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Research Article
Ovarian Development of African Sharptooth Catfish (*Clarias gariepinus*, Burchell 1822) from Delhi Segment of River Yamuna

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Abstract

Background and Objective: An alarming increase in the population of *Clarias gariepinus*, an air-breathing catfish and its devastating role in the Indian aquatic system make it a topic of great concern in scientific fraternity. In view of the development of its management strategies, the present study was conducted to elucidate the ovarian development of this catfish procured from the river Yamuna in Delhi region. **Materials and Methods:** Macroscopic analysis of ovary, gonad somatic estimations, ova-diameter and fecundity estimation were analyzed to determine spawning period of the fish. The morphology of developing gonad (ovary) of *C. gariepinus* is described using light microscopy. Data was subjected to Mean ± Standard Error (SE) of the mean for oocyte diameter and nucleus diameter; Pearson correlation method and regression coefficient were calculated to study various aspects of fecundity. **Results:** Based on macroscopic analysis of ovary, gonad somatic estimations, ova-diameter and histological analysis, the fish was found to exhibit continuous gametogenesis indicating that it breeds throughout the year. The ova-diameter and Gonadosomatic index showed parallel fluctuations throughout the year viz. maximum (1.20 mm, 19.33) marking the spawning period; minimum (0.10 mm, 0.15) indicating the immature stage. All the histoarchitectural stages of the oocyte i.e., chromatin-nucleolus stage, peri-nucleolus stage, primary yolk stage, secondary yolk stage, tertiary yolk stage, migratory nucleus stage, mature stage and spent stage were observed throughout the year. The fecundity was estimated to be in the range of 17,219-3,11,213 eggs per female, positively correlated with gonad weight ($r = 0.94$), total length ($r = 0.70$) and body weight ($r = 0.79$). **Conclusion:** The continuous gametogenesis features adaptability of this species in Indian waters, thereby, placing ichthyofaunal biodiversity in peril. Efficacious control of alien species and designing of innovative options for their management is only possible through understanding of its breeding cycle.

Key words: *Clarias gariepinus*, invasive alien species, Yamuna, fecundity, gonadosomatic index

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

River Yamuna, the largest tributary of river Ganga supports a blend of local and exotic fish species. Since the recent past, the river has been subjected to various anthropogenic pressures which have led to alteration of the habitat structure, thereby, posing threat to the endemic fish species; invasion of alien species into natural water competing out the indigenous ichthyofaunal diversity. According to Singh *et al.*[1], alien invasive species viz. *Cyprinus carpio* var. *communis*, *C. carpio* var. *specularis, Carassius auratus*, *Oreochromis niloticus*, *Clarias gariepinus*, *Aristichthys nobilis*, *Hypophthalmichthys molitrix*. *Ctenopharyngodon idella*, *Gambusia affinis*, constitute major part of catch at most of the landing stations of river Yamuna. Among these, African sharp tooth catfish *Clarias gariepinus* (Burchell, 1822) is now being increasingly caught from various rivers including Ganga, Yamuna, Sutlej and Godavari[2,3]. The fish has also been reported from Periyar Lake[4,5] and Vembanad Lake in Kerala, India[6].

Despite being a fast grower and highly adapted to the harsh environment, *C. gariepinus* has remained a candidate of debate since its introduction in India due to its voracious predatory behavior. Its projected growth potential has been in general negated by the carnivorous feeding habit which has seriously endangered its cohabitant species. It is reported that *C. batrachus*, one of the most economically important indigenous freshwater fish in Asia[7-9] is now vanishing from the Indian aquatic ecosystem due to the invasion of *C. gariepinus*[10,11]. Highly prized native cichlids like *Etroplus suratensis* and *Etroplus maculatus* in Vembanad lake are facing threat due to the introduction of this exotic fish[6]. It has also been reported that *C. gariepinus* harbors around 18 species of infectious bacteria[12]. Although, *C. gariepinus* was banned by Government of India for culture yet it has been reported in almost all the natural water systems[13].

Knowledge of the reproductive cycle and the factors affecting it are important issues in fish biology[14]. Therefore, in view of the significance of elimination of this alien species from natural habitat, information about its breeding biology is of utmost importance which will eventually help in the development of management strategies. Scanty references in the study with respect to the reproductive performance of this fish in Indian water are available, whereas, such study of Yamuna river in Delhi region is altogether lacking. Keeping this in mind the present study was conducted to investigate different aspects of its ovarian development viz. Gonadosomatic Index (I_G), ova-diameter, histoarchitectural changes in the oocyte and fecundity, which will aid in the determination of its spawning season.

MATERIALS AND METHODS

Fish sampling: Female specimens of *C. gariepinus* were identified from the stock during April, 2014-March, 2016 from landing stations near Wazirabad and Okhla barrage, the two extreme ends of the river Yamuna in Delhi region (28.61°N, 77.23°E). Specimens within the range of 170-1700 g of body weight were studied to understand its breeding cycle. The ovaries were procured from the landing stations and were classified macroscopically into different maturity stages to have an idea of its breeding cycle which can shown in Eq. 1:

$$\text{Gonadosomatic index } (I_G) = \frac{\text{Gonad weight}}{\text{Gutted body weight of fish}} \times 100 \quad (1)$$

Ovum diameter: To determine the ovum diameter, 20 ovaries from each maturity stage were fixed in a 10% formalin solution. Sub-samples were taken from the anterior, middle and posterior regions of the ovary. The diameter of randomly 30 oocytes from each sub-sample was recorded using an ocular micrometer (Erma Inc, Japan).

Histological analysis: For histological studies, ovaries (n = 20 of each maturity stage) were fixed in Bouin's fixative for 24 h. After washing in tap water, the tissues were dehydrated in various grades of alcohol. Then the tissues were embedded in paraffin wax (melting point 60°C). The sections were cut at 5 μm thickness using microtome (Weswox Optic Model MT-1090A 15125), stained with haematoxylin and eosin[15] and were observed under a light microscope.

Fecundity estimation: To evaluate the fecundity of the fish, 40 ripe ovaries from the fish specimens (320-605 mm) length and (90-1700 g) weight were utilized. The gravimetric method was utilized where the external connective tissues and the moisture of the ovary were removed and weight of ovary of each fish was recorded with the help of electronic balance. The paired ovaries were kept in Gilson's fluid for 3 weeks for hardening of the eggs[16]. Sub-sample of 1 g from each ovary was taken; the number of eggs for each portion were sorted out and counted. Number of eggs in 1 g was determined and then multiplied by the total weight of the ovary, which gave the total number of eggs i.e., the fecundity of respective fish.

Statistical analysis: From each reproductive stage, oocyte diameter and nucleus diameter was calculated and expressed as Mean±Standard Error (SE) of the mean. Pearson correlation method and regression coefficient were calculated to test the relationship between fecundity with fish body weight, total length and gonad weight.

RESULTS

Macroscopic structure of ovary: Ovaries of *C. gariepinus* are paired sac-like organs located in the peritoneal cavity into which extends numerous ovigerous folds lined by germinal epithelium. On the basis of gross morphology of the gonad, the whole reproductive cycle of female fish was categorized into five stages[17] i.e., immature stage (stage 1), not ripe stage (stage 2), ripe stage (stage 3), running ripe (stage 4) and regressed stage (stage 5) (Fig. 1). The particulars of the gonadal structure, their morphology and gonadal-somatic indexes (Ig) are given in Table 1. Occurrences of all the stages in every month revealed that the fish breeds throughout the year. However, more numbers of specimens at stage 4 and 5 were recorded during May and June. The percent distribution of different stages during study period has been presented in Fig. 2.

Ova-diameter: The ovum diameter depicted variations in different maturity stages. The frequency distribution of oocytes in each maturity stage is shown in Fig. 3. Availability of variable sizes of ova (0.3-1.2 mm) in stages 3 and 4 reveals the fish breed multiple times in a year.

Phases of oocyte development-histological studies: The ovary undergoes various stages of development viz. multiplication, growth, maturity, depletion and rest. These cyclic changes occurring in the ovary of the fish are synchronized with its environmental conditions. Based on microscopic analysis of various characteristics of cells, the development of oocytes of *C. gariepinus* is classified following[18-21]:

- **Chromatin-nucleolus stage:** It was included of the smallest oocytes possessing large nucleus surrounded by cytoplasm. These oocytes remained strongly basophilic and were present throughout the year serving as reserve stock for future to replenish the ovary after depletion (Fig. 4a). Mean oocyte diameter and nucleus diameter was 104±7.40 and 78±5.71 μm, respectively (Fig. 5)
- **Peri-nucleolus stage:** There was an apparent increase in the size of the oocyte exhibiting less basophilic cytoplasm; enveloped by a simple squamous follicular epithelium. The unique characteristic identification of this stage is the presence of a ring of nucleoli at the periphery of the nucleus. Two types of peri-nucleolar stages were recognized based on oocyte shape, size, nature of cytoplasm and arrangement pattern of nucleoli viz.

Table 1: Morphology of the gonad during different stages of maturity in *C. gariepinus*

Maturity stages	Morphology	GSI	Ova-diameter (mm)	Months of availability
Immature stage	Delicate, thread like structure	0.15-0.31	0.10-0.20	Throughout the year
Not ripe stage	Small and reddish brown in color	0.40-0.75	0.20-0.50	Throughout the year
Ripe stage	Swollen, granular in appearance, almost ripe, dark green brown in color with blood vessels ramifying over the surface	1.07-4.17	0.30-1.00	Throughout the year
Running ripe	Light green ova in running ripe condition i.e., the eggs could easily come out from the genital after pressing the abdomen indicating the spawning period	4.41-19.33	0.30-1.20	Throughout the year
Regressed stage	Ova extruded from the vent and ovary appears dark brown red in color	0.43-1.23	0.40-1.00	Throughout the year

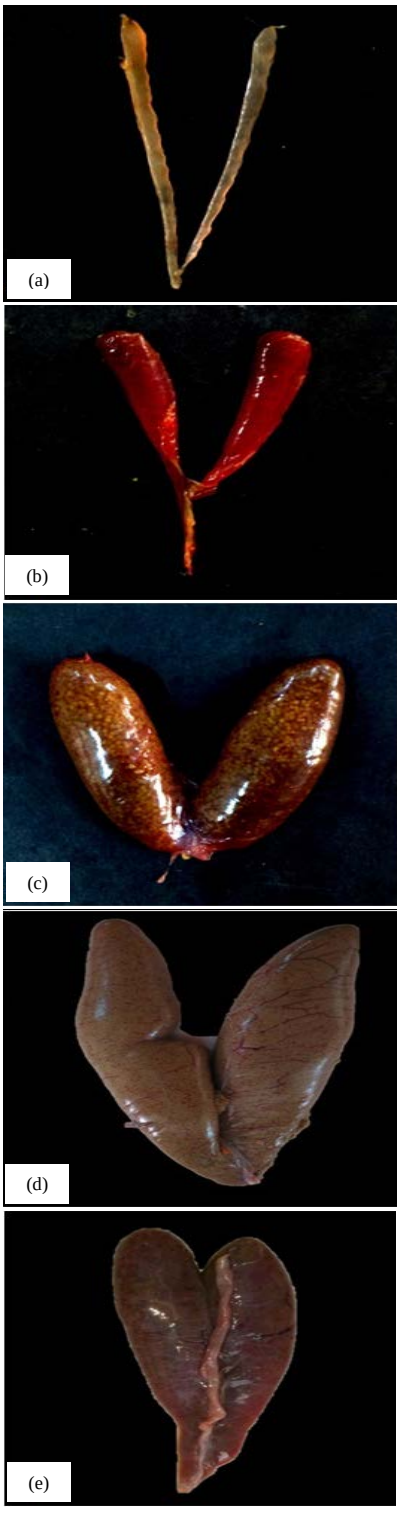

Fig. 1(a-e): View of the ovary of *C. gariepinus* during different reproductive phases (a) Immature (stage 1), (b) Not ripe (stage 2), (c) Ripe (stage 3), (d) Running ripe phase (stage 4) and (e) Regressed phase (stage 5)

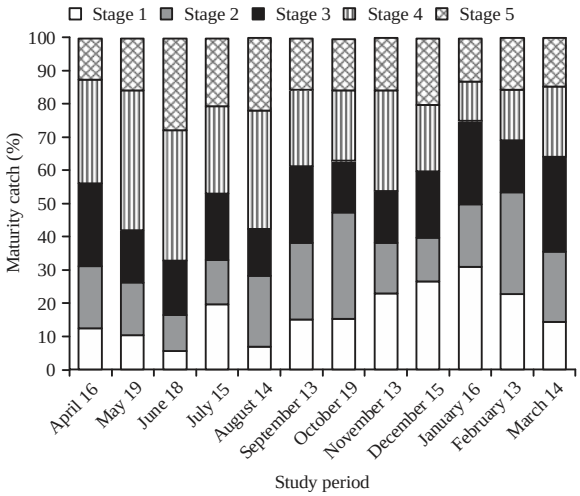

Fig. 2: Monthly variations is percent catch of different maturity stages of *C. gariepinus* during the study period

early peri-nucleolar stage with small size, polygonal shape, basophilic cytoplasm and scattered nucleoli (Fig. 4a), late peri-nucleolar stage with large, spherical in shape, less basophilic, ring of uniformly arranged nucleoli (Fig. 4b). Mean oocyte diameter and nucleus diameter was 238±19.33 and 89±5.04 μm, respectively (Fig. 5)

- **Primary yolk stage:** The size of oocytes (358.16±14.42 μm) had become larger. This stage is marked by the presence of cortical alveoli. These appear as unstained spherical structure usually at the periphery of the oocyte (Fig. 4c). These vacuoles indicated the beginning of growth phase or beginning of vitellogenesis. Large numbers of peripheral nucleoli are present around the centrally located germinal vesicle. The oocyte is marked by a distinct thin vitelline envelope beneath the follicular epithelium. Mean nucleus diameter ranged 114.4±7.6 μm (Fig. 5)
- **Secondary yolk stage:** The development progressed, the oocyte becomes larger in size (536±2.81 μm), yolk spheres, granules or globules increased in number, beginning to move into the ooplasm, marking mid-vitellogenic phase (Fig. 4d). It possesses similar follicular layer as in primary yolk stage. Mean nucleus diameter ranged 92.62±7.65 μm (Fig. 5)
- **Tertiary yolk stage:** Oocytes with mean diameters of 702.8±18.30 μm and nucleus mean diameter 72.40±4.28 μm (Fig. 5) were observed. Yolk globules progress from the periphery towards the centre of the oocyte where an irregularly shaped nucleus is located. The thickness of zona radiata and follicular epithelium has increased (Fig. 6a)

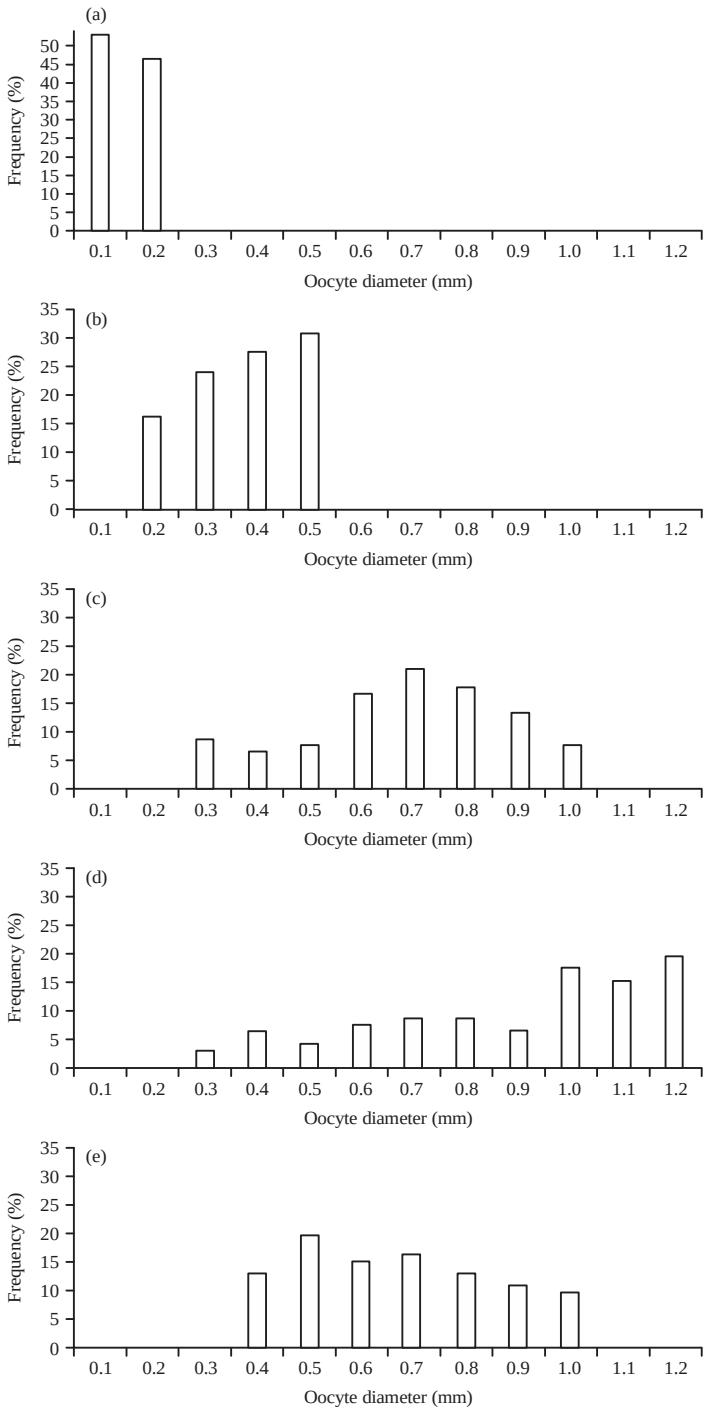

Fig. 3(a-e): Frequency distribution of oocyte diameters in different maturity phases in ovaries of *C. gariepinus*, (a) Immature (stage 1), (b) Not ripe (stage 2), (c) Ripe (stage 3), (d) Running ripe phase (stage 4) and (e) Regressed phase (stage 5)

- **Migratory-nucleus stage:** The nucleus had attained a very small size (57.2±3.38 μm) (Fig. 5), with almost imperceptible nucleoli. It migrates towards the animal pole of the egg leaving the major fraction of the egg covered by yolk (Fig. 6b). Oocyte diameter ranged 808.72±6.72 μm
- **Mature stage:** Soon after germinal vesicle breakdown, yolk globules fused with each other and oocyte is ready to ovulate (Fig. 6c). The oocyte has attained maximum size (815.74±20.46 μm) and was surrounded by well-developed zona radiata (8.4 μm) and the follicular epithelium (Fig. 6d)

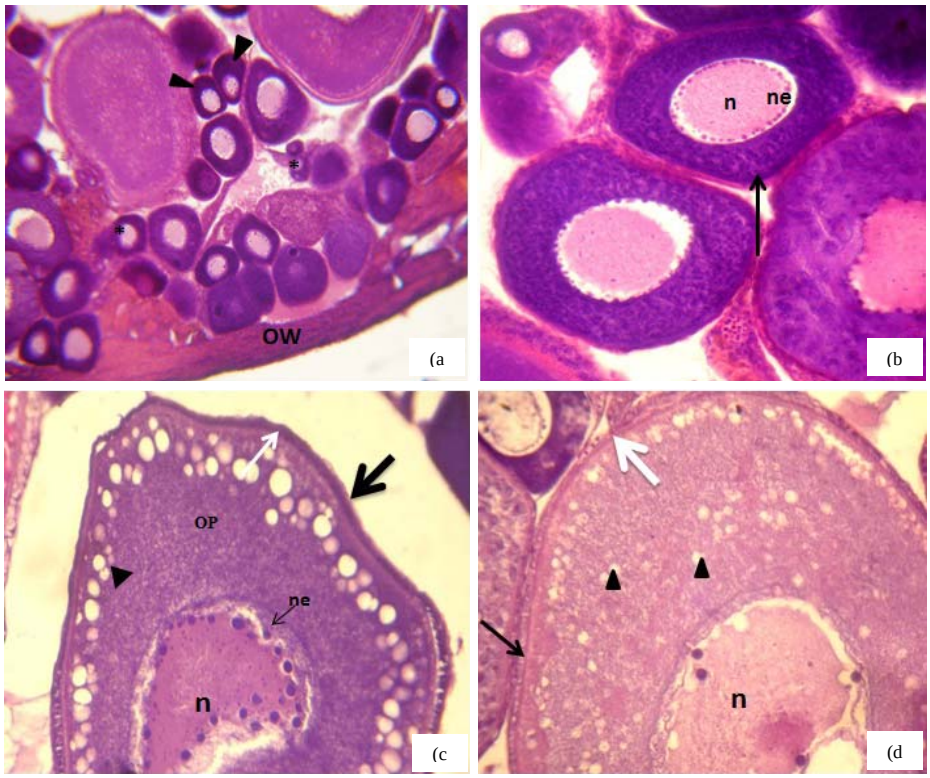

Fig. 4(a-d): Light micrograph of ovary of *C. gariepinus* showing different developmental changes (a) Chromatin-nucleolus stage (*) showing small sized oocyte and early stages of peri-nucleolus stage (arrow heads), enclosed in ovarian wall at 100X, (b) Late peri-nucleolus stage showing large centrally placed nucleus bearing ring of nucleoli enveloped by follicular epithelium at 400X, (c) Primary yolk stage showing cortical alveoli (arrow heads) at the periphery of the oocyte, nucleus with ring of nucleoli at 400X and (d) Secondary yolk stage showing increasing number of yolk granules (arrow heads) towards the nucleus at 400X

n: Nucleus, ne: Nucleoli, OW: Ovarian wall, White arrow: Zona radiata, Black arrow: Follicular epithelium, OP: Ooplasm

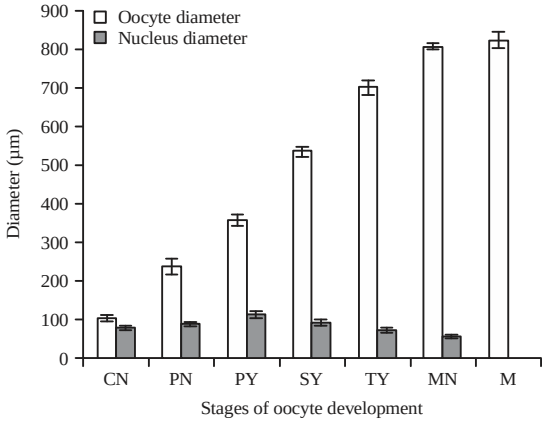

Fig. 5: Mean oocyte and nucleus diameter (µm) of *Clarias gariepinus* during different stages of oocyte development

CN: Chromatin nucleolus stage, PN: Peri-nucleolus stage, PY: Primary yolk stage, SY: Secondary yolk stage, TY: Tertiary yolk stage, MN: Migratory yolk stage, M: Mature stage

- **Postovulatory follicle stage or spent phase:** Irregularly shaped follicles showing resorption of ovular contents can be clearly observed (Fig. 6e)
- **Fecundity:** A total of 40 ripe females of *C. gariepinus*, of 320-605 mm length and 290-1700 g b. wt., respectively were utilized for determining its total fecundity. The resulting fecundity ranged between 17,219-3,11,213 eggs. Fecundity exhibited linear relationship with gonad weight, total length and body weight (Fig. 7a-c) described by linear equation y = 1220x-5294.7, y = 1074.8x-392755 and y = 202.14x-6026, respectively. The coefficient of correlation for the relationship between fecundity and gonad weight, total length and total weight was 0.94, 0.70 and 0.79, respectively, significant at 5% level.

DISCUSSION

Alien species can cause severe changes in ecosystem's functioning and currently recognized as principal agents of

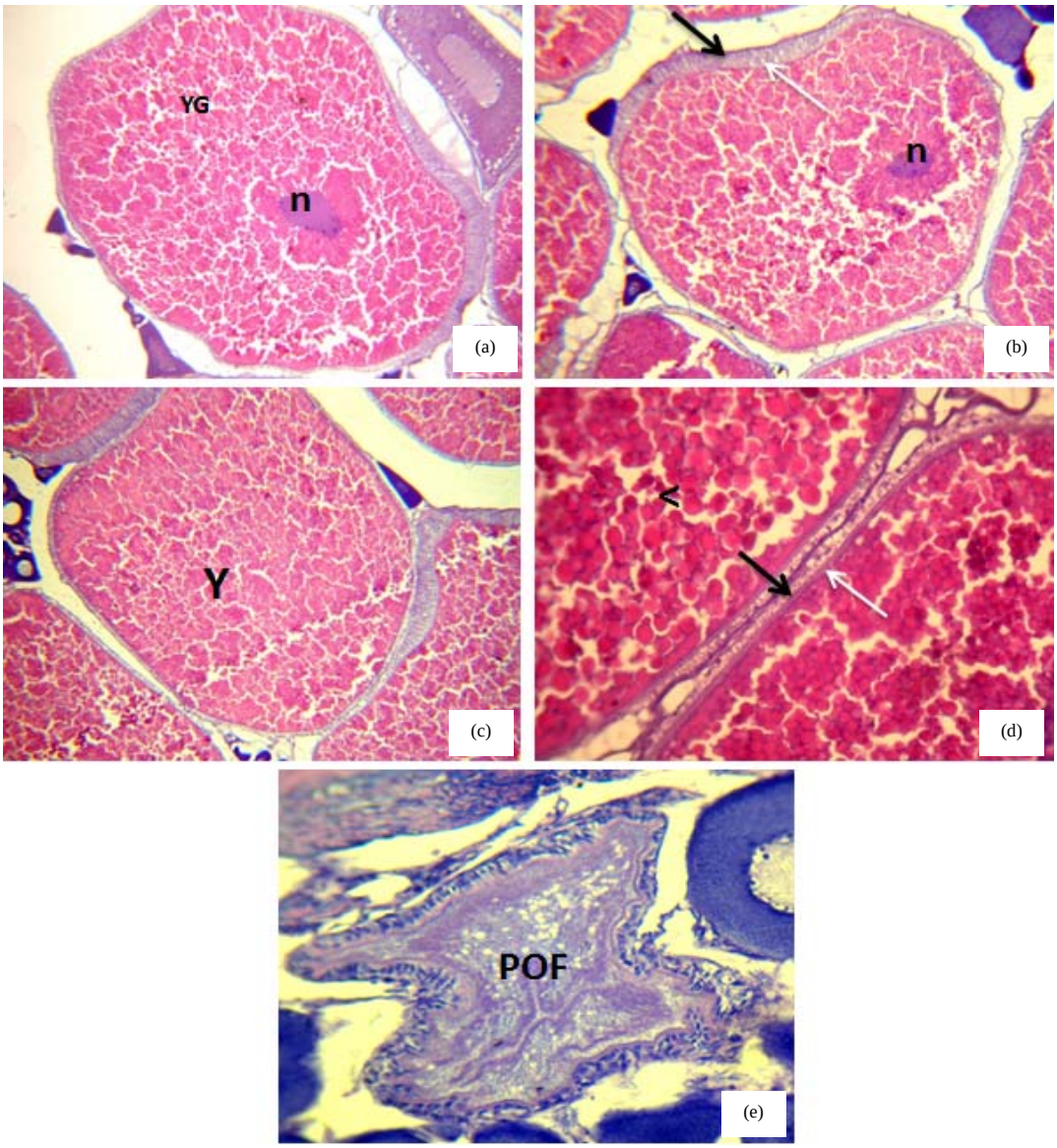

Fig. 6(a-e): Light micrograph of ovary of *C. gariepinus* showing different developmental changes (a) Tertiary yolk stage showing the increasing number of yolk granules which fill the entire ooplasm, small nucleus at 100X, (b) Migratory-nucleus stage displaying the movement of nucleus towards the animal pole at 100X, (c) Mature stage exhibiting degeneration of nucleus and is covered by yolk at 100X, (d) Well developed vitelline membrane (white arrow), follicular epithelium (black arrow) at 400X and (e) Post-ovulatory follicle (POF) at 100X

n: Nucleus, YG: Yolk granules, Y: Yolk, White arrow: Zona radiata, Black arrow: Follicular epithelium

ecological changes[22,23]. Invasion, expansion and pre-eminence of *C. gariepinus* along the stretch of Yamuna river, particularly in Delhi have become a topic of great concern. Therefore, study of its reproductive biology is needed as it is an effective method for recognizing the stocks and life cycle of fishes[24].

According to the macroscopic appearance of ovary, five different forms were observed, which presented the rough estimate of its maturity. Gravid female specimens were recorded during the whole study period, indicating that the species breeds throughout the year. Such a long breeding season is a type of adaptation by this population, which lives in an unstable habitat, to environmental conditions[25]. However, studies performed by Singh *et al.*[26] on *C. gariepinus* collected from culture ponds, revealed the fish breeds throughout the year except from December-February. These

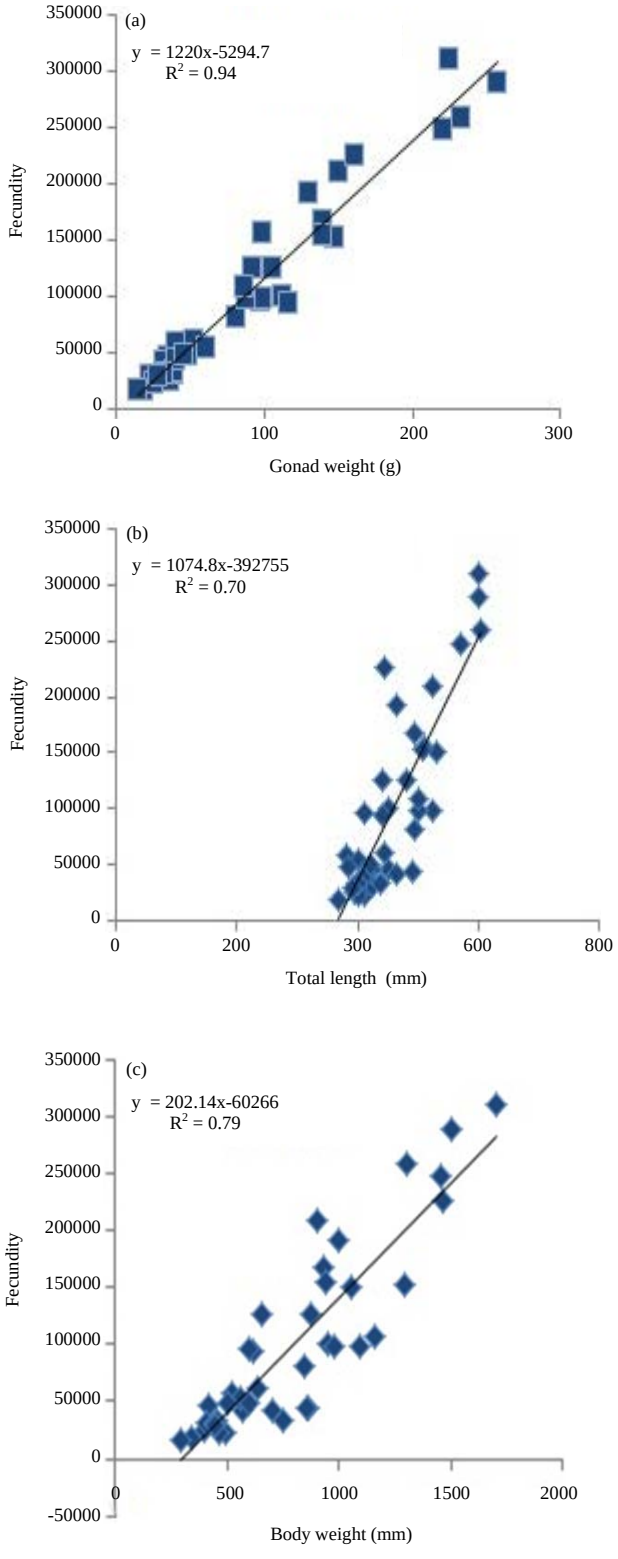

Fig. 7(a-c): Relationship between fecundity of *C. gariepinus* from Yamuna river (a) Gonad weight (g), (b) Total length (mm) and (c) Body weight (g)

slight differences in the reproductive performance of the fish is may be due to differences in physiography of habitat i.e., depth and area of the culture pond is comparatively less where the temperature inclines to the lower side during the cold months, whereas in barrage on the rivers have more open area where continuous mixing of water results in slightly higher temperature especially at the deeper areas. Thus, the population of *C. gariepinus* seems to be well established in Delhi segment of river Yamuna. On the contrary, the fish spawned only during May-August in river Asi, Turkey when the water temperature ranged between 21 and 30°C[17]. The present study confirmed the above observation as a higher percentage of specimens at the ripe stage was encountered during the period from May-July.

The parallel fluctuations in GSI and ova-diameter were noticed, with the sizes of oocytes gradually increasing with ovarian development. Larger-sized groups of oocytes (>0.7 mm) were primarily found in ripe and running ripe stages and smaller sized oocyte (<0.3 mm) were presented in all the maturity stages, displaying heterogeneity in the population of eggs in the gonad. The progressive change observed in the intra-ovarian diameter for a period not less than a year can give an idea of the spawning periodicity of the fish studies[27].

Histomorphological variations observed during the oocyte development such as proliferation, vitellogenesis, maturation and finally ovulation were found similar to the findings in other catfishes by various workers[28-30]. On the basis of histological examination, a range of developmental stages i.e., tertiary yolk, migratory-nucleus and mature stage oocytes with postovulatory oocytes were found throughout the year which revealed *C. gariepinus* possesses asynchronous type of ovarian development in contrast to group-synchronous type as reported by Cek and Yilmaz[31] in African catfish cultured under laboratory conditions. Furthermore, the presence of mature oocytes with postovulatory oocytes confirms the multiple spawning trait of the fish[32].

The fecundity ranged between 17,219-3,11,213 eggs per female, which goes in confirmation of Yalcin *et al.*[17]. The fecundity was affected by many factors, such as the size and age of the female[33], the life history strategy[34], food supply and temperature[35]. The amount of energy available for egg production and the body cavity accommodating the eggs increases with fish size, thereby affecting the fecundity of the fish[36]. According to the present study, the coefficient of correlation for the relationship between fecundity and fish weight (r = 0.89) was more than fish length (r = 0.83), indicating that body weight provides better information of reproductive capacity of fish than its length. The findings of

the present study and other studies performed worldwide on African sharp tooth catfish[37-39] demonstrated the fish has a different spawning period which may be due to different climatic conditions, food availability and area of distribution. The findings of the present study provided baseline information about its breeding biology in Indian waters, particularly Yamuna river, which could be a start to more studies that would contribute and compel the authorities to act in a sagacious manner thereby, managing the stock.

CONCLUSION

The ability of the fish to breed so efficiently and subsequent colonization in the river system of Indian sub-continent has become a major threat to native fish fauna.

Knowing its menacing consequences on native fauna, effective management plans should be undertaken to control this exotic fish which is found almost in every river system of our country.

Considering the fact that this fish breed throughout the year and migrates to the shallower part of the river for maximum survival of the young ones, harvesting all sizes of *C. gariepinus* from the shallow region before it spawns can to some extent reduce its population in the river system.

SIGNIFICANT STATEMENT

Clarias gariepinus is recognized in abundance in rivers of Indian subcontinent, particularly in the river Yamuna, Delhi segment and has been reported as one of the causes for extinction of native species. The findings of the present study on its breeding cycle highlighted that the species has well established itself in the river system by undergoing breeding throughout the year.

This topic is an emerging issue and requires urgent action from the concerned authorities and awareness among fish farmers and consumers.

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