: Cross section of the ovary at chromatin-nucleolus stage I.

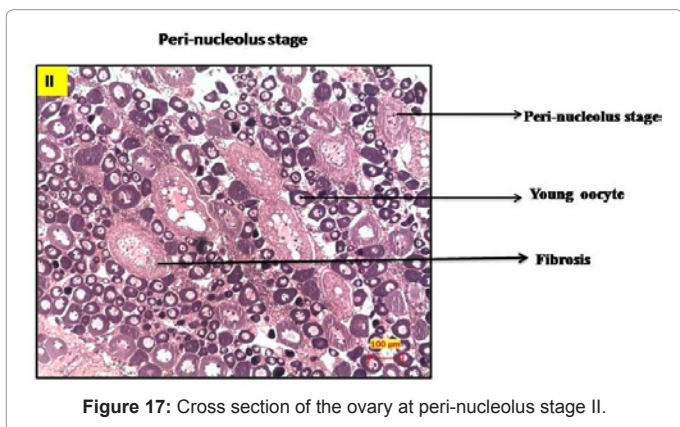


Figure 17: Cross section of the ovary at peri-nucleolus stage II.

generally reddish. Scattered unspawned eggs can be seen. Ovaries that are “Recovering” will appear red and contain scattered eggs, but will not be large or quite as flaccid as very recently spawned ovaries, and should be classified as “Early Developing” (Figure 14).

Observation of ovaries

The development of oocytes of *P. volitans* can be classified into 8 stages (Figure 15) based on cytological characteristics of cells as described below.

Histological observation of the ovaries (Based on the procure of oocyte)

Stage 1: The chromatin-nucleolus stage was comprised of the youngest and smallest oocytes. The large nucleus was surrounded by cytoplasm. The oocytes are remained strongly basophilic, and were deeply stained purple with haematoxylin. Oocyte diameters are ranged between 0.04 to 0.21 mm (Figure 16).

Stage 2: In the pre-nucleolus stage, the cytoplasm had become less basophilic, and stained pale with haematoxylin. At the end of this stage, a number of nucleoli of different sizes were situated in the periphery of the nucleus. Oocyte diameter was ranged from 0.1 to 0.25 mm (Figure 17).

Stage 3: In the primary yolk stage, the size of oocytes has become larger, but they still stained with hematoxylin. Oil-droplets and yolk vesicles began to appear in the cytoplasm. Some yolk globules began to appear in the cytoplasm. Oocyte diameter was ranged from 0.22 to 0.4mm (Figure 18).

Stage 4: In the secondary yolk stage, the accumulation of ova resulted in the rapid growth of oocytes. Yolk globules and oil-droplets rapidly increased in size and number. Oocyte diameter was ranged between 0.32 to 0.84 mm (Figure 19).

Stage 5: In the tertiary yolk stage, yolk globules and oil-droplets are continued to increase in size and number. The nucleus which is located at the centre of the oocyte was spherical, and the nucleus was irregularly shaped. Oocyte diameter was ranged from 0.76 to 1.9 mm (Figure 20).

Stage 6: In the migratory-nucleus stage, the nucleus had moved

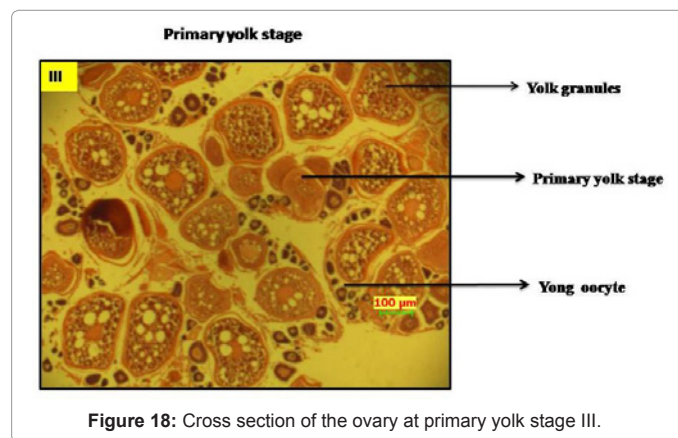


Figure 18: Cross section of the ovary at primary yolk stage III.

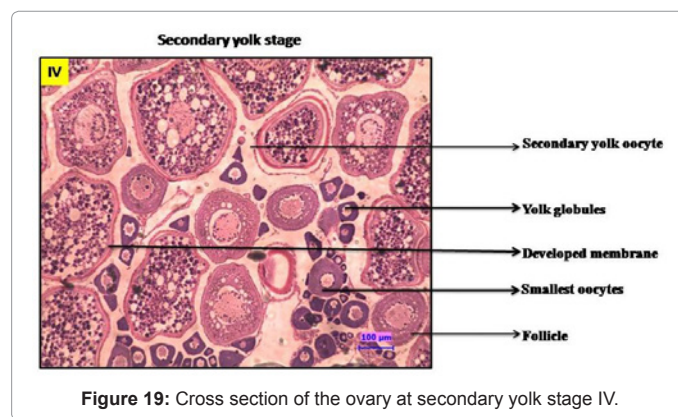


Figure 19: Cross section of the ovary at secondary yolk stage IV.

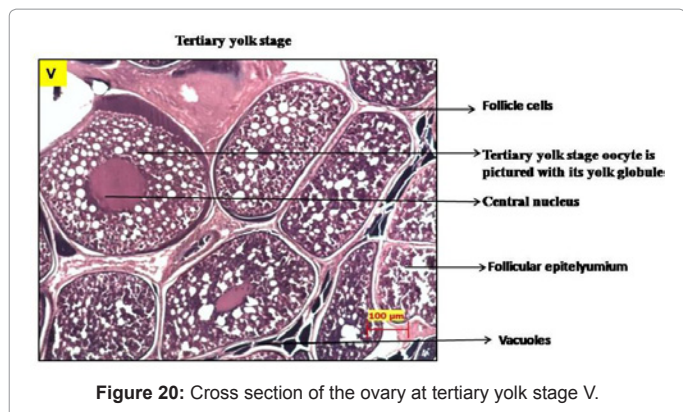


Figure 20: Cross section of the ovary at tertiary yolk stage V.

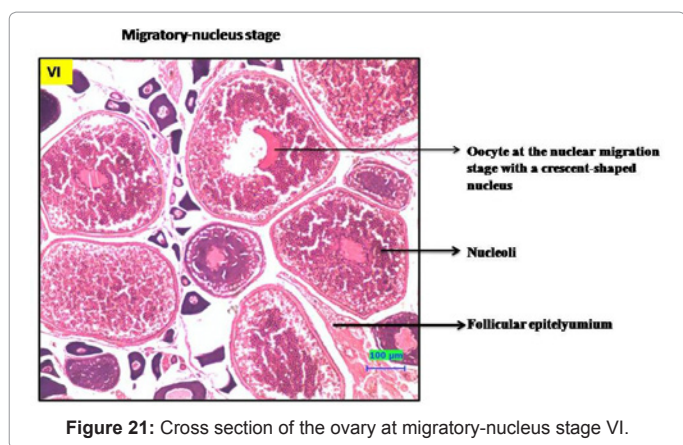


Figure 21: Cross section of the ovary at migratory-nucleus stage VI.

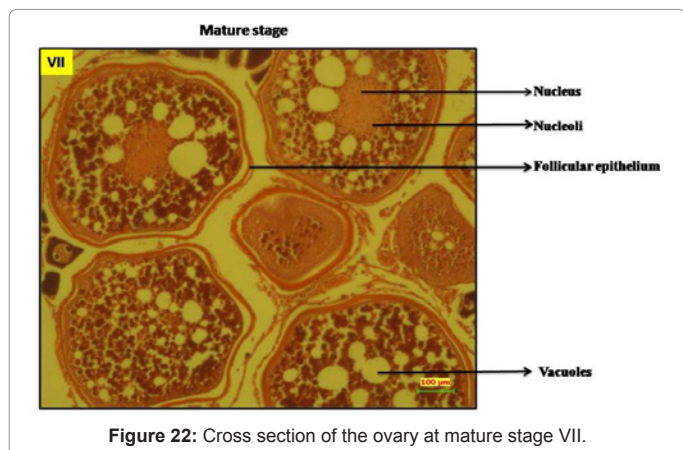


Figure 22: Cross section of the ovary at mature stage VII.

toward the animal pole of the egg, and a few larger oil droplets were found. The oil-droplets first migrated towards the centripetal nucleus. Oocyte diameter was ranged from 1.6 to 2.3 mm (Figure 21).

Stage 7: In the mature stage after germinal vesicle breakdown, yolk globules were fused with each other in the peripheral cytoplasm. Oocyte diameter was ranged from 2.2 to 3.2 mm (Figure 22).

Stage 8: In the postovulatory follicle stage, various types of postovulatory follicles with different morphological features were found. Postovulatory follicles were characterized by a large follicular lumen, formerly occupied by the oocyte. It gradually lost its lumen and was invaded by follicular cells (Figure 23).

The vitellogenic oocytes are continue to expand and reach maximum attainable size before ovulation. The nucleus is well defined in early stage 4 (Figure 21). Yolk vesicles are prominent and usually surround the nucleus in early stage 4 and coalesce towards the centre, when nucleus loses its definition. These yolk vesicles are usually evident in late stage 4 near the oocyte periphery. Acidophilic yolk globules largely replace the basophilic cytoplasm in early stage 4 and become large and well developed in mid stage. The yolk globules coalesce in late stage 4 and present a smooth acidophilic appearance.

Distribution of ova in the ovary

Stage I: Ova in this stage are between 0 and 150 µm in diameter. Majority of oocytes are in 0-50 µm in size. The ova are immature with modes at 0-50 µm and 51-100 µm diameter.

Stage II: Maturing ova with the size from 201 to 250 µm are dominant. The size range of the ova at this stage was from 51 to 300 µm.

Stage III: Largest group of ova with the size from 501 to 550 µm and with secondary modes at 351-400 µm, 401-450 µm, 451-500 µm, 551-600 µm and 601-650 µm.

Fecundity: Fecundity of fishes is usually determined from the number of mature ova in the ovary. In the present study, fecundity of *P. volitans* was determined from the examination of 25 specimens. The fecundity of *P. volitans* (length and width) varied from 13.1×10^3 to 145.7×10^3 with an average number of 75, 547 ova.

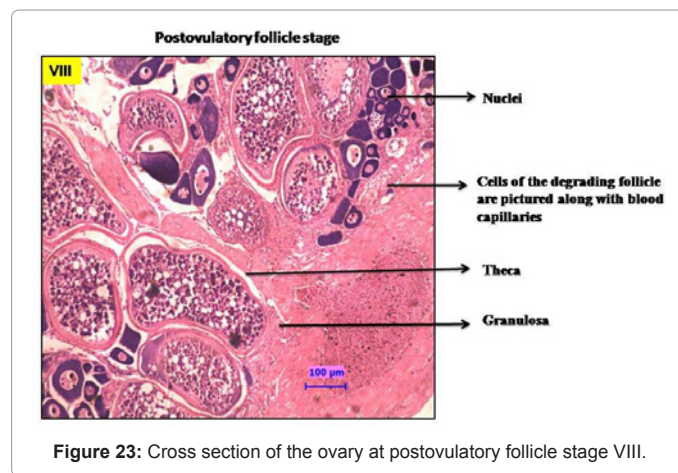


Figure 23: Cross section of the ovary at postovulatory follicle stage VIII.

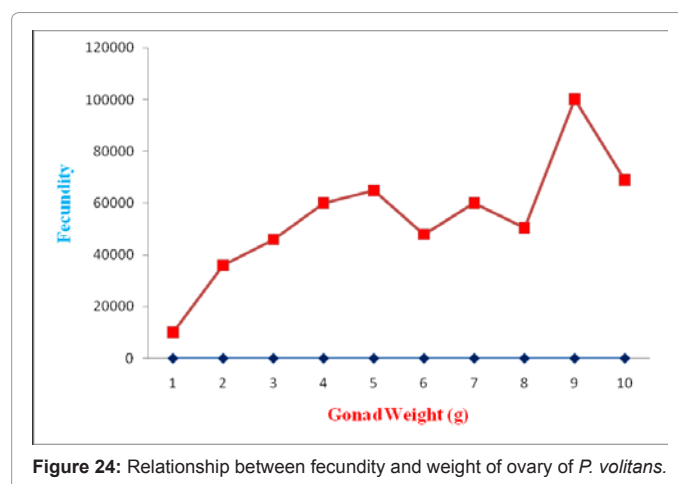


Figure 24: Relationship between fecundity and weight of ovary of *P. volitans*.

Relationship between fecundity and weight of ovary

The number of eggs was plotted against the weight of ovary in a scatter diagram (Figure 24). It was found that the fecundity generally increases with increase in weight of the ovary. The relationship between fecundity and gonad weight in *P. volitans* was linear (Figure 24). The regression of fecundity on gonad weight (GW) can be expressed as

$F=9387.9GW+34026$ with an r^2 value of 0.5723. The values indicated that the correlation was significant.

Relationship between fecundity and total weight of *P. volitans*

The observed values of fecundity for 25 specimens were plotted against the weight of fish in (Figure 25). The relationship between fecundity and weight of fish in female *P. volitans* was linear and it showed a gradual increase of fecundity with increase in total weight. The regression equation of fecundity on total weight can be expressed as $F=11.586TW+72163$ (F =fecundity; TW =Total weight) with an r^2 value of 0.0115.

Relationship between fecundity and total length of *P. volitans*

The number of eggs produced by individuals of *P. volitans* was plotted against the length of fish (Figure 26). In the present study, fecundity showed low correlation coefficient with the total length of the fish. The regression of fecundity and total length can be expressed as $F=677.14TL+56947$ (TL =Total length) and r^2 value was 0.0217.

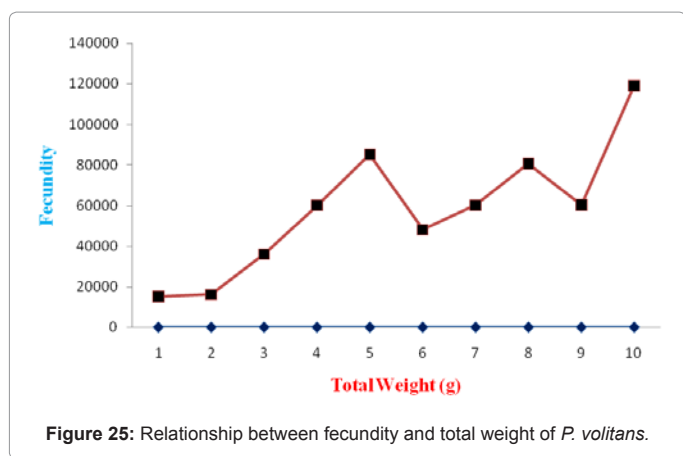


Figure 25: Relationship between fecundity and total weight of *P. volitans*.

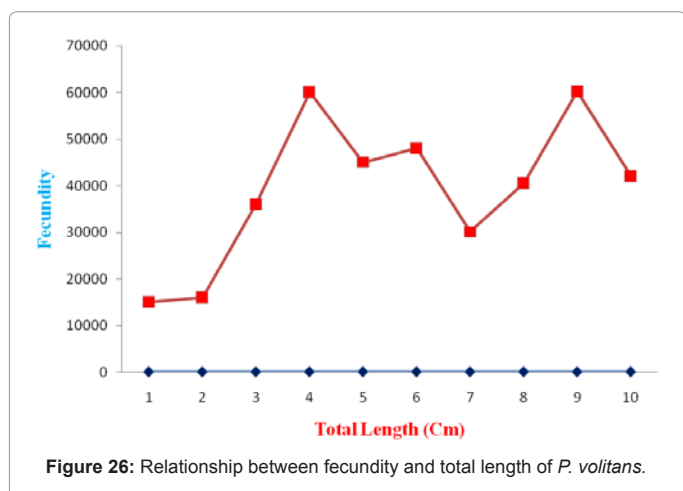


Figure 26: Relationship between fecundity and total length of *P. volitans*.

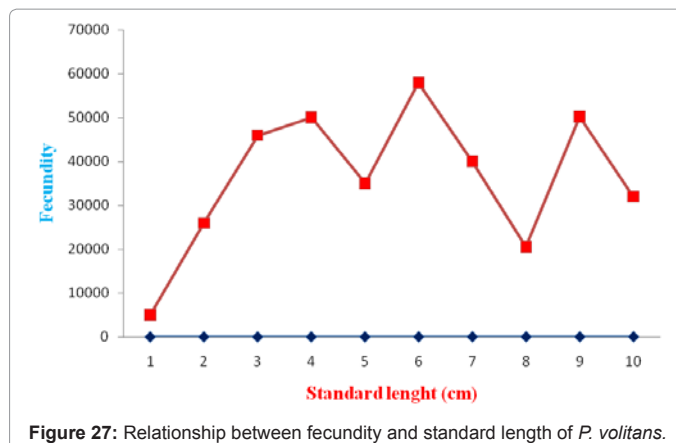


Figure 27: Relationship between fecundity and standard length of *P. volitans*.

Relationship between fecundity and standard length of *P. volitans*

The relation between fecundity and standard length of fish was tested by plotting the observed values in a scatter diagram (Figure 27). In *P. volitans*, it showed a linear regression. The regression of fecundity and standard length can be expressed as $F=556.21SL+63141$ (SL =Standard length) and r^2 value was 0.01.

Discussion

The observations in the present study showed that histological changes in the ovary with the different gonadal developmental stages were recorded in Pterois sp of *P. volitans*. Reproductive studies of the fish require knowledge of the stage of the gonad development in the teleosts. The structural alterations were observed in the *P. volitans* oocytes during the oocyte development by the histological studies. In this study, the oocyte developments of the *P. volitans* were divided into three stages. Reproduction involves changes in growth and development of oocytes during the process of gonad maturation. With the advancement of maturation, oocytes accumulate energy reserves and enlarge further for the onset of embryogenesis. In the present study, *P. volitans* oocyte increase in size from stage I to stage III of gonad maturation. [18] observed that oocyte increased in size with the progression of gonad maturation in *E. malabaricus*. They have reported that the size of the oocyte increased from 0.28 to 0.41 mm with the advancement of vitellogenesis. The above results are similar to the observations made in the present study on *P. volitans*. [19] have noticed that in *E. tukula*, oocyte diameter increases from immature stage (120 μ m) to ripe stage (552 μ m), which is very similar to *P. volitans*. The egg diameter of *E. morio* was found to be less than 1 mm [12]. [20] have found that in *E. guttatus*, with the maturation of the gonads, the egg diameter varied between 0.70 and 0.90 mm. [21] have also observed similar trend in oocyte cyclic development in the immature oocyte (54 μ m) to ripe oocytes (897 μ m) in red grouper, *E. morio*. In the present study, the largest oocyte diameter was 650 μ m in the ripe stage ovary of *P. volitans*. [22-23] observed the largest oocyte diameter was 600 μ m in *P. volitans*. [24] reported eggs of 0.92 mm diameter in *E. striatus*.

Fecundity information of a species is essential for estimating seed production capacity and spawning of the species concerned. Fecundity of the individual fish is determined from the total number of mature ova that are destined to be shed at the ensuing spawning season. In the present study, *P. volitans* gonad weight in relation to the total fecundity showed a significant linear relationship. [25] observed similar relationship between gonad weight and fecundity in *P. volitans*

and *E. bleekeri*. [26] reported that the fecundity is very closely related to the weight of the gonads in *E. aeneus*. The total body weight of *P. volitans* showed a low correlation coefficient with the fecundity. [27] also observed similar relation with total body weight and fecundity in *E. malabaricus*. It may be due to the fact that weight of the ripe gonads in relation to the total body weight of the fish is small. Fecundity in *P. volitans* showed linear relationship with total length and standard length of the fish. It has shown low correlation coefficient, r^2 of 0.0217 and 0.01 respectively compared to the gonad weight ($r^2=0.5841$). Tessy (1994) made similar observations in *E. diacanthus* and *E. bleekeri*. [26] have also observed low coefficient of correlation with the fecundity and standard length in the grouper *E. aeneus*. However, [28] found correlation with the standard length and fecundity in *P. volitans* from the Pacific Ocean.

In the present study, the average fecundity of *P. volitans* estimated was 75,547. Highest fecundity recorded in the present study was 1,45,755. This observation agreed with previous reports; [29] estimated the total potential fecundity in *E. tauvina*, as 258.9 million. [30] found that fecundity of *P. volitans* in the Pacific Ocean ranged from 63,000 to 2,33,000. Bouain and Siau (1983) reported that for equal sizes (standard length=44 cm), *E. saeneus* (Fecundity=0.64 million) was fecundity more than *E. guaza* (F=0.60 million) and *E. salexandrinus* (F=0.43 million). Estimates of potential fecundity in *E. tauvina* ranged from 0.85 million for a fish of 35.1 cm long to 2.9 million for a fish of 62.3 cm long [31]. Hamsa et al. [25] reported that the average fecundity of *P. volitans* was 57,458 and the highest fecundity was 1,65,000.

The condition factor (K) is a measure of fish energy reserves. Condition factor values follow internal variations and seasonal cycles [32]. In the present study, condition factor values are in the range of 1.15 to 1.61 in *P. volitans*. Condition factor has increased in *P. volitans* from stage I to stage III of gonad maturation. [16] reported an increase of conditional factor with the advancement of maturation in *Mugil cephalus*. [33] have also observed an increase of condition factor with the progress of the reproductive season in the fish, *Diplodus puntazzo*. The state of maturity of a fish may be determined by the size of the ovaries. Gonado-somatic index (GSI) indicates the stage and readiness of the ovary for maturation and spawning. Throughout maturation, the GSI values of *Dentex dentex* females were much higher than males implying a greater proportion in body reserves were allocated to the gonads [34]. Gonadosomatic index has been used by many earlier investigators like [35] explain the degree of ripeness of the ovary in a number of fishes. In the present study, the values of GSI for *P. volitans* have showed increasing trend from immature (0.06%) to ripe stage (3.06%). [36] also observed GSI values increasing from 0.43% to 5.2% with the maturation of gonads in *E. malabaricus*. The GSI values obtained in the present study correlated well with the GSI values observed by [37,38] in various size groups of *P. volitans*. [21] noticed greatest variations in the mean gonadosomatic index of female red grouper, *E. morio* from 0.27% to 2.14 % in maturing and ripe running stages.

Histological changes in the ovary of several species of groupers have been shown to correspond well with changes in the GSI. The GSI value increases with corresponding histological changes were also noticed in *E. morio* [39] and in *E. merra* [40]. The present study clearly indicates that the GSI values of female *P. volitans* have also showed similar increasing trend associated with histological changes.

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