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OOCYTE DEVELOPMENT IN THE FRESH WATER SPOTTED SNAKEHAD, *CHANNA PUNCTATA* (BLOCH, 1793)

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Abstract:
Teleosts possesses well defined breeding or reproductive cycle. On the basis of shape, size, colour of the ovary different phases viz., resting phase, early maturing phase, prespawning phase, spawning phase and spent phase are recognized in teleosts. From the end of resting phase to spawning phase developing oocytes passes through different stages. During this journey vitellogenesis and maturation is important event for oocyte development. Various stages of female gametes of *Channa punctata* were analyzed. Mature *Channa punctata* were collected by lakes in and around Nagpur city. Different phases of ovarian maturation were confirmed by calculating gondosomatic index (GSI). Ovarian tissues were fixed in Bouin's fixative, dehydrated in graded alcohol, cleared in xylene, embedded in paraffin wax and thus blocks were prepared. Blocks were cut in 6-7 µm thick sections. Sections were stained with Haematoxylene and Eosin (HE). Oocytes morphology was studied in present work. Morphometric analysis of different oocytes was carried out with help of Tcapture version 4.3.0.602 software.
Keywords: *Channa punctata,* oocytes.

Introduction:

A pair of folded sac like pharyngeal outgrowth provide all the species of the *Channa* viz., *C. punctata, C. marulius, C. striata and C. gachua* a tenacity to survive outside the water for considerable period (Chakrabarty, 2006). Oogenesis of teleosts has been thoroughly studied. The various stages of oocytes is based on peculiar morphological, histological, physiological and/or biochemical cell characteristics (i.e. oocyte size, shape, quantity and distribution of different cytoplasmic as well as nuclear inclusions (Casadevall et al., 1993; Tyler and Sumpter, 1996). Histological studies of gonad of species reveal gonadal development (West, 1992). Ovarian cycle and spawning season of *Ophiocephalus punctatus* inhabiting Jammu water was documented (Malhotra et al., 1978) where six stages of oocytes were reported by following method of Srivastava and Rathi (1970). Variation in gonadal cycle, spawning time and spatial behaviour of this fish were well documented (Swaroop, 1954; Belsare, 1962; Malhotra et al., 1978).

Materials and Methods:

Matured female C. *punctata* of 150-200 gm weight were procured from various water bodies in and around Nagpur. Fishes were acclaimatized in aquaria for one week. After sacrificing fishes, ovaries were dissected out and fixed in Bouin's fluid. After fixation, ovaries were cutted in pieces and cutted tissues were washed and transferred to 70% alchohol and dehydrated in graded alcohol, cleared in xylene and embedded in paraffin wax at 60^{0}C- 62^{0}C. Blocks of tissues were trimmed and serial sections of these blocks were cut on Cambridge (rocking) microtome at 6-8 µm thickness in transverse plane. The sections were fixed on clean slide and later stained by Haematoxylene- Eosine procedure (Lillie, 1965). Sections were observed in Karl Zeiss microscope and photographed by Tucsen USB 2.0 H series camera. Morphometric analysis of different oocytes was carried out with help of Tcapture version 4.3.0.602 software.

Gonado-somatic index was calculated by formula:

$$GSI = (Wg \times W^{-1}) \times 100$$

Where:
Wg-Weight of gonads (g), W- Body weight (g).

Results were expressed as the mean and standard error of mean (SEM). Difference between means were analysed by one-way ANOVA and student T-test. Level of significance was set at $P \leq 0.05$.

Observation:

Based on GSI, morphology of ovaries and presence of oocyte present identified five phases of reproductive cycle viz., Resting phase (Mid December- February), Early maturing phase (March- April), Prespawning phase (May), Spawning phase (June – October) and Spent phase (November- Mid December). GSI of resting phase was lowest (0.58± 0.04) (Table 1, Graph 1). GSI was slightly higher in early maturing phase (1.99±0.9) (Table 1, Graph 1). GSI was further significantly rises in prespawning phase (4.49±0.89) ($p < 0.05$) (Table 1, Graph 1). GSI was maximum in spawning phase and significantly higher than prespawning phase ($p < 0.05$) (14.41± 0.73) (Table 1, Graph 1). In spent phase, GSI was lowered significantly to 0.60 ±1.03. No significant difference observed between GSI of resting and spent phase ($p < 0.05$).

Oogenesis initiated with development and growth of oocytes. Overall six oocyte development stages were identified for *C. punctata*.

Primary oogonia: With 16.52 ± 1.4 µm these were smallest oval cells (Fig. 1a) (Table 2, Graph 2). Primary oogonias either present singly or they present in cluster. Cytoplasm is strongly basophilic.

Chromatin nucleolus oocytes: These oocytes develop from primary oogonias therefore they were observed in proximity of primary oogonias. They were slightly larger (36.47± 1.7µm) (Table 2, Graph 2). They had a large and lightly stained nucleus. A single nucleolus located towards nuclear membrane (Fig. 1b).

Early perinucleolar oocyte: Further growth (47.62 ± 0.87 µm) was observed in early perinucleolar oocyte (Fig. 1c) (Table 2, Graph 2). It had slightly less basophilic cytoplasm. Larger, regular rounded nucleus with several small nucleoli were seen towards peripheral margin of nucleoplasm (Fig. 1c).

Late perinucleolar oocyte: Significant growth observed in late perinucleolar oocyte (64.45 ±0.87) (Fig. 1d) (Table 2, Graph 2). In late perinucleolar oocyte, nucleus become irregular and multiple cortical alveoli appears towards periphery of cytoplasm (Fig. 1d).

Vitellogenic oocyte: Vitellogenic oocyte is larger than early oocytes stages (130.46 ± 1.12) (Table 2, Graph 2). Shrinkage in nucleoplasm observed in vitellogenic oocyte (Fig. 1e). Cytoplasm of vitellogenic oocyte was filled with cortical vesicles (Fig. 1e).

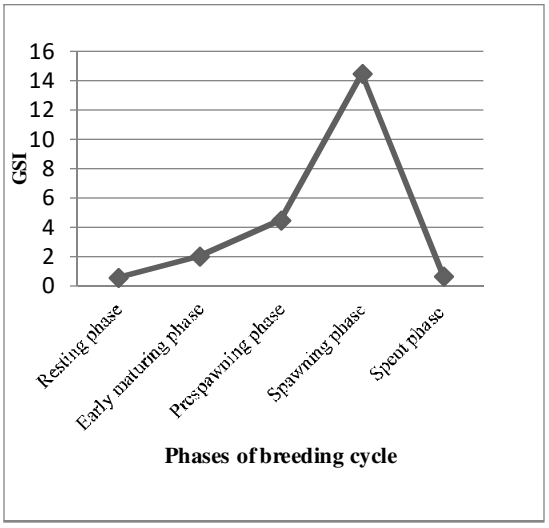
Ripe oocyte: They were largest oocytes (284.99± 0.92) (Table 2, Graph 2). Cytoplasm of ripe oocyte was completely filled with cortical vesicles as well as yolk granules. Disintegration of nucleus is complete in ripe oocyte. The cytoplasm of ripe oocyte was more voluminous and had a grainy appearance.

Table 1: GSI in various phases of breeding cycle.

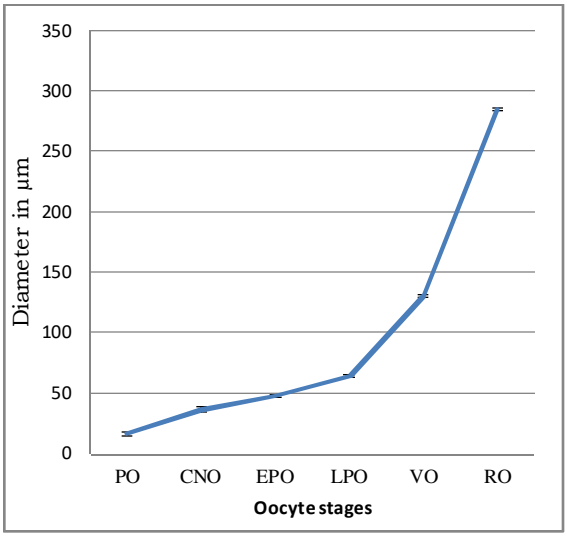
Phases	**GSI**
Resting phase	0.58 ± 0.04
Early maturing phase	1.99 ± 0.9
Prespawning phase	4.49 ± 0.89
Spawning phase	14.49 ± 0.73
Spent phase	0.60 ± 0.02

Table 2: Morphometric analysis of oocytes stages in *C. punctata*

Oocyte stage	Diameter in µm
Primary oogonia	16.52± 1.4
Chromatin nucleolus oocyte	36.47± 1.7
Early perinucleolar oocyte	47.62 ± 0.87
Late perinucleolar oocyte	64.45 ± 0.76
Vitellogenic oocyte	130.46 ± 1.12
Ripe oocyte	284.99 ± 0.92



Graph 1: Oscillation in GSI during various phases of breeding cycle



Graph 2: Diameter (µm) of oocytes in *C. punctata* (PO- Primary oogonia, CNO-Chromatin nucleolus oocyte, EPO-Early perinucleolar oocyte, LPO-Late perinucleolar oocyte, VO-Vitellogenic oocyte, RO-Ripe oocyte)

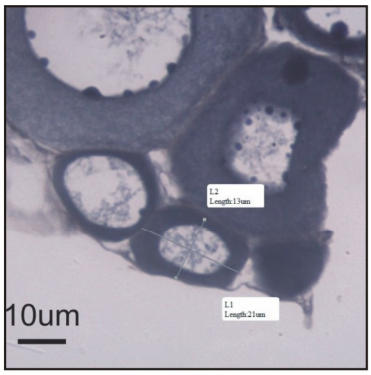


Fig. 3a

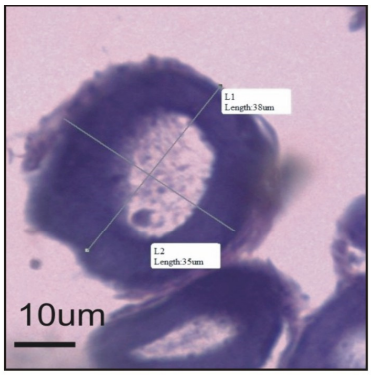


Fig.3b

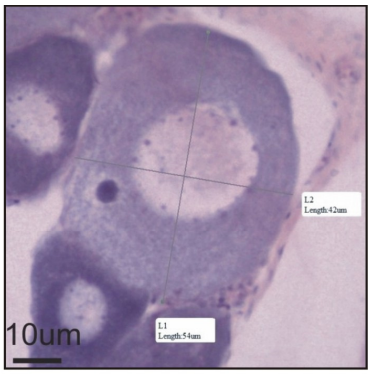


Fig. 3c

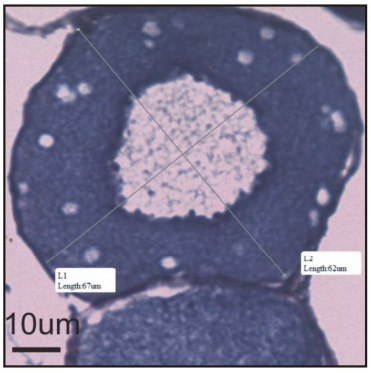


Fig. 3d

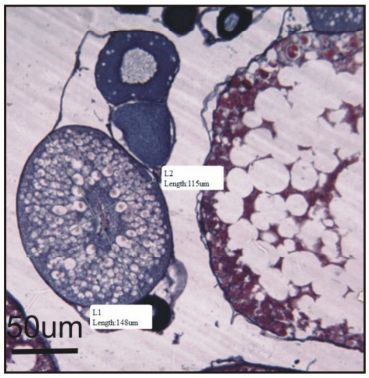


Fig. 3e

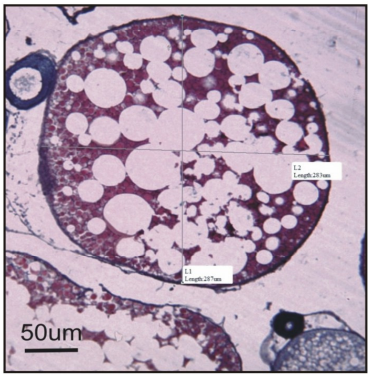


Fig. 3f

Fig. 3 Various oocyte stages in *C. punctata.*
Fig. 3a.Primary oogonia.
Fig. 3b Chromatin nucleolus oocyte.
Fig. 3c Early perinucleolar oocyte.
Fig. 3d Late perinucleolar oocyte.
Fig. 3e Vitellogenic oocyte.
Fig. 3f. Ripe egg

Discussion:

Recently, oocyte stages in teleosts is studied in perspective of oogenesis. This approach to study the oocyte stages widely used by many authors (Saeed et al., 2010; Higashino et al., 2000). Five to eight stages of oocyte were reported in teleost fish (Fishelson et al., 1996; Unal et al., 1999; Gokee et al., 2003). Seven stages of oocytes were reported in *Mystus tengara* (Mayor et al., 1988) and in *Dicentrachus labrax* (Brandao et al., 2003) respectively. Six oocyte stages were reported in present study.

GSI is correlated with gonadal development (Rae and Calvo, 1995; Zimmerman 1997; Koya et al., 1998). Maximum GSI was reported during spawning phase. Similar reports were describe in *Cynoglossus arel* and *C. lida* (Rajguru, 1992), *Potogonotothen tesselata* (Rae and Calvo, 1995) and in rainbow trout, *Oncorhynchus mykiss* (Sharma and Bhat, 2014).

Cystovarian type of ovary was exhibited by *C. punctata* as central lumen of ovary continue posteriorly with lumen of oviduct (Hoar, 1969). Semi cystovarian type of ovary present in Salmoniformes whose gonad open to the coelomic cavity, with oocytes being expelled by funnels (Narahara, 1981)

Different terminology were ascribe to different stages of oogenesis. In spite of using Roman numerals to describe oocyte stages terminology used in *Oncorhynchus mykiss* was used in present study (Sharma and Bhat, 2014).

Present study confirm the subsequent growth of oocyte from primary oogonia to ripe oocyte. Each stage is significantly larger than previous oocyte stage ($P<0.05$). Similar results were found in common carp (Shirali et al., 2012),

Oreochromis niloticus (El–Saba et al., 2013) and in *Oncorhynchus mykiss* (Sharma and Bhat, 2014)

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