

## Study of gonad morphology and histology of goby, *Parachaeturichthys ocellatus* (Day 1873) from the creeks of Mumbai

Bindu Panicker

Department of Biology, Maharashtra College of Arts, Science and Commerce Mumbai, Maharashtra India

### Abstract

The gonad morphology and histology of goby fish *Parachaeturichthys ocellatus* (Day 1873) was studied from July 2010 to September 2011 from the creeks of Mumbai. This study was aimed to understand the reproductive aspect of *P. ocellatus*. The gonad maturity of the fish based on macroscopic and microscopic observation was divided into five stages as 1. Immature 2. Developing 3. Mature 4. Ripe 5. Spent. In male *P. ocellatus* main reproductive organ testis showed regular changes in the testicular cycle with spermatogenic activity with various stages of cells such as spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa. The accessory sexual organ, seminal vesicles appeared at the base of the testes and these opened into the spermatic duct. The gonads and accessory organ in female *P. ocellatus* consisted of a pair of ovaries, a common oviducts and genital papillae. The pattern of oocyte maturation in *P. ocellatus* in immature stage showed asynchronous development since many different stages of ova development were observed simultaneously. Histological studies of ripe stage revealed that all the oocytes mature together and there is hardly any oocytes in any other stage which confirms that *P. ocellatus* is a single spawner. Atretic oocytes were predominantly observed in spent ovary in the state of degeneration. The present study will be helpful in wise management of fishery of *P. ocellatus* from the creeks of Mumbai.

**Keywords:** morphology, histology, spermatogenesis, oogenesis

### Introduction

The study of gonad morphology at the gross anatomical, histological and fine structural levels has become increasingly important in identifying annual reproductive cycles, the length of breeding seasons, the onset of reproductive maturity, spawning rhythms, fecundity and various other aspects of reproductive biology (Parenti and Grier, 2004<sup>[21]</sup>). The validation of gonad maturation stages with histology permits better determination and understanding of the process of gonad maturation by revealing the details of oocyte and sperm development, which present less ambiguity in assigning maturity status (Mendonca *et al.*, 2006)<sup>[17]</sup>. The study of histology of gonads is therefore necessary in the studies of reproductive biology. *Parachaeturichthys ocellatus* is native to Western Indian Ocean and Western Central Pacific ocean at 30° E - 80° E and 45° S - 30° N. It was identified by Day (1873) from the creeks of Mumbai. It was found in plenty in the creeks of Mumbai. Though in terms of value, goby fishes cannot compete with commercial fishes but during monsoon which is a lean period for commercial fisheries; goby fishes do fetch a good price. The local communities residing in the vicinity of the creeks rely on the creek fisheries for their nutritional requirement. A survey of literature revealed no study on gonad morphology and histology of *P. ocellatus*. Hence this study was then undertaken to understand gonad morphology and histology of *P. ocellatus* in particular.

### Materials and Methods

Samples of *P. ocellatus* were collected from the creeks of Malad, Vasai, Thane and Mahul creek every fortnight during the period from June 2010 to September 2011. The gonads from the dissected fish were excised. The length and weight of the gonads were noted and gonad development stage was

determined by visual examination. The stage of maturity and colour of the gonads were recorded. The gonads and fish were then preserved in 5% formaldehyde separately for further studies.

### Morphology of gametes

Developmental stages of gonads were determined from gross visual examination of preserved gonads. Macroscopic and microscopic observation were used following the standard laid down by International council for exploration of sea (Lovern and Wood, 1937)<sup>[16]</sup> and described by Jayashankar (1991b). Macroscopic determination of maturity stages: All gonads were assigned to their developmental stages, based on size, colour, shape, consistency, vascularisation, tunic transparency, and degree of swelling of gonads.

### Histological and staining methods

The gonads fixed in 10% formalin were washed in water to remove excess fixative from the tissues. Processing of the tissues was carried out according to the method suggested by Epple (1967)<sup>[8]</sup>. The tissues were cleaned by tetrahydrofurane and were pre-embedded with xylene-paraffin and then later fully embedded in pure paraffin wax. This was then poured into a mould and cooled in a freezer. The cooled wax block with the gonads was sliced into very thin ribbons at 4-5 micrometre thickness using a microtome. The sections were mounted on a slide and were processed further following the procedure suggested by Humason (1967)<sup>[11]</sup> and Morrison (1990)<sup>[19]</sup>. Two staining methods were used: Haematoxylin Eosin and Toulidine blue. The sections were deparaffinised in xylene and hydrated through graded series of alcohol. Fifty percent of the slides were stained in aqueous Dalafield's haematoxylin for 10 minutes. Following dehydration through alcohol series, the sections

were counterstained with alcoholic eosin (90%) and then subjected to changes for clearing alcohol by xylene. The remaining fifty percent of deparaffinized slides were rehydrated with deionised water and stained with 0.04% Toluidine blue solution for 10minutes. The slides were rinsed with three changes of deionised water and dehydrated with 95% absolute alcohol. All the slides were finally mounted in DPX Mount ant and observed under a microscope. Pic to micro graphs of the different maturity stages were taken using a digital camera.

**Results and Discussions**

In the present study of gonad development in *P.ocellatus* a general pattern similar to many teleost fishes was observed.. For the present study five points scale has been used to classify stages of development in both male and female fishes to understand the phases of maturation of gonads in *P.ocellatus*.

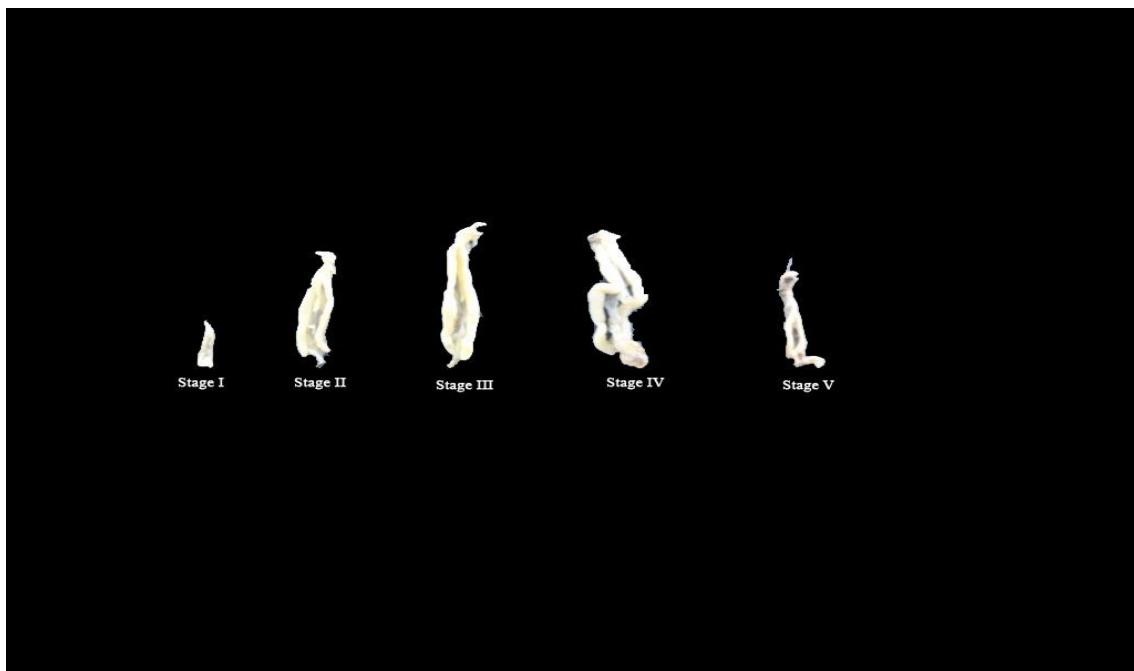
**Gonads and accessory organs in male *P.ocellatus***

The gonads and accessory organs in a mature fish consist of pair of testes, seminal vesicles, sperm ducts and genital papillae. The changes in morphology and histology of these organs reflect on the various stages of maturity leading to

reproductive activities. In fact many of these changes occur in a cyclic manner.

The morphology of testis of male *P.ocellatus* in different stages of maturity is seen in Plate no.1. The five stages of maturity of the gonads and accessory reproductive organs were identified based on morphological and histological observations during the gonadal cycle. The description of the observation of male gonads in different stages of maturity are given in Table no 1.

The creamy white pair of testes in *P.ocellatus* was narrow, elongated and tubular. Each of the testis continued into sperm ducts; the two sperm ducts joined towards the posterior end to form a common duct which opened at the terminal end into the genital papillae. There was a pair of accessory reproductive organ called seminal vesicles. The seminal vesicles appeared as paired organ and were in late stages usually much larger than the testis. At maturity the vesicles showed the presence of vesicular fluid. In some species like *Gobius cobitis* and *Bathygobius fiscus* similar findings were recorded while in *Buenia jeffreysii* and *Acentrogobius cyanomus* seminal vesicles were smaller than testis (Fishelson, 1991) [9]. The seminal vesicles of *P.ocellatus* were glandular structures lined by cuboidal epithelium.



**Fig 1:** Male *P.ocellatus*, testes in different stages of maturity: Stage I-Immature, Stage II- developing, Stage III-Mature, Stage IV-Ripe, Stage V-Spent.

**Table 1:** The macroscopic and microscopic stages of maturity of male testes of *P. ocellatus*.

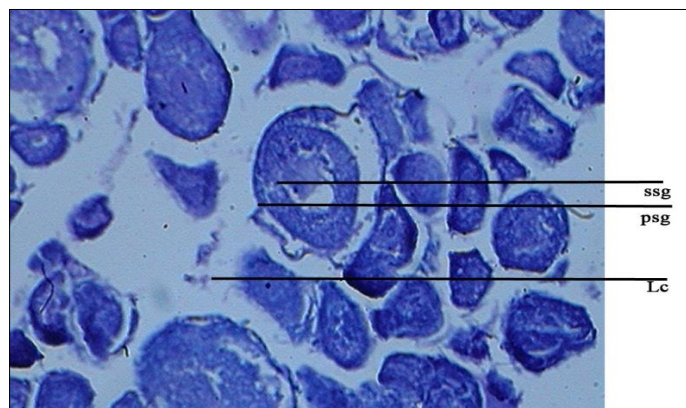
<b>Stage I Immature</b>	
Macroscopic Observation	Small thin thread like white testes occupying 1/10 <sup>th</sup> of the body cavity and weighing 0.010-0.025g. No seminal vesicles seen.
Microscopic observation Plate no.2a	The lobules contain numerous seminiferous tubules of different sizes. The seminiferous tubule contains primary and secondary spermatogonia. Narrow and clear lumen observed. No spermatogenic activity observed.
<b>Stage II Developing</b>	
Macroscopic Observation	Elongated, thickened and white testes occupying 1/6 <sup>th</sup> of the body cavity and Weighing 0.035-0.070g. Seminal vesicles were visible in the posterior end of the testes.
Microscopic Observation Plate no. 2b	The lobules contain many seminiferous tubules. Some of the tubules shows primary and secondary spermatogonia while some germinal cysts along with spermatogonia have primary and secondary spermatocytes. The lumen is prominent. Dispersed Leydig cells are seen.
<b>Stage III Mature</b>	

Macroscopic Observation	Large, elongated, thickened and creamy white testes with crenulated edges occupying 1/5 <sup>th</sup> to 1/4 <sup>th</sup> of the body cavity. And weighing 0.120-0.250g. Seminal vesicles were longer than the length of the testes
Microscopic observation Plate no.2c	Few spermatogonia are observed. Sperm atocytes and spermatids are observed on the walls of the seminiferous tubule. Large number of spermatozoa are observed in the lumen. The spermatids are attached to sertoli cells. Leydig cells are observed.
Stage IV Ripe	
Macroscopic Observation	Thick, swollen, mature and creamy white testes occupying 1/3 <sup>th</sup> of the body cavity and weighing 0.250-0.275g. Milt expulsion took place on slight pressure. The seminal vesicles grew past the testes.
Microscopic observation Plate no.2d	The lumen appears large and is completely filled with spermatozoa. Few spermatocytes are observed. Leydig cells are distinct.
Stage V Spent	
Macroscopic Observation	Shrunken, flat, opaque, compact and white testes reduced to 1/5-1/8 of the body cavity and weighting 0.050-0.010g. No milt expulsion on pressure. The seminal vesicles decreased in size and were not visible in the late spent stage
Microscopic observation Plate no.2e	The spermatozoa are shed. There is lot of empty spaces in the lumen. Few sperm atogonia are seen in the empty tubules. The lobules decrease in size.

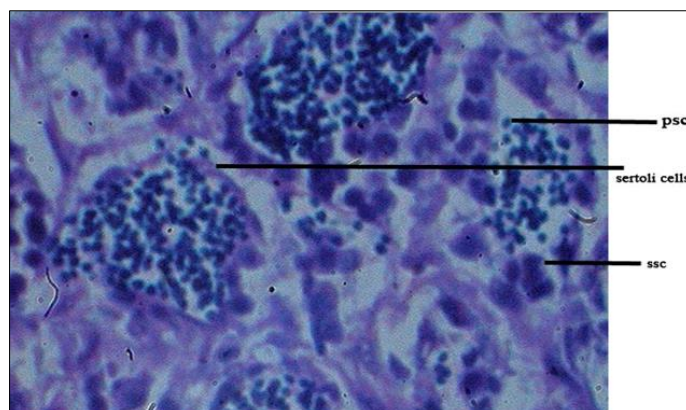
The histological observation of male *P.ocellatus* in different stages of maturity is shown in Plate no. 2a to 2e. The histological study of the testis showed that it was made of two layered wall, outer peritoneum and inner tunica albuginea. The walls were thick in immature testis and were thin in matured one. The testis was mainly composed of numerous lobules which were separated from each other by a thin connective tissue having dispersed interstitial cells or Leydig cells and blood capillaries. The lobules extended from periphery towards the centre. Each lobule was characterized by the presence of seminiferous tubules which contained spermatogenic cells. Various stages of spermatogenic cells were observed such as sperm atogonia, primary spermatocytes, secondary spermatocytes, spermatids, and spermatozoa. The seminiferous tubules opened into the

lumen of the lobule which showed presence of mature sperms. The inner margins of the seminiferous tubules showed sertoli cells. The seminal vesicles are accessory organ of male reproductive system. Seminal vesicles are glandular structures lined by cuboidal epithelium, muscles fibres and blood capillaries. The vesicles contain vesicular fluid.

The morphological and histological structures of male gonads and accessory organs of *P.ocellatus* as described above are similar to those observed in other species of gobies like *Gobius pagnellus* (Miller, 1961) [18], *Oxyeleotris marmoratus* (Suwanjarat *et al.* 2004), *Boleophthalmus boddarti* (Gore, 2007) [10] and *Padogobius martensi* (Cinquetti and Rinaldi, 2009) [6] and *Periophthalmus papilio* (Lawson, 2011) [14].

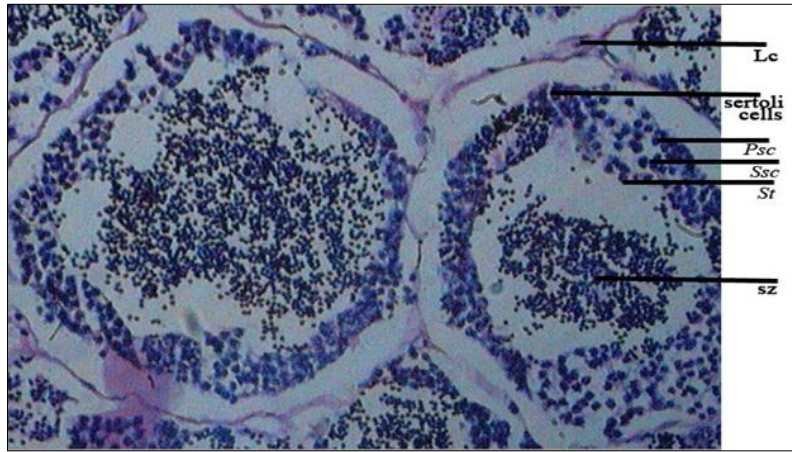


**Plate 2a:** Male *P.ocellatus*, C.S of Testis showing stage I-Immature stage, TB 40X (ssg- secondary spermatogonia, psg- primary spermatogonia, Lc-Leydig cells)

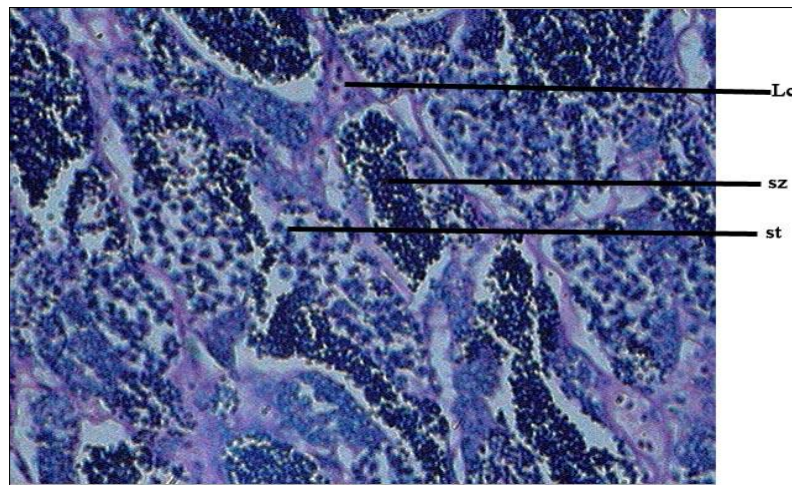


**Plate 2b:** Male *P.ocellatus*, C.S of Testis showing stage II-Developing stage, TB 40X (psc-primary spermatocyte, ssc-secondary spermatocyte)

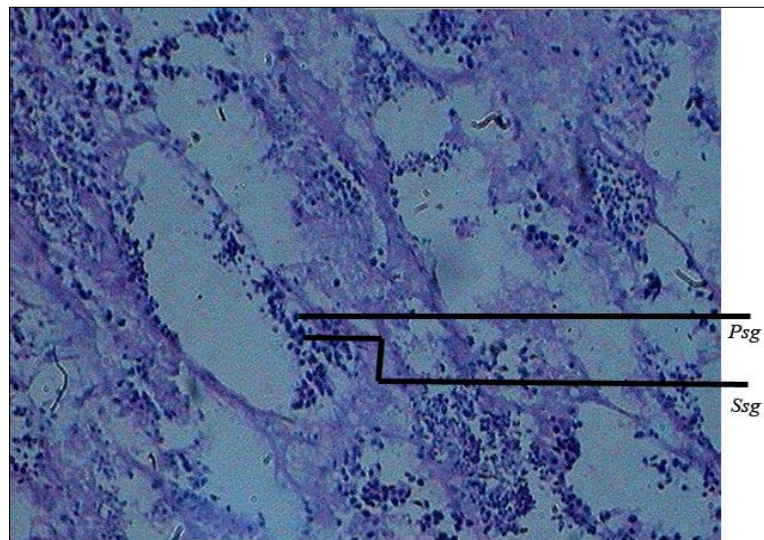




**Plate 2c:** Male *P. ocellatus*, C.S of Testis showing stage III-Mature stage, TB 40X (Lc-Leydig cell, psc-primary spermatocyte, ssc-secondary spermatocyte, st-spermatids, sz-spermatozoa)



**Plate 2d:** Male *P. ocellatus*, C.S of Testis showing stage IV-Ripe stage, TB 40X (Lc-Leydig cells, sz-spermatozoa, st-spermatids)

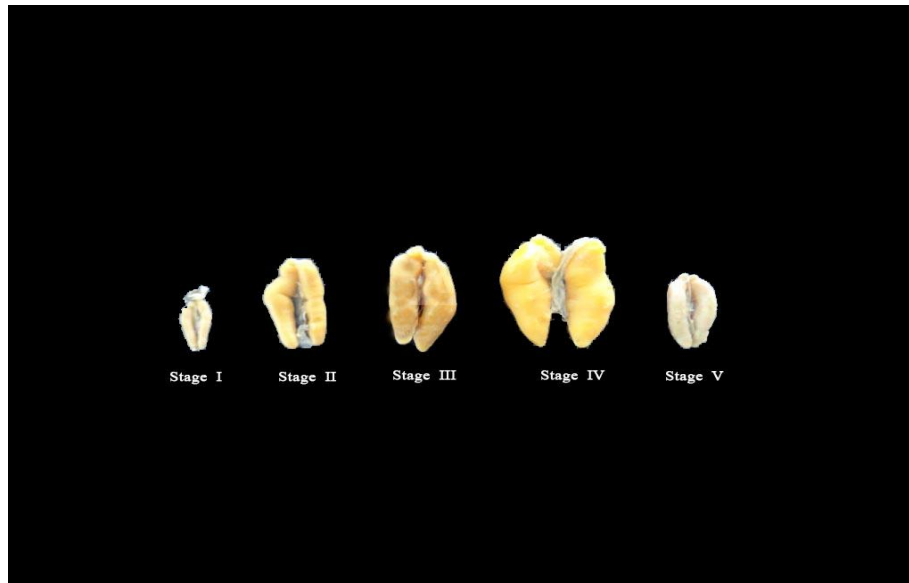


**Plate 2e:** Male *P. ocellatus*, C.S of testis showing stage V-Spent stage, TB 40X (psg-primary spermatogonia, ssg-secondary spermatogonia)

**Gonads in female *P. ocellatus***

The female gonads in different stages of maturity are shown in Plate no. 3. During the present study of *P. ocellatus* five stages of ovarian maturity were identified. The gonads and accessory organ in female *P. ocellatus* consist of a pair of ovaries, a common oviducts and genital papillae. The observation of morphology and histology of gonads of

female *P. ocellatus* is described in Table no.2. The ovarian cycle seem to be reflected in the morphological and histological changes in the ovaries. Pair of ovaries in *P. ocellatus* were fusiform, located ventral to the kidneys and suspended from the coelomic wall by meso-ovarian ligament. The two ovaries fused posteriorly forming a common oviduct which opened into a genital papilla.



**Fig 2:** Female *P. ocellatus*, ovaries in different stages of maturity.

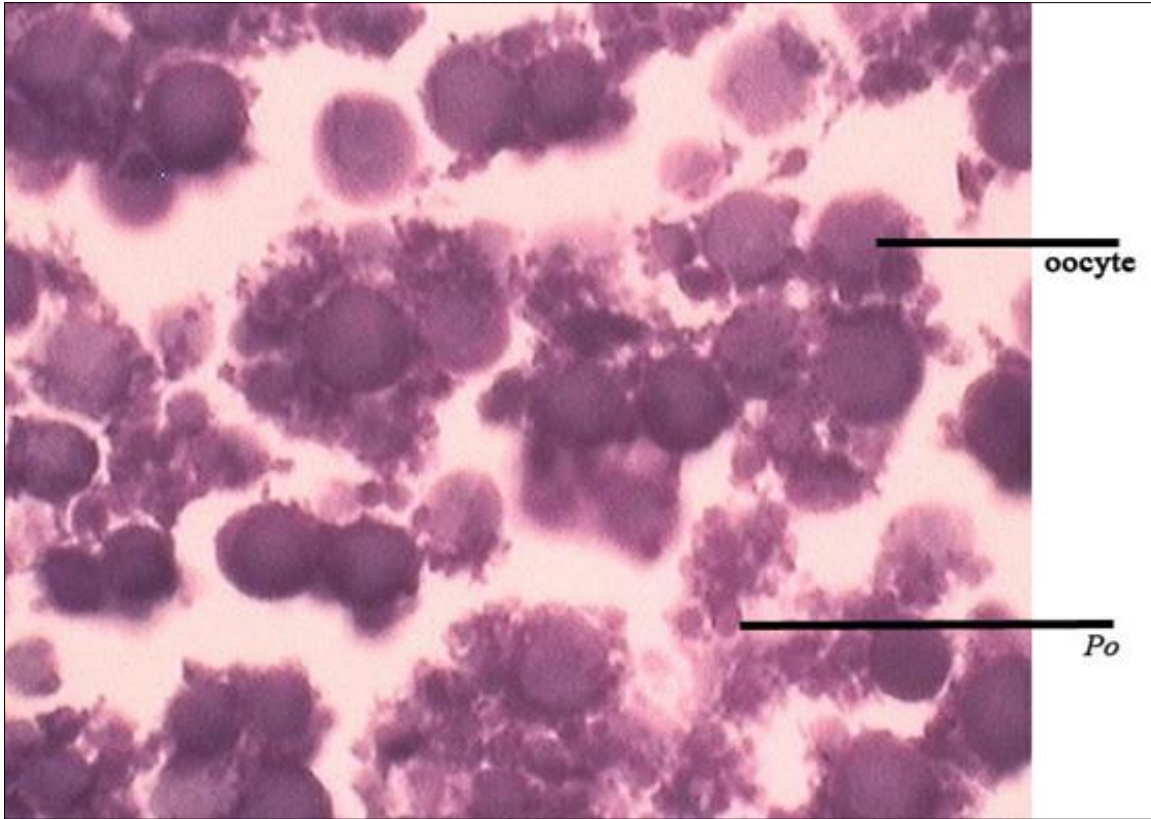
**Table 2:** The macroscopic and Microscopic Observation of different maturity stages of development of ovary

<b>Stage I Immature</b>	
Macroscopic observation	Small, slender, thin, light yellow ovaries occupying 1/8 to 1/6 of the body cavity and weighing 0.015-0.295g.
Microscopic observation Pre- vitellogenic and Perinuclear stage Plate no. 4a	The oocytes of different sizes are seen in the ovary. The cytoplasm is homogenous and is stained. Oocytes are surrounded by rows of follicular cells. The diameter of oocyte varies between 0.01-0.25mm
<b>Stage II Developing</b>	
Macroscopic Observation	Thickened, elongated and clear yellow ovaries occupying 1/4 to 1/2 of the body cavity and weighing 0.2-1.005 g. Network of blood capillaries are seen on the surface of the ovary.
Microscopic observation Early vitellogenic, yolk vesicles stage Plate no. 4b	The oocytes are spherical, oval or polygonal in shape. They show moderate amount of chromophobic cytoplasm, medium in size nucleus with many nucleoli near the nuclear membrane. Numerous yolk vesicles were seen in the cytoplasm surrounding the nucleus of larger oocytes. Smaller yolk vesicles are seen surrounding the nuclear membrane. Larger vesicles are seen towards the periphery which fills the major part of the cytoplasm towards the later stages. The size of the oocyte varies between 0.06-0.35mm
<b>Stage III Mature</b>	
Macroscopic Observation	Highly thickened, swollen, orange ovaries occupying 2/3 <sup>rd</sup> of the body cavity and weighing 0.824-1.590g. A heavy network of blood capillaries appeared on the ovarian surface.
Microscopic observation Late vitellogenic stage Plate no. 4c	Large mature oocytes seen. The nucleus contains many nucleoli. The nuclear membrane is indistinct. Yolk granules, lipid globules and cortical alveoli are present in the ooplasm. The follicular layers and zona pellucida are well differentiated. The size of the oocyte varies between 0.11-0.55mm.
<b>Stage IV Ripe</b>	
Macroscopic Observation	Large, swollen and orange ovaries occupying the entire space of the body cavity and weighing 1.620-3.580g. The gonopore was swollen and on gentle application of pressure the eggs flowed out. Dense network of blood vessels are seen on the ovaries.
Microscopic observation Ripe, yolk globule stage Plate no.4d	The large numbers of mature oocytes with maximum diameter are seen. They are spherical or polygonal in shape. The yolk globules and lipid droplets fill the entire ooplasm as the intra vesicular and inter vesicular yolk deposition takes place. The vacuolated large yolk globules are found in the centre. Peripheral nucleus with disintegrated nuclear membrane and dispersed nuclear material can be observed. The size of the oocyte varies between 0.5-0.70mm
<b>Stage V Spent</b>	
Macroscopic Observation	Flaccid hard yellow ovaries occupying 1/3 <sup>rd</sup> of the body cavity and weighing 0.195-0.575g. The blood vessels were prominent and dark.
Microscopic observation Spent stage Plate no. 4e	The ovarian wall is thick and shows empty follicles. Post ovulatory follicles, immature oocytes and unspawned oocytes are observed. They appear in the active state of reabsorption. Follicles are contracted and tunica wall appears folded. The size of decreased oocyte ranges from 0.06-0.35mm. Atretic oocytes observed in this stage.

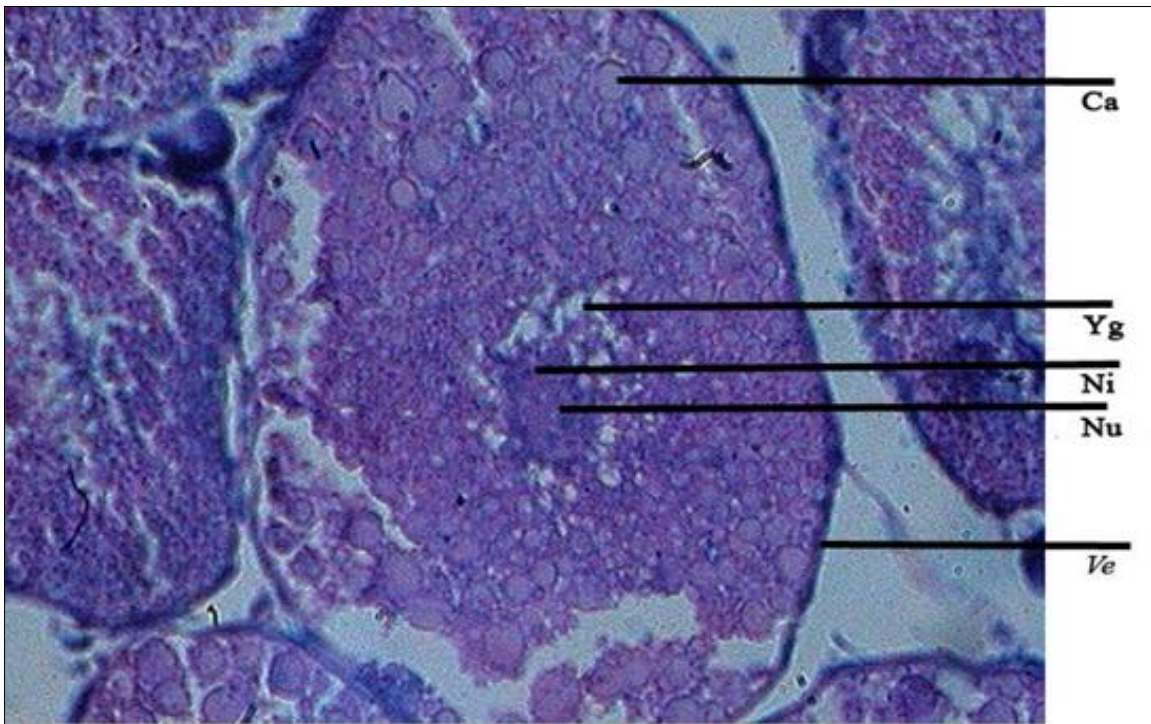


Histological observation of ovary in different stages of maturity is shown in plate no.4a to 4e. Each ovary was sheathed by a thin peritoneal membrane. The ovarian wall was thin in early stages and thickened progressively in the later stages of development. The second layer enveloping ovary the tunica albuginea consisted of connective tissue, smooth muscle fibres and blood vessels.

The inner most layer, the germinal epithelium project into the lumen of the ovary in the form of lamellae. The ovary contain oocytes of various stages of proliferation from germinal epithelium. The oogonia or germ cells occur as clusters or nests in the lamellae. The younger germ cells were found towards the main matrix while the advanced stages were located towards distal end of the lamellae.

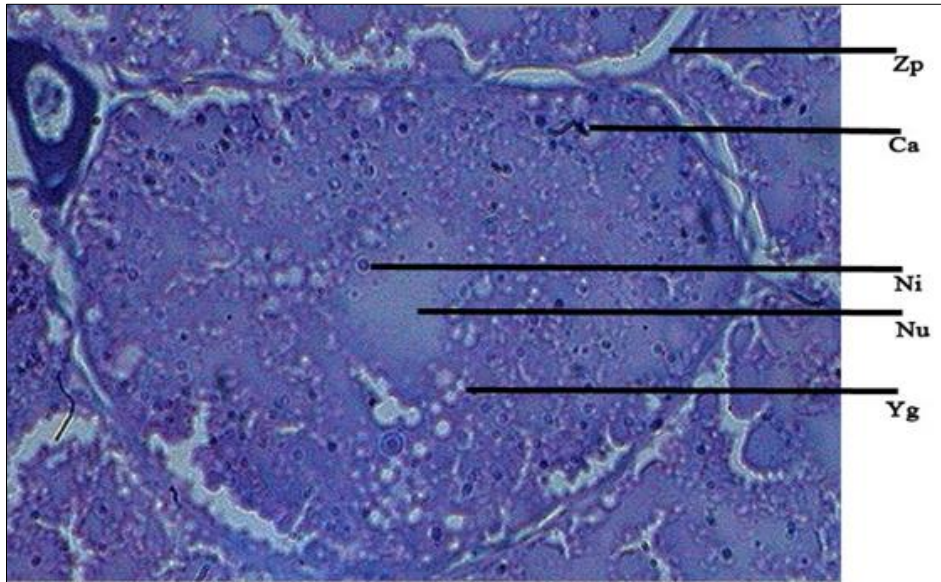


**Plate 4a:** Female *P.ocellatus*, C.S of ovary showing stage I, Immature stage, H&E 40X (Po- primary oocyte)

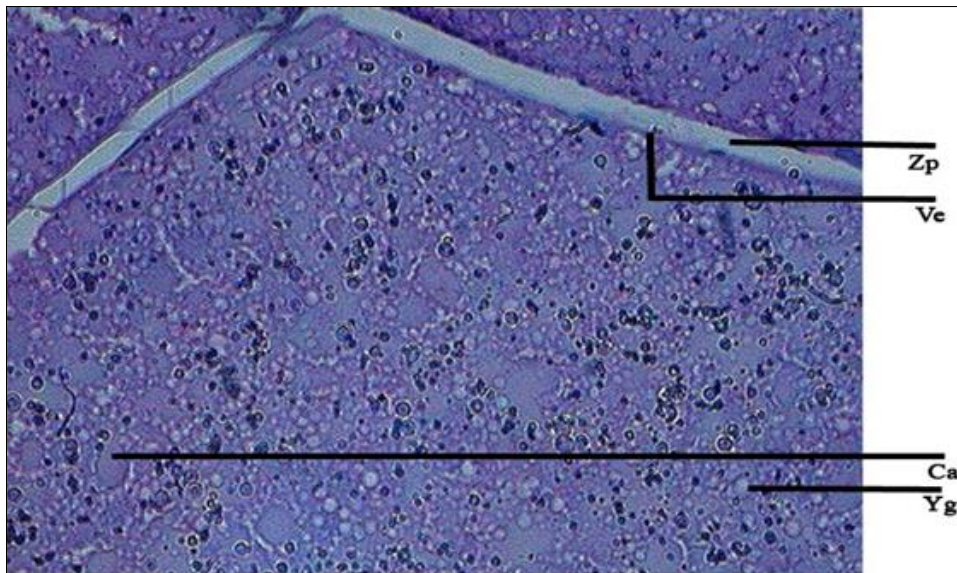


**Plate 4b:** Female *P.ocellatus*, C.S of ovary showing stage II, Developing stage, TB 10X (Ca-Cortical alveoli, Yg-Yolk globules, Ni-Nucleoli, Nu-Nucleus, Ve- Vitelline envelope)





**Plate 4c:** Female *P.ocellatus*, C.S of ovary showing stage III, Mature stage TB 40X (Zp- Zona pellucida, Ca- Cortical alveoli, Ni-Nucleoli, Nu- Nucleus, Yg- Yolk globule)



**Plate 4d:** Female *P.ocellatus*, C.S of ovary showing stage IV, Ripe stage TB 40X (Zp-zona pellucida, Ve-vitelline envelope, ca- cortical alveoli, Yg- Yolk globule)



**Plate 4e:** Female *P.ocellatus*, C.S of ovary showing stage V, Spent stage H&E 40X (Ao-Atretic oocyte, Yg- Yolk globule)

The pattern of oocyte maturation in *P.ocellatus* is similar to that observed in other teleost fishes (Bara, 1960) [4]. In *P.ocellatus* immature ovaries were small slender and light yellow. The Plate no. 4a showed that the oocytes were immature and devoid of yolk. The oogonia in different stages of maturity occurred in clusters in the germinal epithelium exhibiting asynchronous growth. Similar features were observed in many species of goby by Thacker and Grier (2005) [27].

In developing stage the ovaries were thick, elongated and yellow. The vascularisation of ovary was seen in the later stages. The histological preparation showed major developmental changes in the oocyte in this stage. Large number of oocyte in different stages of development was observed. The nucleus was visible in the centre of the developing oocyte. The nucleoli could be seen dispersed towards the periphery of the nucleus. The vitelline membrane was distinct. The ooplasm was peripheral and showed lipid droplets cortical alveoli and small yolk granules in larger oocytes suggestive of endogenous vitellogenesis. Towards the end of this stage ovaries were completely filled with oocytes of different sizes which were clearly visible. This developing stages of ovary was in agreement with many species of gobies like *Oxyeleotris marmoratus* (Boonyoung *et al.*, 2003) [5], *Boleophthalmus boddarti* (Gore, 2007) [10], *Periophthalmus papilio* (Lawson, 2010) [14].

In the third maturing stage an indistinct nuclear membrane with many nucleoli at the periphery could be observed. The chorion or zona pellucida and vitelline membrane was visible. The entire ooplasm was filled with yolk granules and lipid globules. The cortical alveoli though present in peripheral ooplasm were hardly visible in later stages. Similar observation of maturing ovary was recorded in goby *Padogobius martensi* by Cinquetti and Rinaldi (2009) [6]. The increasing number of nucleoli in the later stages is an indication of yolk formation suggestive of special role for nucleoli in the formation of rRNA (Al Mokhtar *et al.*, 1981) [3]. This stage was considered more appropriate to estimate absolute fecundity since the number of oocytes was very close to the number of eggs released (Nunez and Duponchelle, 2008).

Histological observation of ripe stage showed that the oocytes contained ooplasm completely filled with large yolk globules and lipid droplets restricting the nuclear material towards the periphery of the ooplasm thus leading to the formation of an animal pole. Inner to zona pellucida, vitelline membrane was seen. Similar features were observed in many teleost fishes by Nunez and Duponchelle (2009). The multiple spawners are distinguished from single spawners by the presence of vitellogenic oocytes of different sizes (Nunez and Duponchelle, loc.cit.). In *P.ocellatus* the fully grown vitellogenic oocytes were of uniform size, without any small oocytes between them indicating that the fish is not a multiple spawner.

The ovaries of *P.ocellatus* in Spent stage were flaccid and small. During the initial phase the vascularisation was visible. The histological preparation showed that ovaries contained remnants of some oocytes, post ovulatory follicles and few atretic oocytes. The vitelline membrane appeared to be disintegrated. Hardly any yolk could be seen probably indicating its reabsorption. Similar observation was recorded in the zebra fish *Danio rerio* (Koc *et al.* 2008). The occurrence of residuary mature oocyte and empty follicle in a state of reabsorption was observed in *Mystus seenghala*

(Sathysane san, 1961), *Boleophthalmus boddarti* (Gore, 2007) [10] and *Periophthalmus papilio* (Lawson, 2010) [14]. Nunez and Duponchelle (2008) have reported that the ovary with post-ovulatory follicles and atretic oocytes evolve into a resting stage with thicker ovarian wall and large empty spaces within the lamellae. The authors observed that the ovaries remained in this stage until next breeding season when they evolve into stage II.

### Atretic oocyte

The process of degeneration (atresia) is a consistent feature of teleost ovary (Agarwal 1988) [1]. The atretic oocytes in spent ovary have been recorded by Rajalakshmi (1966) [23] in *Gobius guiris* and Rahemo and Al-Shatter (2012) [22] in *Barbus luteus* and *Varicorhinus trutta*. On the contrary atretic oocytes in different stages of development were observed by Al Daham and Bhatti, (1979) [2] and Ravaglia and Maggese (2002) [24]. In the *P.ocellatus* atretic oocytes were predominantly observed in the spent ovary in the state of degeneration.

### Conclusion

The study of morphology and histology of gonads of both male and female confirms the different stages of gonad maturity in *P.ocellatus*. Histological study of male *P.ocellatus* showed spermatogenic activity with cells in different stages of development in mature stage. Maximum number of matured spermatozoa was observed in the mature and ripe stages. Histological studies of ripe ova revealed that all the oocytes mature together and there is hardly any oocytes in any other stage which confirms that *P.ocellatus* is a single spawner unlike other gobies which may spawn many times in a year.

### Acknowledgement

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