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Original article

Ovarian development and histological observations of threatened dwarf snakehead fish, *Channa gachua* (Hamilton, 1822)

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ABSTRACT

Channa gachua were monthly sampled throughout a year and the histological analysis of their ovaries was done to determine the changes occurring in ovarian development. Based on histological examination of the ovaries, the oogenic process of *C. gachua* undergoes distinct cyclic and seasonal morphological changes. Five different developmental stages were identified under three major categories: pre-spawning (immature, maturing, mature), spawning (ripe-running) and post-spawning (spent). The peak spawning period of *C. gachua* was noticed during December - February. The gonadosomatic index (GSI) and ova diameter ranged from 0.79 to 3.61% and 543–1123 μm respectively. The highest mean GSI (3.61 ± 0.16) and oocyte diameter (1123 ± 55 μm) were observed in December indicating that during this month the gonadal development reached maturity.

1. Introduction

Murrels commonly called snakeheads due to the presence of large scales on their head belong to the family Channidae. *Channa gachua*, commonly called dwarf snakehead is an important food fish and is one of the most suitable channa species for aquarium due to its beautiful colouration and small size (Talwar and Jhingran, 1992). Even though it is widely distributed in Asia, it has declined drastically (vulnerable) in India (CAMP, 1998) and endangered in some Asian countries like Singapore (Lim and Ng, 1990). Although, this species is economically important, information on the reproductive physiology and histomorphological changes of ovary of *C. gachua* in captive conditions remains limited.

For proper management of aquaculture practices, a detailed study of gonad maturation is important, since such studies are aimed in understanding the annual changes of the population (Thorpe et al., 1990; Jobling et al., 2002; Tomkiewicz et al., 2003; Shein et al., 2004). The reproductive cycles in teleost occur during a particular phase; some breed once in a year as annual breeders and others as monsoon breeders (Zuckerman, 1962).

Freshwater murrels being seasonal breeders exhibit clear changes in the gonads during breeding season (Marimuthu et al., 2001). Cyclic gonadal changes have been studied in a few Channa species viz. *Channa marulius* (Parameswaran and Murugesan, 1976), *C. punctata* (Srivastav and Srivastav, 1998) and *C. striata* (Al Mahmud et al., 2016).

Histological studies on fish reproduction to determine peak period of spawning and to understand effective methods for increasing efficiency of broodstock and ultimately increasing the fish production are prerequisite. The detailed information on changes in the ovaries of the dwarf snakehead during the reproductive cycle is important to provide useful data for the management of this species. Hence, this study was performed to examine the ovarian developmental stages of dwarf snakehead, *C. gachua* during its annual maturation cycle under captive conditions.

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2. Materials and methods

2.1. Sample collection

The experimental fishes (12–20 cm in total length, 15–50 g in weight) were collected from Thamirabarani River (8.44°N, 77.44°E) by a cast net during the months of December 2008–February 2009. The fishes were acclimatized to captive conditions by maintaining them in earthen ponds (3 × 3 × 1 m) filled with chlorine free tap water (dissolved oxygen 5.2 mg l^{-1}, temperature 28 ± 2 °C, and pH 6.4–7.1) at Center for Aquaculture Research and Extension (CARE) Aquafarm. The pond was provided with plenty of aquatic plants (*Hydrilla verticilata*). The fishes were fed on formulated diets (60% chicken intestine, 17% ground nut oil cake, 11% rice bran, 10% tapioca, and 2% vitamin and mineral mixture) following Haniffa et al. (1999), twice daily (morning and evening) until satiation.

2.2. Gonadosomatic index

Ovary samples were obtained from 5 female dwarf snakehead specimens every month captured using drag net from April 2009 to March 2010. Fish were measured (nearest 0.1 cm) and weighed (nearest 0.1 g) and were dissected to remove the ovaries. Ovarian samples were weighed (nearest 0.1 g) and fixed in 10% buffered formalin prior to histological analysis. Gonadosomatic index (GSI) was calculated using the following formula:

$$GSI = \frac{GW}{TW} \times 100$$

where

GW = Gonad weight

TW = Total body weight.

2.3. Histology

Gonad sections were collected from the mid-part of the ovary monthly for histological analysis. These sections were fixed in 10% buffered formalin for further laboratory analysis. After washing in running water, the samples were dehydrated in an ascending series of ethanol (70%, 90% and absolute ethanol) and clarified with xylene. The samples embedded in paraffin blocks were trimmed and 5 μm thick sections taken were stained using haematoxylin and eosin followed by periodic acid Schiff (PAS) reaction. The maturity stages of ovary of *C. gachua* were determined using the modified gonad maturity scale developed by Arockiaraj et al. (2004). The histological sections were examined under the light microscope (Nikon microscope - U III E-400 Eclipse). Diameter of eggs was measured using Magnus Pro Microscope Software with accuracy level of 0.01 mm.

One-way analysis of variance (ANOVA) was used to analyze the data, followed by comparison of means using Duncan's multiple range tests. Statistically significant differences were accepted at $p < 0.05$.

3. Results and discussion

Stages of oocyte development similar to those described in most teleost fishes (Encina and Granado-Lorencio, 1997) were identified and described in *C. gachua*. The three maturity stages of oocyte development of *C. gachua* determined based on morphological features are described in Table 1. First stage was pre-spawning stage which was divided into three sub-stages viz, (a) immature, (b) maturing, (c) mature. Second stage was spawning or ripe-running stage and the third was post-spawning or spent stage. Mature ovaries (stage Ic) were frequently observed after October and were abundant in December to February signalling the period of spawning. Females with empty ovaries, spent fishes (stage IIIe) were seen between March and May indicating the recovery period. These different stages corresponded with those described macroscopically for *M. montanus* (Arockiaraj et al., 2004). However, microscopic examination of ovary sections reported by Al Mahmud et al. (2016) revealed four stages of maturity in striated snakehead, *Channa striata*. The female fish showed significant ($P < 0.05$) weight changes in the ovaries corresponding to the three gametogenic stages (pre-spawning, spawning and post spawning) during different months of the year (Table 2).

To determine the GSI values, 60 female dwarf snakehead samples (weight range = 30–47 g) were used. The GSI of *C. gachua* was significantly different during the sampling months ($P < 0.05$). The GSI was minimum in April (0.79 ± 0.11%), began to increase in June and reached the stage of complete sexual maturity in December (3.61 ± 0.16%) where the ovaries were ripe and mature (Table 2, Fig. 1). Our result showed highest GSI in winter months (December, January and February) which could be the possible spawning season of *C. gachua*. Moreover, the mean ova diameter gradually increases in size from March onwards and rapidly reaching the maximum size (1123 ± 55 μm) in December (Fig. 2). During the sampling months, the oocyte diameter was significantly different ($P < 0.05$). The highest proportion of "spent" gonads was noticed in March - May, where the GSI values declined rapidly after spawning.

In contrast to our reports, Al Mahmud et al. (2016) reported the minimum GSI value during the month of September and higher values from April to July for female *Channa striata*. Similarly, highest values of GSI were recorded during April- July in *Channa bleheri* (Rinku et al., 2013) and during May - August in *C. punctatus* (Sunita et al., 2011; Lalta et al., 2011). The highest/lowest values of GSI were due to the active somatic energy accumulation/depletion (Encina and Granado-Lorencio, 1997). The variations are probably due to the fact that the changes in energy are usually more than

Table 1
Ovarian condition of different maturity stages and comparison with histological examination in *Channa gachua*.

Stage and period	Macroscopic appearance	Histological examination
I. Pre-spawning		
(a) Immature (June-July)	Ovary small, thin ribbon like transparent, whitish gray, granular in appearance occupying half of the abdominal body cavity, eggs very minute distinct under microscope	Monolayer follicle phase in primary oocyte development with stage I present, ovigerous lamellae from the tunica albuginea were evident (Fig. 3)
(b) Maturing (August-September)	Ovary straight; ova visible through the capsule; ova orange yellow in colour	Oogonia, chromatin nucleolar and perinuclear oocytes growing rapidly; cortical alveoli start to appear in few oocytes (Fig. 4)
(c) Mature (October-November)	Ovary increases in size; forms lobes and is the largest organ in the abdominal cavity; ova yellowish-orange in colour	Ovaries dominated by late perinuclear oocytes and primary yolk vesicle; few secondary yolk vesicle oocytes present (Figs. 5–7)
II. Spawning		
(d) Ripe-running (December-February)	Ovary fully distended and fills the abdominal cavity; oocyte orange yellow and easily shed on application of slight pressure on the ovary	Ovary dominated by tertiary oocytes; fully mature eggs with yolk globules; few previtellogenic stages begin to grow for the subsequent season (Figs. 8 and 9)
III. Post-spawning		
(e) Spent (March-May)	Ovary flaccid and often haemorrhagic if spawning was successful; few oocytes visible giving the ovary a speckled appearance	Post-ovulatory follicles, oocytes undergoing atresia and type I and II atretic oocytes, (Fig. 9)

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Table 2
Annual changes in ovary (GSI and ova diameter).

Month	Wt of the fish (g)	Ovary wt (g)	Ovary length (mm)	GSI (%)	Ovary diameter (μm)
January	44 ± 2.3	1.472 ± 0.24	32 ± 1.6	3.22 ± 0.32	1054 ± 42
February	45 ± 1.6	1.49 ± 0.12	28 ± 1.4	3.31 ± 0.24	1002 ± 53
March	30 ± 0.8	0.312 ± 0.09	22 ± 1.7	1.04 ± 0.16	543 ± 61
April	34 ± 1.3	0.27 ± 0.67	17 ± 1.0	0.79 ± 0.11	621 ± 23
May	32 ± 2.2	0.29 ± 0.14	19 ± 0.8	0.9 ± 0.03	654 ± 44
June	40 ± 0.8	0.72 ± 0.03	22 ± 1.2	1.87 ± 0.23	789 ± 39
July	45 ± 3.3	0.89 ± 0.07	24 ± 0.9	1.97 ± 0.13	843 ± 12
August	42 ± 2.0	0.82 ± 0.21	23 ± 1.1	2.01 ± 0.09	879 ± 33
September	38 ± 1.4	0.912 ± 0.09	26 ± 1.3	2.42 ± 0.13	912 ± 13
October	32 ± 2.9	0.826 ± 0.07	28 ± 1.7	2.58 ± 0.21	963 ± 44
November	36 ± 3.7	1.02 ± 0.09	30 ± 1.3	2.83 ± 0.08	978 ± 26
December	42 ± 2.1	1.52 ± 0.11	34 ± 1.6	3.61 ± 0.16	1123 ± 55

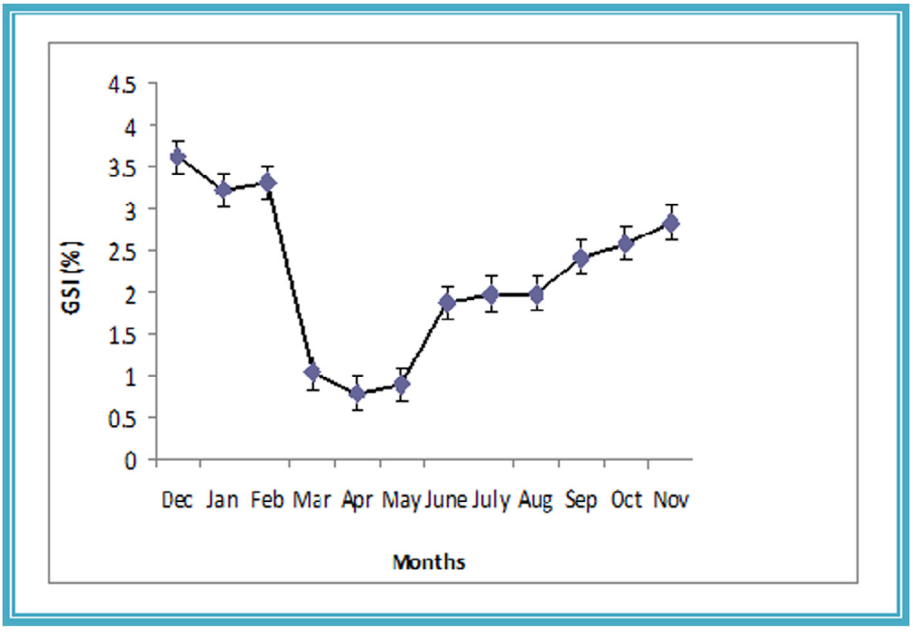

Fig. 1. Seasonal changes in the average Gonado-somatic index (GSI) of *C. gachua*.

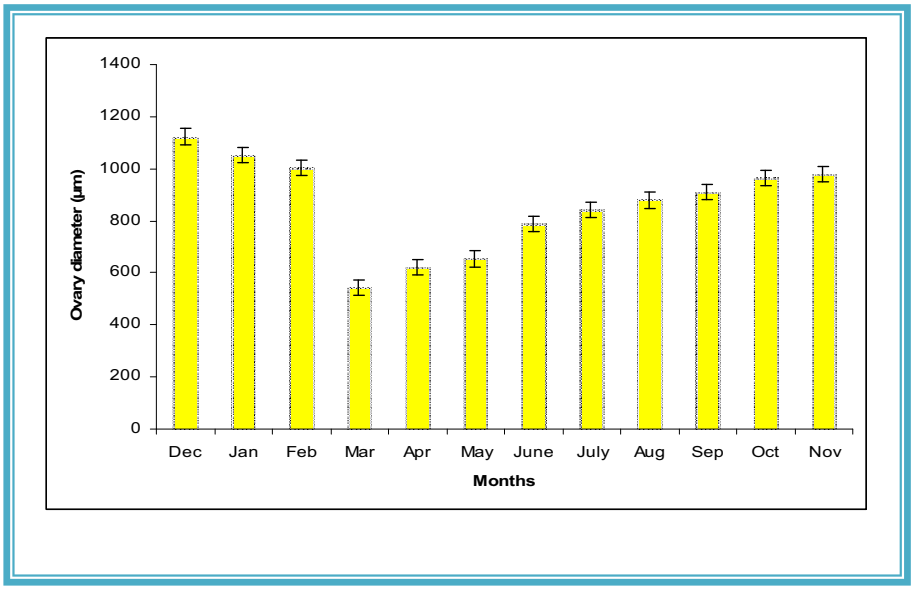

Fig. 2. Seasonal changes in the average Ovary diameter of *C. gachua*.

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the seasonal weight variations as was reported by Scott et al. (1980). In the current study, highest oocyte diameter was observed in December, indicating that the maturation of oocyte reached peak in this month.

3.1. Histological features of oocyte developmental stages

Based on the shape, weight, changes in the nuclear and cytoplasmic components of oocytes observed during histological analysis, five different oocyte developmental stages were distinguished immature, maturing, mature, ripe running and spent (Table 1). The oogenesis process was classified based on the oocyte size and staining, presence of follicular layer, number of nucleoli and the distribution of cytoplasmic inclusions. In a majority of teleost fishes, five-eight stages of oogenesis have been reported (Nagahama, 1983; West, 1990; Isisag, 1996; Gokçe et al., 2003). Arockiaraj et al. (2004) described five stages in the gonad of *Mystus montanus*. Similarly, Kader et al. (1988) made similar observations in "*Gobioides rubicundu*" = *Odontamblyopus rubicundus*.

Figs. 3–9 shows the histological appearances of different stages of ovarian development described in Table 1. The ovigerous lamellae was observed in ovary parenchyma with abundant follicles at different stages of development (Fig. 3), embedded in a connective tissue mass. A single layer of follicular cells was seen surrounding each developing oocyte. Early perinucleolar oocytes were most immature and polygonal in shape (Fig. 5). However, late perinucleolar oocytes became larger in size with the progress of oocyte development and varied in shape from polygonal to oval (Fig. 5). The yolk vesicles which were first seen at the periphery of the oocyte slowly spread towards the central nucleus (Figs. 6 and 7). The light pink stained yolk granules which were first observed in the outer cortex showed gradual increase in size and number (Fig. 7). The oocytes were greatly increased in diameter at this stage. As the yolk granules moved towards the inner cortex, they fused with lipid droplets and appeared deep pink with haematoxylin and eosin staining (Fig. 8).

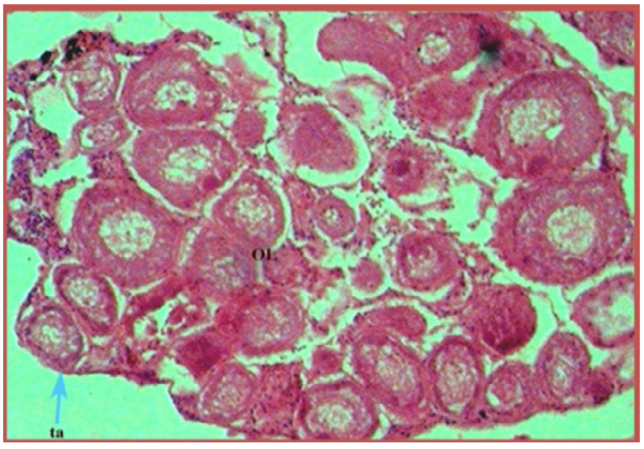

Fig. 3. Transverse section through the ovary illustrating oogenesis; Ovegerous lamellae (ol) from tunica albuginea (ta).

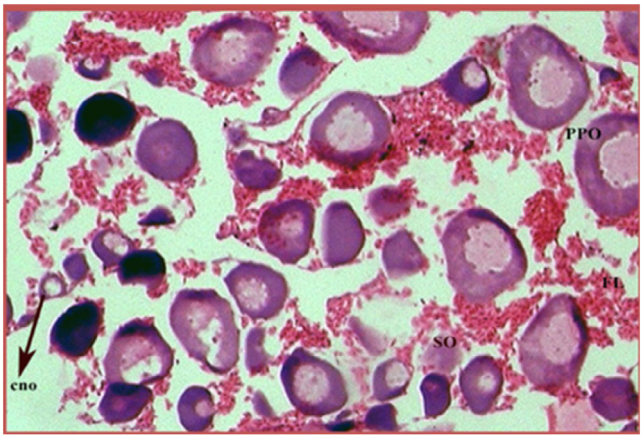

Fig. 4. Primary oogonia (po), secondary oogonia (so) and chromatin nucleolar oocytes (cno) adhered in ovarian nests together with follicle cells (fc), pre-perinucleolar oocytes (ppo) were outside the nests.

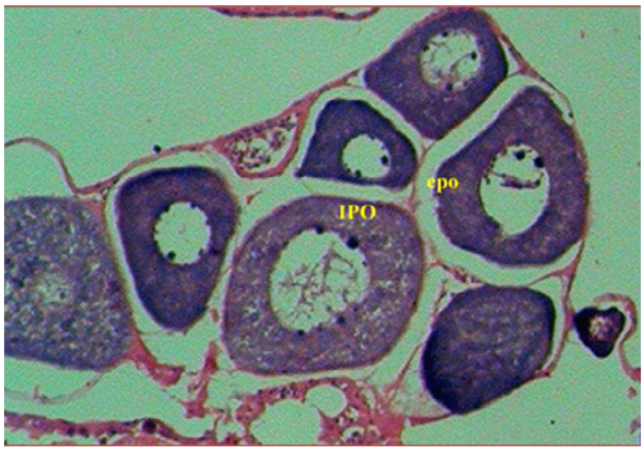

Fig. 5. Early perinuclear oocytes (epo), late perinuclear oocytes (lpo).

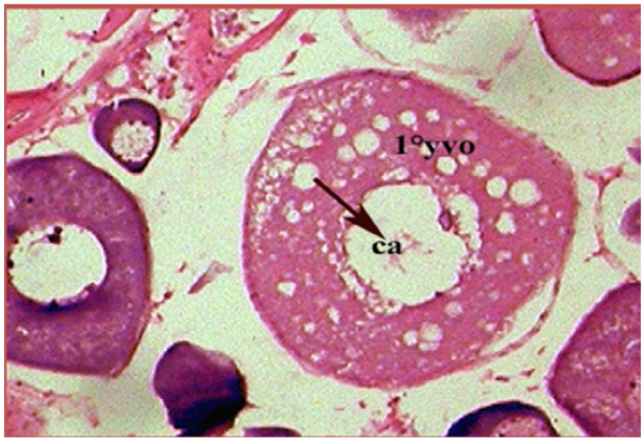

Fig. 6. Cortical alveoli (ca) in the primary yolk vesicle oocyte (1° yvo), chromatin nucleolar oocytes (cno), pre-perinucleolar oocytes (ppo).

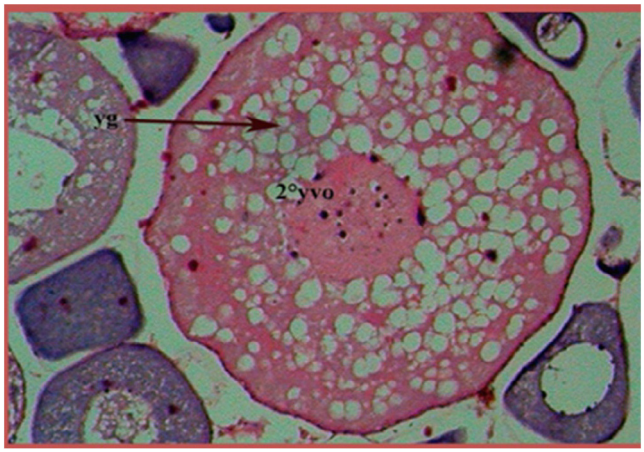

Fig. 7. Yolk granules (yg) and the cortical alveoli in secondary yolk vesicle oocyte (2° yvo).

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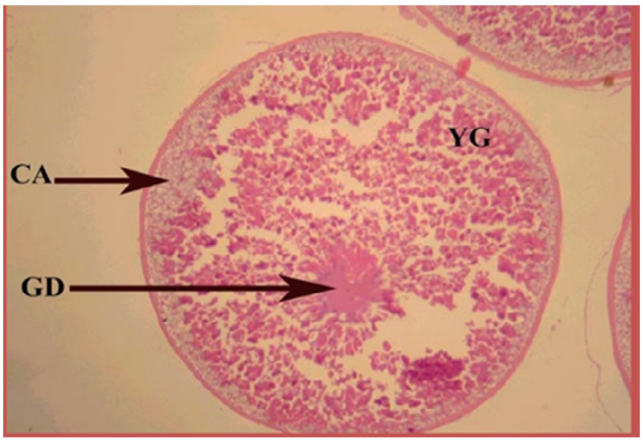

Fig. 8. TS through a tertiary yolk vesicle illustrating the central location of the yolk globules (YG), cortical alveoli (CA), and eccentric germinal disk (GD).

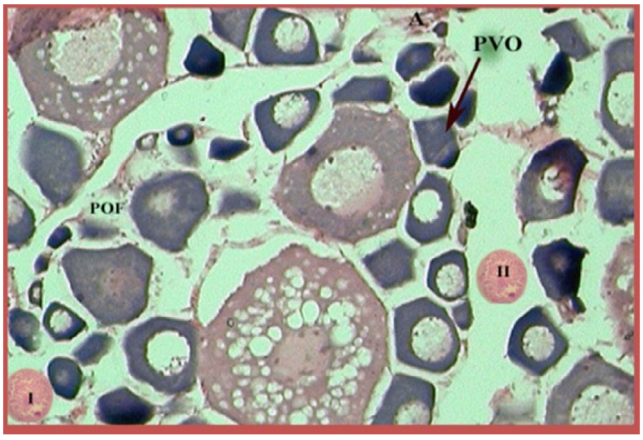

Fig. 9. TS through an ovary illustrating post ovulatory changes, post ovulatory follicles (POF), atretic oocytes (Type I & II) and a cohort of previtellogenic oocytes (PVO).

The presence of oocytes in different phases of development and of postovulatory follicles after winter months indicated that the dwarf snakehead prefer winter spawning (Fig. 9). Two stages of atretic oocytes in the *C. gachua* ovaries were detected in the transverse sections: Type I were relatively large (Fig. 9) and the loose, convoluted layer of granulosa cells were formed inside the thecal cell layer; however, the Type II were somewhat compact, and spherical outer thecal cell layer was seen (Fig. 9). In *C. gachua* the spawning season differed from *M. montanus* (Arockiaraj et al., 2004). *M. montanus* mainly breeds from October to December. In the present investigation, *C. gachua* breeds from December to February.

The application of histological studies in gonad developmental is consistent and widely accepted (Tomkiewicz et al., 2003). The process of ovarian development of *C. gachua* also follows the same basic progression as that described in other teleostean species.

4. Conclusion

Our results clearly demonstrated the GSI values of females showed significant difference between different months for Tamirabarani River populations. The increasing GSI values appeared from August to February with the peak in December, indicating the onset of the reproductive season. The GSI value declined rapidly from 3.31 to 0.79 after spawning. Therefore it was confirmed that the fish spawned once in a year with peak spawning from December to February. It can be concluded that the current study will contribute to better understand the cyclic and seasonal ovarian changes of *C. gachua* which in turn will help in conservation plans and captive maturation of this valuable species. The current study will also be suitable for selective breeding under captivity and sustainable fishery management of *C. gachua* in its natural habitats.

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