



Oocyte growth, gonadosomatic index, hepatosomatic index and levels of reproductive hormones in goldspot mullet *Planiliza parsia* (Hamilton, 1822) reared in captivity

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ABSTRACT

Goldspot mullet *Planiliza parsia* (Hamilton, 1822) is a commercial food fish in South-east Asian countries. Availability of hatchery produced seed is the major bottleneck in the development of aquaculture of this species. Therefore, an attempt was made to develop captive broodstock of *P. parsia* and understand its reproductive maturation. Broodstock reared for eighteen months were sampled and six maturation stages were defined (I-VI) in both the sexes. Ovarian biopsy, gonad histology, gonadosomatic index (GSI), hepatosomatic index (HSI) and reproductive hormones assay were performed in different maturation stages. Diameter of pre-vitellogenic, vitellogenic, post-vitellogenic and ripe oocytes was less than 369.80, 444.38, 477.41 and 665.5 μm , respectively. In the female, the highest value ($p < 0.05$) of GSI (18.31 ± 1.15), HSI (1.84 ± 0.84), 17 β -estradiol (E2) ($898.45 \pm 0.35 \text{ pg ml}^{-1}$), follicle stimulating hormone (FSH) ($81.14 \pm 40.48 \text{ ml U ml}^{-1}$) and vitellogenin (Vtg) were found at stage IV, whereas luteinising hormone (LH) attained its peak at V stage. In males, the concentration of testosterone (T) increased gradually from stage I ($7.60 \pm 0.43 \text{ pg ml}^{-1}$) to VI ($40 \pm 2.89 \text{ pg ml}^{-1}$). Histological observations revealed synchronous type of oogenesis and cystic type of spermatogenesis. The results of this study will help in the captive seed production of this species.

Keywords: Captive maturation, Histology, Ovarian stages, Reproductive hormones, Vitellogenin

Introduction

In fish, as in all vertebrates, the brain-pituitary-gonad (BPG) or hypothalamic-pituitary-gonad (HPG) axis regulates the reproductive function (Kumar *et al.*, 2021). Preoptic-hypothalamic neurons of the brain produce the gonadotropin releasing hormone (GnRH) that stimulates the pituitary gland to synthesise and release the gonadotropins (GtH) (Follicle stimulating hormone, FSH and Luteinising hormone, LH), which, in turn, stimulates gonadal activity (steroidogenesis and gametogenesis) (Kumar *et al.*, 2021). In addition to the GnRH stimulatory system, neurons secreting dopamine (DA) have been identified as an inhibitory factor over the reproductive axis (Peter *et al.*, 1978). Gonad development and corresponding changes in sex steroid level are vital in understanding the spawning pattern of fish (Lee and Yang, 2002; Manosroi *et al.*, 2003). In the course of gonad development, vitellogenesis and final oocyte maturation (FOM) are regulated by GtHs, which mediates their actions through steroid hormones secreted by follicular cells of oocyte (Nagahama, 1994). Estrogen is an important steroid hormone regulating reproductive functions in teleosts (Nagahama and Yamashita, 2008). In female fish, 17 β -estradiol (E2) is estrogen, which regulates ovarian

development through vitellogenesis (Nagahama and Yamashita, 2008). LH controls the synthesis and release of maturation inducing steroid (MIS), which regulates FOM and ovulation in fishes (Nagahama and Yamashita, 2008). Fish under captive conditions generally fail to spawn due to the inhibitory tone of dopamine on the release of the LH and mullets are especially prone to this issue (Aizen *et al.*, 2005).

The goldspot mullet *Planiliza parsia* (Hamilton, 1822) is a euryhaline fish that thrives well in freshwater, brackishwater and seawater (Riede, 2004). It is widely distributed in the coastal waters of tropical and sub-tropical regions (Talwar and Jhingran, 2001) and it is a commercially important fish in South-east Asia, Central and South America (Alam *et al.*, 2008). It is a migratory species that spawns in seawater during December to February months (Talwar and Jhingran, 2001) or November to March (Begum *et al.*, 2010). Due to its commercial importance for aquaculture, several studies have been conducted on reproductive biology of *Liza* sp. (*L. parsia*, Begum *et al.*, 2010; Renjini and Bijoy, 2011; Bose and Chakrabarti, 2014; Bir *et al.*, 2016). Maturation of male and female *P. parsia* in brackishwater pond system at the salinity of 7.0 and 7 ± 1.5 ppt was reported by Alam *et al.* (2008)

and Kumar *et al.* (2020), respectively. However, there are no reports on histological changes in gonads at different maturation stages and corresponding changes in sex hormones in pond reared goldspot mullet *P. parsia*. Therefore, this study was carried out to understand the gonad development and hormonal cycle in different maturation stages of captive reared broodstock of *P. parsia* in order to facilitate the artificial propagation of this species.

Materials and methods

Captive broodstock development

Adult *P. parsia* having bodyweight of 30 to 70 g were collected from a brackishwater canal at Sunderban, West Bengal and transported in an open container with oxygen bubbling to Kakdwip Research Centre (KRC) of ICAR-Central Institute of Brackishwater Aquaculture (ICAR-CIBA), West Bengal, India. Fishes were treated with five ppm KMnO_4 and stocked at a density of 0.25 fish m^{-2} in a tide-fed brackishwater pond (1200 m^2) of KRC and reared for 18 months. After 18 months of rearing, size of fish ranged from 50-120 g. During rearing, fishes were fed twice daily with a pellet diet (32% crude protein and 8% lipid) prepared at KRC; fish were fed at the rate of 3% of fish total biomass. During the spawning season (December-February), physico-chemical parameters of broodstock pond water were measured with a probe (HACH-HQ40d) based analyser.

Ovarian biopsy and maturation stages

During the spawning season of three months (December-February), sampling at an interval of 15 days were carried out in broodstock pond with a drag net. Sampling was carried out six times during the spawning season. In each sampling, more than 50 fish were sampled from the broodstock pond. To assess the gonad development and stage of maturation, ovarian biopsy and macroscopic observation of gonad were made. The fishes were anaesthetised (phenoxyethanol 0.3 ppm), oocytes were collected through *in vivo* biopsy of ovarian tissue (2 mm dia) (Shehadeh *et al.*, 1973) and the diameter of 30 oocytes was measured immediately under a trinocular microscope (Radical RXLr-5, India) supported with the software ProgRes Capture 2.7. Further, to visualise the position of germinal vesicle (GV), oocytes were cleaned with a cleaning solution (ethanol: formalin: acetic acid=6:3:1 v v⁻¹) and observed under the microscope (Kumar *et al.*, 2018). Following biopsy sampling, the fishes were dissected for macroscopic examination of gonads (Kumar *et al.*, 2015). Based on mean oocytes diameter and macroscopic observation of gonad, female fishes were grouped into six different maturation stages, such as I (immature), II (early maturing), III (maturing),

IV (late maturing), V (mature) and VI (ripe/hydrated). Male fish were also anaesthetised and dissected out to make macroscopic observations of testis development. Based on this, male fish were grouped into six different maturity stages from I to VI.

A total of 12 female fishes from each maturity stage *viz.*, I to VI were sampled to collect oocyte, blood, and ovarian tissue. Samples collected from four fishes were pooled for individual maturity stages. Similarly, 12 male fishes from each maturity stage *viz.*, I to VI were sampled to collect blood and testicular tissue. Blood sample collected from four fishes were pooled for individual maturity stages. Hormone assay in different maturity stages of male and female serum samples were performed in triplicate (n=3). Western blot analysis of vitellogenin (Vtg) was executed in pooled sample of individual maturity stages of females.

Tissue fixation and serum separation

The total length (cm) and weight (g) of the individual fish from different maturity stages of both sexes were measured. Blood samples from different maturity stages were collected by puncturing the heart and allowed to clot for 1 h at 4°C and centrifuged for 10 min at 5000 rpm (4°C). The serum was separated and stored at -40°C until further analysis (one week). Following blood collection, fish were dissected out to collect gonad tissues. Gonad tissues were fixed in 10% neutral buffered formalin (NBF) for histological analysis. Ovary tissues were homogenised in 5% lysis buffer (20 mM tris HCl, containing 10% of 0.1mM phenyl methane sulfonyl fluoride, PMSF, pH 8). Homogenate was centrifuged (12,000 rpm, 20 min at 4°C) to collect the supernatant, and the supernatant was stored at -40°C for Vtg semi quantification. Total protein content in serum and ovarian samples was estimated using a modified Lowry protein assay kit (Thermo scientific 23240).

Gonadosomatic index, condition factor and hepatosomatic index

Ovary and testis were dissected out and measured for total length (cm) and weight (g). Gonadosomatic index (GSI) of different maturity stages was calculated as: $\text{GSI (\%)} = (\text{Weight of gonad (g)} / \text{Weight of fish (g)}) \times 100$ (Yuen, 1955). Similarly, hepatosomatic index (HSI) was calculated as: $\text{HSI (\%)} = \text{Weight of liver (g)} / \text{Weight of fish (g)} \times 100$. The condition factor or Ponderal index (K) for each maturity stages was calculated as: $\text{K} = \text{Final mean body weight (g)} / \text{Cube of mean total length (cm}^3) \times 100$ (Clark, 1934).

Gonad histology

Gonad tissue fixed in NBF was dehydrated gradually in increasing ethanol concentrations (70-100%), followed

by dipping in acetone and cleaning in xylene. The tissues were embedded in paraffin wax and cut into 5 μm thickness with the aid of microtome (Thermo, HM325). The tissue sections were stained with haematoxylin and eosin, as described by Roberts (1989), cleared in xylene, mounted in DPX and observed under trinocular microscope.

Hormone assay

Different sex hormones such as FSH, LH, testosterone (T) and E2 were analysed by enzyme linked immunosorbent assay (ELISA). Assay was performed using commercially available enzyme immunosorbent assay (EIA) kits (Cayman Chemical Company, USA). All the used kits were having 100% specificity. Assays were performed following the assay kit protocols. The absorbance of samples and standard was read at 450 nm for FSH and LH and at 415 nm for T and E2 using a microplate reader (BIORAD). Data were quantified against standard curve that was linearised through 4-parameter logistic fit for % B/B₀ (Bound sample/Maximum bound).

Statistical analysis

The comparison of all the studied variables between different maturity stages was done by one-way analysis of variance (ANOVA). All the statistical analyses were performed with SPSS 20.0 for windows. Comparisons were made at 5% probability level.

Results

Physico-chemical parameters of water

Mean values of measured physicochemical parameters viz. temperature, pH, dissolved oxygen and salinity were $19\pm 2.5^\circ\text{C}$, 8.2 ± 0.5 , 4.9 ± 1.5 ppm and 7 ± 1.5 ppt, respectively during the sampling months.

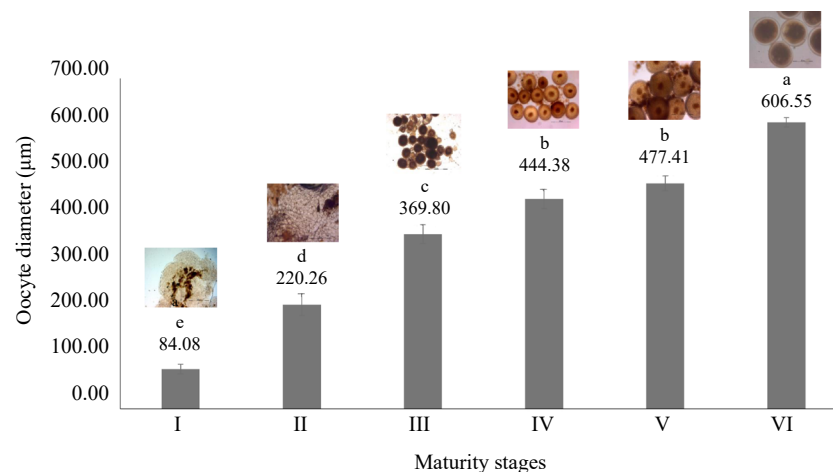


Fig. 1. Oocytes and their mean diameter ($\mu\text{m}\pm\text{SE}$) at different maturity stages of captive reared *P. parsia*. (a) Small oogonial cells, (b) clubbed oocytes, (c) free/separated oocytes, (d) oocytes with centric germinal vehicle, (e) oocytes with eccentric/migrating germinal vehicle, (f) hydrated oocytes (10X). Data bar represent mean \pm SE. Different alphabetical superscripts (a, b, c, d and e) on bar diagram denotes significant differences among different maturation stages ($p<0.05$)

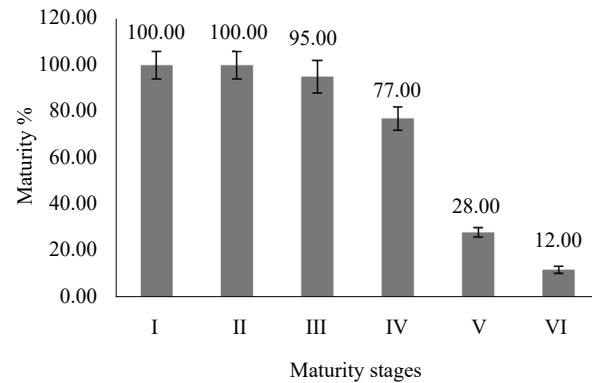


Fig. 2. Percentage of different maturity stages in captive reared stock of *P. parsia*

Observation of ovarian tissue immediately after cannulation

In the present study, six different maturity stages of gonad development namely, I (immature), II (early maturing), III (maturing), IV (late maturing), V (mature) and VI (ripe) were observed based on macroscopic observation of gonad and size of the oocyte. Oocytes of different maturity stages and their size are described in Fig. 1 and Table 1. The percentage of different maturity stages in captive reared stock of *P. parsia* is shown in Fig. 2.

GSI and HSI

The value of GSI increased gradually as maturation progressed. In both the sexes, significantly ($p<0.05$) high value of GSI (male: 1.23 ± 0.06 ; female: 18.31 ± 1.15) was estimated at stage VI. The GSI value of six different maturity stages of the female is depicted in Fig. 3a. The mean value of GSI ($\pm\text{SE}$) for the six different maturity stages

Table 1. Macroscopic and microscopic (histological) description of ovarian development of stages of *P. parisa*

Maturity stages	Oocyte stages	Oocyte dia. (μm)	Macroscopic and histological description
I (<100)	Oogonia	20-100	Ovary thin, thread like and translucent in structure. Ovarian biopsy showed presence of mass of oogonia cells (Fig. 1a). Histologically oogonia cells were characterised by basophilic cytoplasm and light coloured large nucleus. Oogonia cells were without distinct boundaries (Fig. 5a).
II (100-350)	Chromatin	100-275	Ovary was thicker and translucent in structure. Ovarian biopsy showed presence of clubbed oocyte cells (Fig. 1b). Histological observation displayed presence of many chromatin nucleolar oocytes and few perinucleolar oocytes (Fig. 5b).
	Perinucleolar	275-300	
III (350-400)	Larger chromatin nucleolar	150-275	Ovary fleshy and pinkish in colour with longer left lobe. Tissue collected through ovarian biopsy showed presence of distinct and separated oocytes (Fig. 1c). Histologically observation showed the presence of chromatin nucleolar, perinucleolar and few cortical alveolar oocytes (Fig. 5c). Chromatin nucleolar oocytes characterised by presence of strongly basophilic cytoplasm (dark blue staining with haematoxylin) with centrally located large nucleus and single basophilic nucleolus (Fig. 5d), perinucleolar oocytes were polygonal in shape with less basophilic cytoplasm, single large nuclei and multiple nucleoli on the periphery of nuclear membrane (Fig. 5e), cortical alveolar oocytes characterised by presence of few vacuoles in the cytoplasm (Fig. 5f).
	Larger perinucleolar	275-350	
	Cortical alveolar	350-400	
IV (400-450)	Early-vitellogenic	400-410	Ovary orange-yellow in colour with thin blood vessels on ovarian wall. Oocytes obtained after ovarian biopsy characterised by distinct egg chorion, centrally located germinal vehicle (nucleus) and lipid droplets. Oocytes were discernible with naked eyes (Fig. 1d). Histological observation showed the presence of centrally located nucleus, uniform distribution of yolk granules/globules and oil globules in cytoplasm, and cortical alveoli/yolk vesicle in the periphery of the cytoplasm (Fig. 5g). Yolk granules acidophilic (stain pink with acidic dye <i>i.e.</i> eosin), with deposition in cytoplasm centripetal (Fig. 5h). At this stage, two distinct layers of egg chorion (theca and granulosa) appeared. Size of oil globules and yolk granules were $28.53\pm 4.35 \mu\text{m}$ and $9.89\pm 0.02 \mu\text{m}$, respectively.
	Vitellogenic	410-440	
	Post-vitellogenic	440-450	
V (450-500)	Eccentric	450-470	Ovary yellow in colour with prominent blood vessels. Oocytes obtained after ovarian biopsy characterised by presence of eccentric germinal vehicle (migratory germinal vehicle) and fused lipid droplets (Fig. 1e). Microscopic observation revealed the increase in number and diameter ($12.35\pm 1.5 \mu\text{m}$) of yolk granules. Coalesce of lipid droplets was intense around the nucleus (Fig. 5i). Theca and granulosa layer were more distinct (Fig. 5j).
	GVBD	470-500	
VI (500-600)	Hydrated (ripe)	>500	Ovary completely filled the body cavity and with slight pressure eggs come out. Oocytes obtained from ovarian biopsy had homogeneous mass of yolk protein and clear egg chorion. Homogenisation of yolk protein due to intense fusion of lipid droplets and yolk protein (Fig. 1f). Histological observation showed the presence of migrating amoeboid shaped nucleus towards animal pole and absence of follicular cells around oocytes indicates eggs are ready for spawning (Fig. 5k). At this stage, thickening of chorion and differentiation of <i>zona radiata</i> was clear (Fig. 5l). Fusion of yolk granules was intense to form homogeneous mass of yolk (homogenisation of yolk granules). Fusion of small oil globules / vacuoles with each other forms large vacuole, which is characteristic of pelagic eggs (Fig. 5l). At this stage, diameter of oocytes was $606.55\pm 9.83 \mu\text{m}$.
VII (spent)	--	---	Spent stage is not represented here because this fish does not spawn naturally in captive condition.

of male was 0.15 ± 0.03 , 0.63 ± 0.07 , 0.94 ± 0.05 , 0.85 ± 0.05 , 0.97 ± 0.01 , 1.23 ± 0.24 , respectively. Significantly ($p<0.05$) highest (1.84 ± 0.84) and lowest (0.33 ± 0.13) HSI values were observed at stage IV and I, respectively (Fig. 2a).

Hormone assay

E2 level in serum changed with ovarian developmental stages and the significantly highest ($p<0.05$) level of E2 was observed at stage IV (894.30 ± 49.02 pg ml⁻¹), which did not vary significantly with that of III and V stages (Fig. 3b). FSH profile showed a similar trend as that of E2, with a peak value at stage IV (81.14 ± 40.48 ml U ml⁻¹) (Fig. 3c). The LH concentration increased gradually and peak at stage V (41.34 ± 12.23 ml U ml⁻¹) (Fig. 2c). In males, the concentration of T increased gradually from stage I (7.60 ± 0.43 pg ml⁻¹) to VI (40 ± 2.89 pg ml⁻¹) (Fig. 2d). In females, T was undetectable at stage I; however, it reached the maximum value (32.5 ± 3.13 pg ml⁻¹) at stage II (Fig. 3d).

Expression of Vtg

Western blot analysis of Vtg protein in serum and ovarian tissue, after SDS-PAGE under reducing conditions, exhibited a positive reaction to the polyclonal

antibody against sea bream Vtg (rabbit anti-seabream vitellogenin) (Fig. 4a and c). In serum, the signal value of Vtg was poor at I, II and III developmental stages. In contrast, it was high at IV, V and VI stages (Fig 5b). A similar trend was noticed in ovarian tissues (Fig 5c). This analysis revealed antibody specificity to 170.58 ± 2.58 kDa (~170 kDa) molecular weight protein. Nevertheless, an additional faint band of low molecular weight (95, 72, 46 and 29 kDa) was detected in serum and ovarian samples of IV, V and VI maturation stages. To validate the western blotting procedure, expression of β -actin protein as control was performed. Based on expression of Vtg, maturation stage I, II and III are named as pre-vitellogenic stage, stage IV as vitellogenic stage, stage V as post-vitellogenic stage and stage VI as ripe/hydrated stage.

Macroscopic and histological observation of gonad

The detailed description of macroscopic and microscopic (histological) changes in ovary and testis of different maturity stages are shown in Tables 1 and 2; Fig. 5 and 6), respectively. Oocytes of pre-vitellogenic (I, II and III), vitellogenic (IV), post-vitellogenic (V)

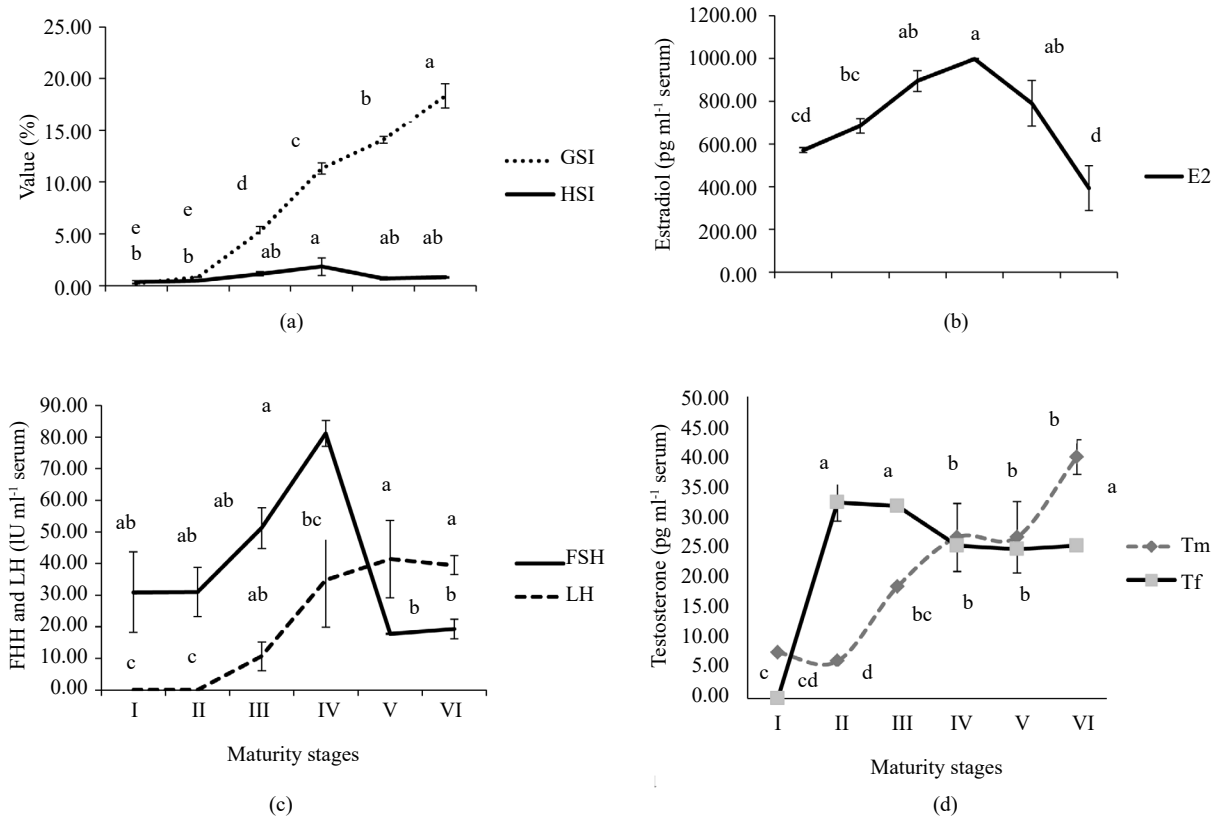


Fig. 3. (a) Gonadosomatic index (GSI); Hepatosomatic index (HSI), (b) Estradiol (E2), (c) Follicle stimulating hormone (FSH), Luteinising hormone (LH) and (d) Testosterone (T) in different maturity stages (I to VI) of captive reared *P. parsia*. Data line represent mean±SE. Different alphabetical superscripts on line diagram denotes significant differences among different maturation stages ($p<0.05$)

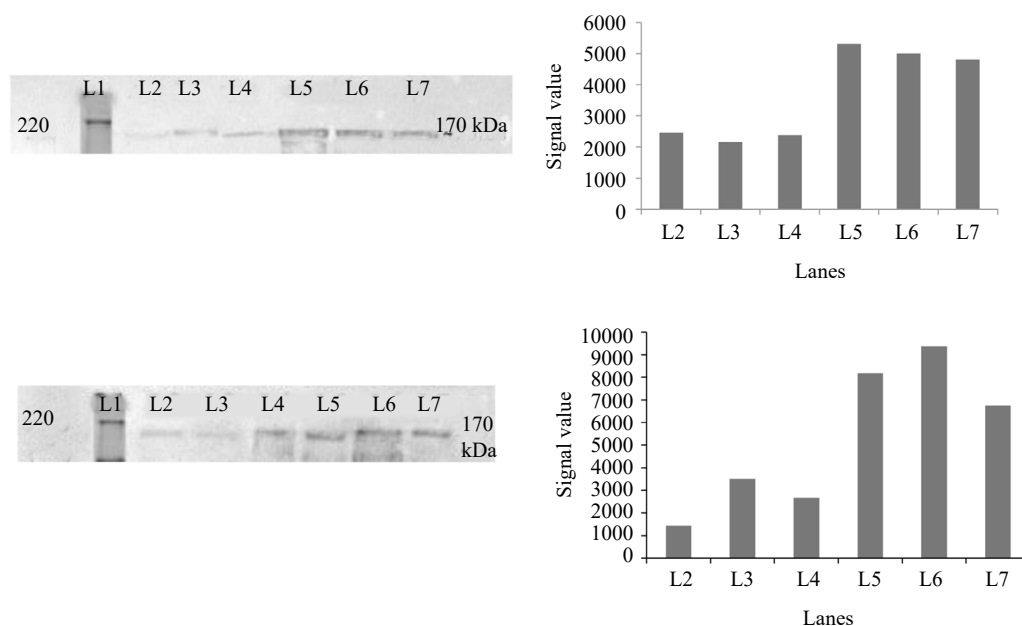


Fig. 4. Western blot analysis showing expression of vitellogenin (Vtg~170 kDa) protein in *P. parsia*. Lane (L) 1-Molecular weight marker and other lanes L2, L3, L4, L5, L6 and L7 represent different maturity stages from I to VI, respectively. (a) Expression of Vtg in serum of different maturation stages of female *P. parsia*; (b) Corresponding signal value of serum Vtg expression in *P. parsia*; (c) Expression of Vtg in ovarian tissue of different maturation stages of female *P. parsia*; (d) Corresponding signal value of ovarian tissue Vtg expression in *P. parsia*

Table 2. Macroscopic and microscopic (histological) description of testicular developmental stages of *P. parisa*

Maturity stages	Macroscopic and histological description
I	Testis at this stage was thin, thread like and translucent. Histological sections revealed that testis is composed of many lobules. Primordial germ cells and spermatogonial cells seen in the peripheral and central parts of lobules, respectively (Fig. 6a). Lobules were separated from each other by interstitial compartment, which has connective tissue, leydig cells, blood vessels, fibroblasts and collagen fibres (Fig. 6b). Cluster of spermatogonia seen on the inner margin of the lobules, surrounded by basophilic sertoli cells (Fig. 6b). Spermatogonia, measured $6.42 \pm 0.85 \mu\text{m}$ in dia. At this stage lumen of lobules generally empty.
II	Macroscopic observation showed translucent, thread like, fleshy and thicker testis. At this stage, most of central lobules were filled with equal proportion of spermatogonia and spermatocyte cells, whereas peripheral lobules had only spermatoginal cells (Fig. 6c, d).
III	Testis light pink in colour with smooth and fleshy texture. Histological observation of this stage showed predominantly spermatocytes, spermatids and few spermatozoa in the lumen (Fig. 6e).
IV	Testis white in colour with smooth texture. Histological observation showed approximately equal proportions of spermatocytes ($3.36 \mu\text{m} \pm 0.26$ dia), spermatids (1.99 ± 0.52 dia) and spermatozoa ($0.86 \mu\text{m} \pm 0.24$ dia) (Fig. 6f).
V	Macroscopic and microscopic observations showed that this stage was similar to stage IV, except for numerous spermatozoa (Fig. 6g).
VI	This is oozing stage of maturation. Testis white in colour, milt extrude out on gentle pressure on the abdomen. At this stage, lobules were distended and all the lobules were filled with developed spermatozoa (Fig. 6h, i).

and ripe/hydrated (VI) stage had a mean diameter of 369.80, 444.38, 477.41 and 606.55 μm , respectively. Sera cleaned oocytes in stage I, II and III had no clear nucleus. However, centric germinal vesicle (GV), migrating GV and germinal vesicle breakdown (GVBD) was noticed in the oocytes of stage IV, V and VI, respectively.

Discussion

The ovarian developmental stages in fish are classified based on the oocyte growth (Honji *et al.*, 2009; Lubzens *et al.*, 2010) and it varies from five to nine stages (Shinkafi *et al.*, 2011). In the current study, six different

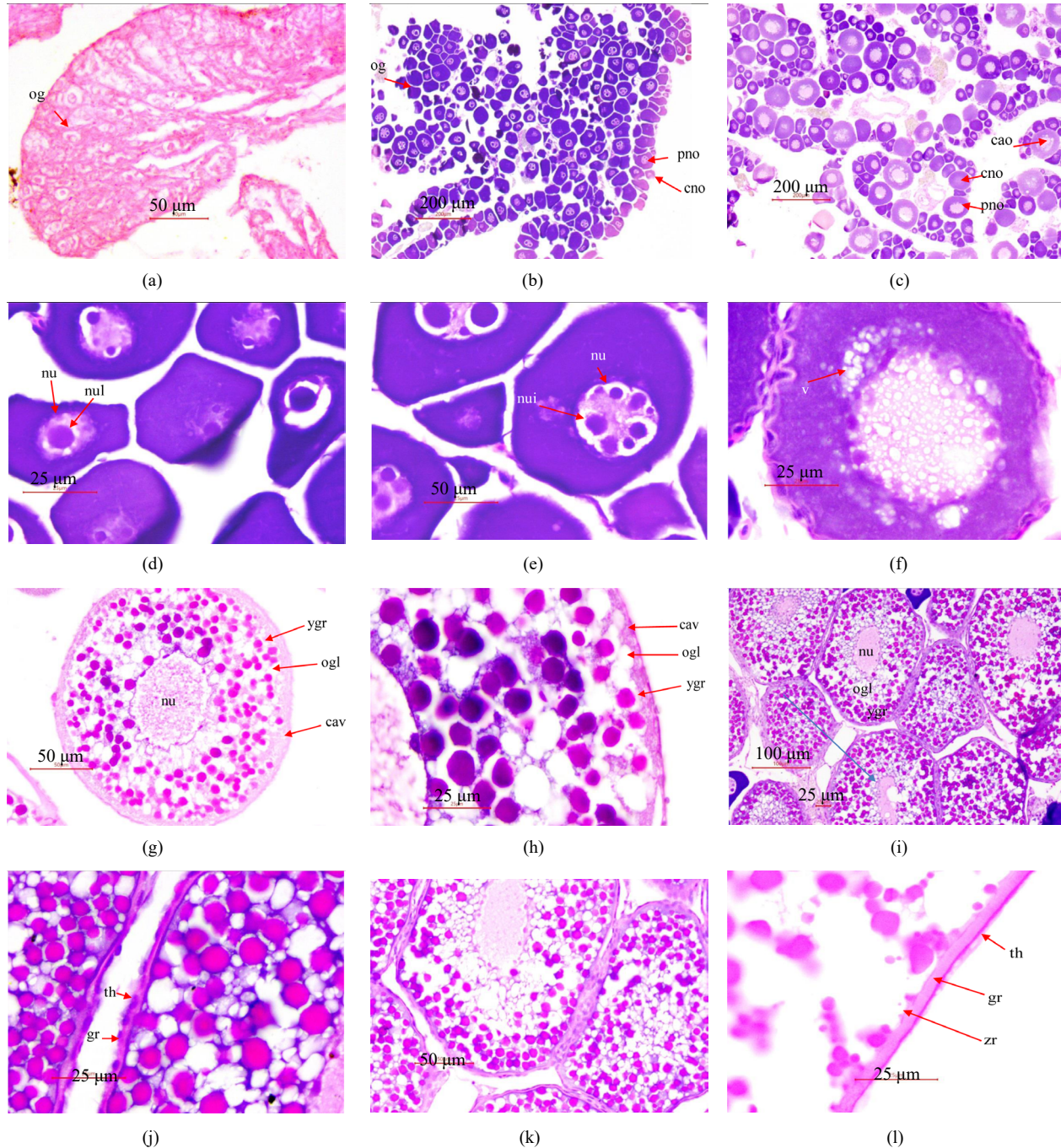


Fig. 5. Histological sections of *P. parsia* ovary at different maturity stages. (a) Stage I: Ovary filled with cluster of oogonia cells (og) (H&E; x400); (b) Stage II: Dominated with chromatin nucleolar oocytes (cno) and few perinucleolar oocytes (pno) (H&E; x100); (c) Stage III: filled with chromatin nucleolar, perinucleolar and few cortical alveolar oocytes (cao) (H&E; x100); (d) Chromatin nucleolar oocytes, characterised by the presence of centrally located large nucleus (nu) with single basophilic nucleolus (nul) (H&E; x1000); (e) Perinucleolar oocytes, characterised by the presence of multiple nucleoli (nli) on the periphery of nucleus membrane (100X); (f) Cortical alveolar oocytes, characterised by the presence of vacuoles (v) in the cytoplasm (H&E; x1000); (g) Stage IV: Cytoplasm of oocytes filled with yolk granules (ygr), few oil globules (ogl) and periphery had cortical alveoli (cav) (H&E; x400); (h) Stage IV oocyte at higher magnification showing cortical alveoli, separated yolk granules and oil globules (H&E; x1000); (i) Stage V: Yolk granules number and diameter increased, and oil globules fused intensely around nucleus (H&E; x200); (j) Theca (th) and granulosa (gr) layer were distinct at the stage V (H&E; x1000); (k) Stage VI: Oocytes characterised by presence of migrating amoeboid shaped nucleus (anu), large vacuole (star) due to intense fusion of oil globules, homogeneous mass of yolk caused by fusion of yolk granules (arrow) (H&E; x200); (l) Thickening differentiation of chorion into outer theca cell (tc), middle granulosa cell (gc) and inner zona radiata (zr) (H&E; x1000)

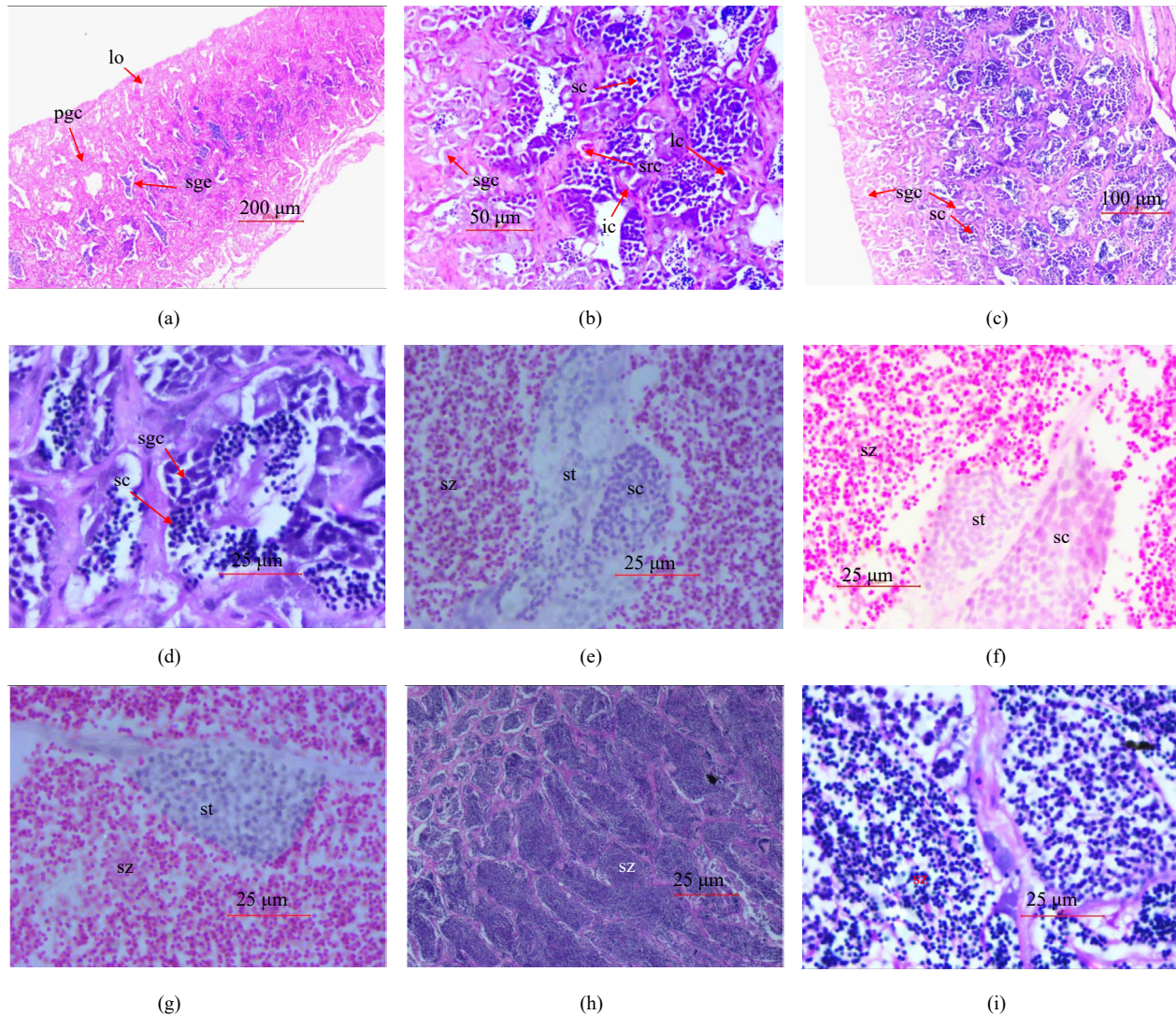


Fig. 6. Histological sections of *P. parsia* testis at different maturity stages. (a) Stage I: Testis lobe composed of many lobules (lo), peripheral lobules filled with primordial germ cells (pgc), and a few central lobules have spermatogonia cells (sgc) (H&E; x100); (b) Lobules separated from each other by interstitial compartment (ic), which has leydig cells (lc); cluster of spermatogonia cells surrounded by sertoli cells (src) and few spermatocytes cells (sc) present inside lobules (H&E; x400); (c) Stage II: Peripheral lobule had only spermatogonia cells whereas central lobules had equal proportion of spermatogonia and spermatocyte cells (H&E; x200); (d) At higher magnification spermatogonia and spermatocytes cells were distinct (H&E; x1000); (e) Stage III: Lobules were predominated with spermatocytes, spermatids (st), and few spermatozoa (sz) in the lumen (H&E; x1000); (f) Stage IV: Equal proportions of spermatocytes, spermatids and spermatozoa were seen (H&E; x1000); (g) Stage V: Predominated with spermatozoa cells and few spermatids (H&E; x1000); (h) Stage VI: Lobules were completely filled with well-developed spermatozoa cells (H&E; x100); (i) Well developed spermatozoa shown at higher magnification (H&E; x1000)

maturation stages were observed based on macroscopic and microscopic observation of gonad, which is similar to the maturation stages of *M. cephalus* (Assem *et al.*, 2008). However, it differs from the view of Albieri and Araujo (2010), who described four different maturation stages in *Mugil liza*. We observed that the left lobe of *P. parsia* ovary was longer than right one in accordance with the observation made by Das *et al.* (2014) and Kumar *et al.*

(2015) in *M. cephalus*. In most teleosts, oocyte diameter increases with the advancement of maturation that leads to an increase in ovarian size, weight and GSI (Taranger *et al.*, 1981; Lubzens *et al.*, 2010; Norberg *et al.*, 2010). In the current study, a maximum GSI of 18.31 was recorded in a female at the VI stage of ovarian development. Maximum GSI of 14.71 and 15.76 was reported by Begum *et al.* (2010) and Bose and Chakrabarti (2014) in

P. parsia female, respectively. In our study, the value of GSI was positively correlated to OD ($GSI=24.27OD+165.05$; $R^2= 0.911$), which is similar to the finding of Das *et al.* (2014) and Kumar *et al.* (2015) in *M. cephalus*. Fully ripe eggs of *P. parsia* in captivity measured $606.55\pm 9.83 \mu\text{m}$, which is very similar to other closely related species such as *L. macrolepis* (700 to 740 μm ; James *et al.*, 1982), *L. ramada*, (600 μm ; Mousa, 2010), *M. cephalus* (600 μm , Yousefian *et al.*, 2009; 635 μm , Kumar *et al.*, 2015) and *L. parsia* (590 μm , Begum *et al.*, 2010). In the current study, almost all the females reached the pre-vitellogenic stage, 77% of fish attained vitellogenic stage, 28% of fishes achieved post-vitellogenic and 12% reached the hydrated/ripe stage of sexual maturation. There is no published report to support the current finding. The role of the liver in vitellogenesis (Cowan *et al.*, 2017) and change in its weight during the breeding cycle (Hismayasari *et al.*, 2015) are well known in fish. The HSI is an indicator of energy reserve of the fish liver (Skjæraasen *et al.*, 2010). In this study, maximum HSI found during vitellogenic phase (IV) of oocyte development. This indicated the maximum Vtg production in the liver during this stage. A similar observation was made by Pham and Nguyen (2019) and Nunes *et al.* (2011) in rabbitfish, *Siganus guttatus* and Atlantic sardine, *Sardina pilchardus*, respectively.

Histology of fish testis has been studied extensively (Munoz *et al.*, 2002; Chevalier *et al.*, 2011). In *P. parsia*, the testis is a paired elongated organ located in the abdominal cavity and composed of many lobules. The lobules of *P. parsia* testis were separated from each other by the interstitial compartment. This compartment is made up of connective tissue, which has myoid, leydig, blood and fibroblasts cells, whereas sertoli cells and germ cells were present inside lobules. This observation is similar to the histology of other teleost testes (Rutaisire *et al.*, 2006) and *L. parisa* (Chakrabarti, 2014). In general, male fish have five different maturation stages (Chevalier *et al.*, 2011), based on the relative proportion of varying germ cells such as spermatogonia, spermatocytes, spermatids and spermatozoa. In the present study, histological observation showed six different maturation stages of male *P. parsia*, whereas Chakrabarti (2014) reported five distinct maturation stages. According to terminology of Blazer (2002), stage I and II are similar to pre-spermatogenic stage; stage III is similar to early-spermatogenic stage; stage IV is similar to mid-spermatogenic stage whereas stage V and VI are similar to late-spermatogenic stage. In teleosts, two types of spermatogenesis (cystic and non-cystic) were described by Mattei *et al.* (1993). Cystic type of spermatogenesis is more common in teleosts (Rutaisire *et al.*, 2006). In the current study, cystic type of spermatogenesis was noticed in the testis of *P. parsia*, where spermatozoa are released

into the lumen of seminiferous lobules. A similar type of spermatogenesis was noticed in *L. ramada* (Halfawy *et al.*, 2007) and *M. cephalus* (Kumar *et al.*, 2015). Vitellogenic growth involves sequestering (from the blood), packaging and deposition of hepatically derived vitellogenin to oocytes (Patino and Sullivan, 2002). In our study, vitellogenic oocytes (stage IV) are characterised by the centripetal deposition of yolk granules in the oocytes, similar to that of *L. ramada* (Halfawy *et al.*, 2007). The role of follicle cells in the oocytes is to transfer nutrients from the blood to developing oocytes. In *P. parsia*, these follicular cells were prominent during the pre-vitellogenic (I, II and III) and vitellogenic (IV) stages and absent in germinal vesicle migration (stage V) and hydrated stages (stage VI), which indicates the excess nutritional requirement during pre-vitellogenic and vitellogenic stages. The presence of cortical alveolar oocytes marks the beginning of vitellogenesis in fish (Wallace and Sellman, 1981; Albieri and Araujo, 2010). In our study, cortical alveolar oocytes were noticed at stage III (mean diameter: 369.80 ± 19.81), which indicated the inception of incorporation of Vtg into the oocytes. Thickening of vitelline membrane and fusion of yolk granules at stage V indicates the end of vitellogenesis in *P. parsia*. The present finding is in accordance with Shabanipour and Heidari (2004) in *Liza aurata*. At this stage, an amoeboid shaped eccentric germinal vesicle was seen, and oocytes were ready for germinal vesicle breakdown (GVBD) and ovulation. This finding is in line with the observation made in previous related studies on *L. ramada*, *L. aurata* and *M. cephalus* (Shabanipour and Heidari, 2004; Halfawy *et al.*, 2007; Kumar *et al.*, 2015). In the present study, overall histological observation of the ovary indicated synchronous type of oocyte development. Similar observation was made by Bose and Chakrabarti (2014) in *L. parisa* and Albieri and Araujo (2010) in *Mugil liza*. In this study, the E2 profile also supported the synchronous type of ovarian development, where E2 level remained high during III and IV stages and low during mature (V) and ripe (VI stages), which is similar to the observation of Kumar *et al.* (2015) in *M. cephalus*. Percentage of ripe stage of maturation was very low (around 12%) and we could not find spent stage of ovarian maturation in our study, which could be due to the absence of spawning in captive stock.

The BPG axis controls reproduction, gonad development, maturation, ovulation and spawning in teleosts (Levavi Sivan *et al.*, 2010; François *et al.*, 2017). In teleosts, it is well known that oocyte growth and FOM are regulated by gonadotropic hormones (FSH, LH) secreted from the pituitary gland. These hormones mediate their actions *via* steroid secreting cells of oocytes (Nagahama *et al.*, 1994). Changes in concentrations of

sex hormones during the reproductive cycle have been studied for various freshwater and marine teleost species (Sisneros *et al.*, 2004). In this study, FHS and E2 levels were highest during vitellogenic stage, which would have been required for the vitellogenesis and oocyte growth. After vitellogenesis, LH level was significantly increased at stage V, which might be essential for FOM. This finding is similar to that of other fishes (Reading and Sullivan, 2017). However, the LH level did not show further rise after stage V, which might be due to the inhibitory role of dopamine on the release of LH in captivity. This would have inhibited the natural spawning of *P. parsia* in captivity, which is similar to the observation of Zohar and Mylonas (2001) in other fishes. *P. parsia* requires seawater salinity for spawning (Talwar and Jhingran, 2001); therefore, another possible cause of failure of spawning in captivity could be the low salinity in broodstock pond (7 ± 1.5 ppt). Alam *et al.* (2008) had preliminary success in induced breeding of *P. parsia*. They increased the salinity from 7 to 20 ppt and administered Ovaprim, which has salmon gonadotropin-releasing hormone analogue and domperidone (dopamine antagonist). Induced breeding of *P. parsia* using salmon gonadotropin-releasing hormone analogue and domperidone confirmed that dopamine may have inhibitory role on spawning of this fish in captivity. The plasma sex steroid (E2) secreted from the cells of ovarian follicles that stimulate vitellogenesis and control oocytes growth is a valuable indicator of gonad development (Sisneros *et al.*, 2004; Kumar *et al.*, 2015). In the present study, serum E2 level was high in pre-vitellogenic (III) and vitellogenic (IV) stages, and low during germinal vesicle migration (V) and hydrated stage (VI). A similar trend of E2 was noticed in other fish species (Dahle *et al.*, 2003; Pham *et al.*, 2012). In our study, a low level of E2 at GVBD (V) and hydrated stage (VI) indicated that E2 is not an important steroid for FOM and ovulation in *P. parsia*. Increase in E2 level till stage IV, and concomitant increase in GSI, HSI and oocyte diameter confirmed the role of E2 in oocyte growth and vitellogenesis. A similar trend was reported in different fishes (Pham *et al.*, 2012; Adebisi *et al.*, 2013; Moreira *et al.*, 2015). Therefore HSI, GSI and E2 will be suitable indicators for the maturation process and spawning season of *P. parsia*, as used in other fish species (Mylonas *et al.*, 2010; Nunes *et al.*, 2011; Hismayasari *et al.*, 2015; Pham and Nguyen, 2019). In the present study, a drop in FSH and E2 levels after vitellogenic stage (IV) probably reduced the intensity of E2 feedback on BPG axis and allowed the hypothalamus-mediated surge of LH, which indicated the switching off of aromatase activity for FOM and ovulation in *P. parsia*. Almost a similar pattern of E2 profile has been found during the transition from vitellogenesis to FOM stage in medaka, *Oryzias latipes*

(Sakai *et al.*, 1987), Indian major carp (Sen *et al.*, 2002), mullet, *M. cephalus* (Aizen *et al.*, 2005; Das *et al.*, 2014; Kumar *et al.*, 2015), Waigieu seaperch, *Psammoperca waigiensis* (Pham *et al.*, 2012) and Kutum, *Rutilus frisii Kutum* (Sabet *et al.*, 2016). However, the opposite trend was noticed in some other fish species such as gudgeon, *Gobio gobio* (Rinhard *et al.*, 1993); European sea bass, *Dicentrarchus labrax* (Prat *et al.*, 1990) and goldfish, *Carassius auratus* (Kagawa *et al.*, 1983). In teleosts, the first peak of T during oocyte development is for E2 synthesis (Barannikova *et al.*, 2000; Sen *et al.*, 2002) and second peak during final stage of maturation is due to release of excess T into the plasma, when this is no longer needed for aromatisation (Nagahama, 1987; Frederiek *et al.*, 2007). This second peak of T also indicates that oocytes are fully mature and ready for ovulation (Kobayashi *et al.*, 1989). However, in this study, we could find only one peak of T in female *P. parsia* at stage II. This could also be another cause of the spawning failure of *P. parsia* in captivity. In male *P. parsia*, serum T increased gradually and reached its peak value at the final stage of maturation, which is in accordance with the finding of Kumar *et al.* (2015) in *M. cephalus*. Vtg is a complex protein synthesised in the liver under the control of E2 and transported to the ovary through blood (Nagahama *et al.*, 1995; Yaron *et al.*, 2003). The current study showed that the expression of Vtg in both serum and ovary was under the control of serum E2.

Reproductive maturation of *P. parsia* in brackishwater pond was evaluated in terms of HSI, GSI, gonad histology and reproductive hormones. In this study, it was found that only 12% of females reached the ripe stage of maturation. Oocytes of pre-vitellogenic, vitellogenic, post-vitellogenic and ripe/hydrated stage had a mean diameter of 369.80, 444.38, 477.41 and 606.55 μm , respectively. Histological observations revealed that *P. parsia* has synchronous oocyte development and cystic type of spermatogenesis, which is attributed to seasonally spawning fish with a single spawning event. The lack of seawater salinity could be the major cause of spawning failure of *P. parsia* in brackishwater; however the inhibitory role of dopamine could not be overruled. Therefore, the use of dopamine antagonists and seawater is essentially required for spawning of *P. parsia* in captivity. The information generated in this study will be helpful in induced breeding for quality seed production and development of aquaculture of the goldspot mullet.

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References

- Adebiyi, F. A., Siraj, S. S., Harmin, S. A. and Christianus, A. 2013. Plasma sex steroid hormonal profile and gonad histology during the annual reproductive cycle of river catfish *Hemibagrus nemurus* (Valenciennes, 1840) in captivity. *Fish Physiol. Biochem.*, 39: 547-557. doi: 10.1007/s10695-012-9718-x.
- Aizen, J., Meiri, I., Tzchori, I., Levavi Sivan, B. and Rosenfeld, H. 2005. Enhancing spawning in the grey mullet (*Mugil cephalus*) by removal of dopaminergic inhibition. *Gen. Comp. Endocrinol.*, 142: 212-221. doi: 10.1016/j.ygcen.2005.01.002.
- Alam, M. J., Begum, M., Pal, H. K., Islam, M. A. and Mamum, A. A. I. 2008. Preliminary success on hormone induced captive breeding of goldspot mullet, *Liza parsia* (Ham.). *Bangladesh J. Fish. Res.*, 12: 151-156.
- Albieri, R. J. and Araujo, F. G. 2010. Reproductive biology of the mullet *Mugil liza* (Teleostei: Mugilidae) in a tropical Brazilian Bay. *Zoologia*, 27: 331-340.
- Assem, S. S., El-Dahhar, A. A., and Mourad, M. M. 2008. Reproductive biology (histological and ultrastructure) and biochemical studies in ovaries of *Mugil cephalus* from Mediterranean water. *J. Arabian Aquac. Soc.*, 3: 33-58.
- Barannikova, I. A., Diubin, V. P., Baiunova, L. V. and Semenkova, T. B. 2000. Steroids in the reproductive function regulation in fish. *Rossiskii Fiziologicheskii Zhurnal Imeni IM Sechenova.*, 86: 968-978.
- Begum, M., Islam, M. A., Pal, H. K. and Alam, M. J. 2010. Reproductive characteristics of *Liza parsia* (Ham.) inhabiting south-west coast of Bangladesh. *J. Bangladesh Agr.*, 8: 173-178.
- Bir, J., Shahinur, B. M., Rahman, M. D., Sarower, E. M., Aminur, M. R. and Shah, M. S. 2016. Reproductive biology and feeding habit of goldspot mullet, *Liza parisa*. *Am. J. Zool. Res.*, 1: 7-12.
- Blazer, V. S. 2002. Histopathological assessment of gonadal tissue in wild fishes. *Fish Physiol. Biochem.*, 26: 85-101. DOI:10.1023/A:1023332216713.
- Bose, S. and Chakrabarti, P. S. 2014. Distribution of different cells and seasonal variations of gonadotropin cells in the pituitary gland of *Liza parsia* (Hamilton, 1822) in relation to maturation of ovary. *Zool. Stud.*, 1: 33-49.
- Chakrabarti, P. 2014. Seasonal changes in the activity of the thyroid follicles in relation to testicular activities in mullet *Liza parsia* (Hamilton). *Int. J. Fish. Aquat. Stud.*, 1: 40-47.
- Chevalier, C., Conto, C. D. and Jean Marie Exbrayat, J. M. 2011. Reproductive biology of the endangered percid *Zingel asper* in captivity: A histological description of the male reproductive cycle. *Folia Histochemica et Cytobiologica*, 49: 486-496. doi: 10.5603/fhc.2011.0069.
- Clark, F. N. 1934. Maturity of the Californian sardine (*Sardina caerulea*) determined by ova diameter measurements. *Calif. Dept. Fish Game Fish. Bull.*, 42: 1-49.
- Cowan, M., Azpeleta, C. and Lopez Olmeda, J. F. 2017. Rhythms in the endocrine system of fish. *J. Com. Physiol. B.*, 187: 1057-1089. doi: 10.1007/s00360-017-1094-5.
- Dahle, R., Taranger, G. L., Karlsen, Q., Kjesbu, O. S. and Norberg, B. 2003. Gonadal development and associated changes in liver size and sexual steroids during the reproductive cycle of captive male and female Atlantic cod (*Gadus morhua* L.). *Comp. Biochem. Physiol.*, 136: 641-653. doi: 10.1016/s1095-6433(03)00215-0.
- Das, P., Maiti, A. and Maiti, B. R. 2014. Circannual changes in morphological, ultrastructural and hormonal activities of the ovary of an estuarine grey mullet, *Mugil cephalus*. *Biol. Rhythm Res.*, 44: 541-567.
- François, C. M., Florence, P., Frank, G., Alain, G., Celia, R., Solange, M. and Celine, J. G. 2017. A novel action of follicle-stimulating hormone in the ovary promotes estradiol production without inducing excessive follicular growth before puberty. *Sci. Rep.*, 7: 46222.
- Frederiek, J., Munday, L., Westcott, A., Hobbs, V. and Robin Liley, N. 2007. Aromatase pathway mediates sex change in each direction. *J. Biol. Sci.*, 272: 1399-1405. doi: 10.1098/rspb.2005.3097.
- Halfawy, E. L. M. M., Magdy, M., Ramadan Amal, M. and Mahmoud Waheed, F. 2007. Reproductive biology and histological studies of the grey mullet, *Liza ramada*, (Risso, 1826) in lake Timsah, Suez Canal. *Egypt. J. Aquat. Res.*, 33: 434-454.
- Hismayasari, B. I., Marhendra, A. P. W., Rahayu, S., Saidin and Supriyadi, D. S. 2015. Gonadosomatic index (GSI), hepatosomatic index (HSI) and proportion of oocytes stadia as an indicator of rainbow fish *Melanotaenia boesemani* spawning season. *Int. J. Fish. Aquat. Stud.*, 2: 359-362.
- Honji, R. M., Narcizo, A. M., Borella, M. I., Romagosa, E. and Moreira, R. G. 2009. Patterns of oocyte development in natural habitat and captive *Salminus hilarii* Valenciennes, 1850 (Teleostei: Characidae). *Fish Physiol. Biochem.*, 35: 109-123. doi: 10.1007/s10695-008-9239-9.
- James, P. S. B. R., Rengaswamy, V. S., Raju, A., Mohanraj, G. G. and Gandhi, V. 1982. Induced spawning and larval earing of the grey mullet, *Liza macrolepis* (Smith). *Indian J. Fish.*, 30(2): 185-202.
- Kobayashi, M., Aida, K. and Hanyu, I. 1989. Hormone changes during ovulation and effects of steroid hormones on plasma gonadotropin levels and ovulation in goldfish. *Gen. Comp. Endocrinol.*, 67: 301-307.
- Kumar, P., Arasu, A. R. T., Kailasam, M., Sukumarran Krishna, Subburj, R., Tyagraj, G. and Natarajan, M. 2015. Gonadal development and steroid hormone profile of wild caught grey mullet (*Mugil cephalus*). *Biol. Rhythm Res.*, 4: 601-610.

- Kumar, P., Biswas, G., Ghoshal, T. K., Kailasam, M. and Vijayan, K. K. 2018. Embryonic and larval developments of brackishwater catfish, *Mystus gulio* (Hamilton and Buchanan, 1822) induced with human chorionic gonadotropin and consequent larval rearing. *Aquac. Res.*, 49: 2466-2476.
- Kumar, P., Biswas, G., Ghoshal, T. K., Christina, L., Behera, P. and Kailasam, M. 2020. Effect of salinity acclimatisation and administration of hormones on oocyte maturation, ovulation and spawning of goldspot mullet *Liza parsia* (Hamilton, 1822) reared in brackishwater pond. *Indian J. Fish.*, 67(1): 56-63. DOI: <http://dx.doi.org/10.21077/ijf.2019.67.1.90246-08>.
- Kumar, P., Behera, P., Christina, L. and Kailasam M. 2021. Sex hormones and their role in gonad development and reproductive cycle of fishes. In: Sundaray, J. K. and Rather, M. A. and Kumar, S. and Agarwal, D. (Eds.), *Recent updates in molecular endocrinology and reproductive physiology of fish: An imperative step in aquaculture*. Springer, Singapore, p. 12-22.
- Lee, W. K. and Yang, S. W. 2002. Relationship between ovarian development and serum levels of gonadal steroid hormones, and induction of oocyte maturation and ovulation in the cultured female Korean spotted seabass *Lateolabrax maculatus* (Jeom-nong-eo). *Aquaculture*, 207: 169-183.
- Levavi Sivan, B., Bogerd J, Man., Anos, E. L., Gomez, A. and Lareyre, J. J. 2010. Perspectives on fish gonadotropins and their receptors. *Gen. Comp. Endocrinol.*, 165: 412-437. doi: 10.1016/j.ygcen.2009.07.019.
- Lubzens, E., Young, G., Bobe, J. and Cerda, J. 2010. Oogenesis in teleosts: How fish eggs are formed. *Gen. Comp. Endocrinol.*, 165: 367-389. doi: 10.1016/j.ygcen.2009.05.022.
- Manosroi, A., Meng, U. K. and Manosroi, J. 2003. Annual sex hormonal profiles, gonad development and age determination of the Mekong giant catfish (*Pangasianodon gigas*, Chevey). *Aquac. Res.*, 34: 1379-1385. <https://doi.org/10.1111/j.1365-2109.2003.00955.x>.
- Mattei, X., Sliaw, Y., Thiaw, O. T. and Thiam, D. 1993. Peculiarities in the organisation of testis of *Ophidion* sp. (Pisces, Teleostei). Evidence of two types of spermatogenesis in teleost fish. *J. Fish Biol.*, 43: 931-937.
- Moreira, R. G., Honji, R. M., Melo, R. G., Narcizo, M. A., Amaral, S. J., Araujo, C. R. and Hilsdorf, A. S. W. 2015. The involvement of gonadotropins and gonadal steroids in the ovulatory dysfunction of the potamodromous *Salminus hilarii* (Teleostei: Characidae) in captivity. *Fish Physiol. Biochem.*, 41: 1435-1447. doi: 10.1007/s10695-015-0097-y.
- Mousa, M. A. 2010. Induced spawning and embryonic development of *Liza ramada* reared in freshwater ponds. *Anim. Reprod. Sci.*, 119: 115-122. doi: 10.1016/j.anireprosci.2009.12.014.
- Munoz, M., Koya, Y. and Casadevall, M. 2002. Histochemical analysis of sperm storage in *Helicolenus dactylopterus* (Teleostei, Scorpaenidae). *J. Exp. Zool.*, 292: 156-164. doi: 10.1002/jez.1151.
- Mylonas, C. C., Fostier, A. and Zanuy, S. 2010. Broodstock management and hormonal manipulations of fish reproduction. *Gen. Comp. Endocrinol.*, 165: 516-534. doi: 10.1016/j.ygcen.2009.03.007.
- Nagahama, Y. 1987. Endocrine control of oocyte maturation. In: Norris, D. O. and Jones, R. E. (Eds.), *Hormones and reproduction in fishes, amphibians and reptiles*. Plenum Press, New York, India.
- Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. *Int. J. Dev. Biol.*, 38: 217-229.
- Nagahama, Y. and Yamashita, M. 2008. Regulation of oocyte maturation in fish. *Dev. Growth Differ.*, 50: S195-S219. doi: 10.1111/j.1440-169X.2008.01019.x.
- Nagahama, Y., Yoshikuni, M., Yamashita, M. and Tokumoto, T., Katsu, Y. 1995. Regulation of oocyte growth and maturation in fish. *Curr. Top. Dev. Biol.*, 30: 103-145. doi: 10.1016/s0070-2153(08)60565-7.
- Nunes, C., Silva, A. and Soares, E. 2011. The use of hepatic and somatic indices and histological information to characterise the reproductive dynamics of Atlantic sardine *Sardina pilchardus* from the Portuguese coast. *Mar. Coast. Fish.*, 3: 127-144. DOI:10.1080/19425120.2011.556911.
- Patino, R. and Sullivan, C. V. 2002. Ovarian follicle growth, maturation and ovulation in teleost fish. *Fish Physiol. Biochem.*, 26: 57-70.
- Peter, R. E., Crim, L. W., Goos, H. J. T. H. and Crim, J. W. 1978. Lesioning studies on the gravid female goldfish: Neuroendocrine regulation of ovulation. *Gen. Comp. Endocrinol.*, 35 (4): 391-401. DOI: 10.1016/0016-6480(78)90133-8.
- Pham, H. Q. and Nguyen, A. T. 2019. Seasonal changes in hepatosomatic index, gonadosomatic index and plasma estradiol-17 β level in captivity reared female rabbit fish (*Siganus guttatus*). *Aquac. Res.*, 50(8): 2191-2199.
- Pham, H. Q., Nguyen, A. T., Kjorsvik, E., Nguyen, M. D. and Arukwe, A. 2012. Seasonal reproductive cycle in Waigieu seaperch (*Psammoperca waigiensis*). *Aquac. Res.*, 43: 815-830. <https://doi.org/10.1111/j.1365-2109.2011.02894.x>.
- Prat, F., Zanuy, S., Carrillo, M., De Mones, A. and Foptier, A. 1990. Seasonal changes in plasma levels of gonadal steroids of seabass, *Dicentrarchus labrax* L. *Gen. Comp. Endocrinol.*, 78: 361-373.
- Reading, B. J. and Sullivan, C. V. 2017. *Vitellogenesis in fishes. Reference module in life sciences*. Elsevier, Amsterdam, the Netherlands. <https://doi.org/10.1016/B978-0-12-809633-803076-4>.
- Renjini, P. K. and Bijoy Nandan, S. 2011. Length-weight relationship, condition factor and morphometry of goldspot mullet *Liza parsia* (Hamilton, 1822) from Cochin Estuary. *Indian J. Mar. Sci.*, 40(4): 567-571.

- Riede, K. 2004. *Global register of migratory species from global to regional scales. Final Report of the R&D-Project 808 05 081*, Federal Agency for Nature Conservation. Bonn, Germany, 329 pp.
- Rinchar, J., Kestemont, P., Kühn, E. and Fostier, A. 1993. Seasonal changes in plasma levels of steroid hormones in an asynchronous fish the gudgeon *Gobio gobio* L. (Teleostei, Cyprinidae). *Gen. Comp. Endocrinol.*, 92: 168-178. <https://doi.org/10.1006/gcen.1993.1153>.
- Roberts, R. J. 1989. Nutritional pathology of teleosts. In: Roberts, R. J. (Eds.), *Fish pathology*. Bailliere Tindall, London, UK, p. 337-362.
- Rutaisire, J., Muwazi, R. T. and Booth, J. 2006. Ultrastructure description of spermiogenesis and spermatozoa in *Labeo victorinus* Boulenger, 1901 (Pisces, Cyprinidae). *Afr. J. Ecol.*, 44: 102-105.
- Sabet, S. S., Imanpoor, M. R., Fatideh, B. A. and Gorgin, S. 2016. Variations in plasma sex steroid hormones of the wild caspian cyprinid fish, kutum (*Rutilus frisii*). *J. Cell Mol. Res.*, 8(1): 25-34.
- Sakai, N., Iwamatsu, T., Yamauchi, K. and Nagahama, Y. 1987. Development of the steroidogenic capacity of medaka (*Oryzias latipes*) ovarian follicles during vitellogenesis and oocyte maturation. *Gen. Comp. Endocrinol.*, 66: 333-342.
- Sen, U., Mukherjee, D., Bhattacharyya, S. P. and Mukherjee, D. 2002. Seasonal changes in plasma steroid levels in Indian major carp *Labeo rohita*: Influence of homologous pituitary extract on steroid production and development of oocyte maturational competence. *Gen. Comp. Endocrinol.*, 128: 123-134. doi: 10.1016/s0016-6480(02)00060-6.
- Shabanipour, N. and Heidari, B. 2004. A histological study of the zona radiata during late oocyte developmental stages in the caspian sea mugilid, *Liza aurata* (Risso 1810). *Braz. J. Morphol. Sci.*, 21: 191-195.
- Shehadeh, Z. H., Kuo, C. M. and Milisen, I. C. 1973. Validation of an *in vivo* method for monitoring ovarian development in the grey mullet *Mugil cephalus*. *J. Fish Biol.*, 5: 489-496. <https://doi.org/10.1111/j.1095-8649.1973.tb04478.x>.
- Shinkafi, B. A., Ipinjolu, J. K. and Hassan, W. A. 2011. Gonad maturation stages of *Auchenoglanis occidentalis* (Valenciennes 1840) in river Rima, North-Western Nigeria. *J. Fish. Aquat. Sci.*, 6(3): 236-246. DOI: 10.3923/jfas.2011.236.246.
- Sisneros, J. A., Forlano, P. M., Knapp, R. and Bass, A. H. 2004. Seasonal variation of steroid hormone levels in an intertidal-nesting fish, the vocal plainfin midshipman. *Gen. Comp. Endocrinol.*, 136: 101-116. doi: 10.1016/j.ygcen.2003.12.007.
- Skjæraasen, J. E., Nash, R. D. M., Kennedy, J., Thorsen, A., Nilsen, T. and Kjesbu, O. S. 2010. Liver energy, atresia and oocyte stage influence fecundity regulation in North-east Arctic cod. *Mar. Ecol. Prog. Ser.*, 404: 173-183. DOI:10.3354/meps08486.
- Talwar, P. K. and Jhingran, A. G. 2001. *Inland fishes*. Oxford and IBH Publishing, Co. Pvt. Ltd, New Delhi, India, 1158 pp.
- Taranger, G. L., Carrillo, M., Schulz, R. W., Fontaine, P., Zanuy, S., Felip, A., Weltzien, F. A., Wallace, R. A. and Selman, K. 1981. Cellular and dynamic aspects of oocyte growth in teleosts. *Am. Zool.*, 21: 325-343. <https://doi.org/10.1093/icb/21.2.325>.
- Yaron, Z., Gur, G., Melamed, P., Rosenfeld, H., Elizur, A. and Levavi, S. B. 2003. Regulation of fish gonadotropins. *Int. Rev. Cytol.*, 225: 131-185. doi: 10.1016/s0074-7696(05)25004-0.
- Yousefian, M., Ghanei, M., Pourgolam, R., Rostami, H. K. H. 2009. Gonad development and hormonal induction in artificial propagation of grey mullet, *Mugil cephalus* L. *Res. J. Fish. Hydrobiol.*, 4(2): 35-40.
- Yuen, H. S. H. 1955. Maturity and fecundity of big eye tuna in the Pacific. *Spec. Sci. Rep. Fish.*, 150: 28-30.
- Zohar, Y. and Mylonas, C. C. 2001. Endocrine manipulation of spawning in cultured fish from hormones to genes. *Aquaculture*, 197: 99-136. [https://doi.org/10.1016/S0044-8486\(01\)00584-1](https://doi.org/10.1016/S0044-8486(01)00584-1).