Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6447

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (3):** 658-665 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Seasonal changes in the ovary of *Heteropneustes fossilis* (Bloch) in correlation with its reproductive cycle

K. Singh*

Department of Zoology, Campus of Best Moral College, Bedia, Distt. Khargone [M.P.] India

*Corresponding Author E-mail: k.singhsagar@gmail.com

Received: 28.04.2018 | Revised: 25.05.2018 | Accepted: 9.06.2018

ABSTRACT

During the post-spawning period (September to December) the ovary shows early stages of oogeneis viz., chromatin-nucleolus stages, peri-nucleolus while yolk vesicle stage observed in large number. During pre-spawning period (January to April) further growth and development of the oocyte take place while the yolk stages are also observed in abundance. By the end of this period i.e., pre-maturation and maturation stages are seen in the oocytes alongwith a few corpora atretica. During spawning period (May to August), particularly in June and July, most of the ovas are in a mature stage amongst which are also found some early stages of oocyte as well as few corpora atretica. During July month, the post-ovulatory follicles are observed indicating the optimum spawning condition.

Key words: *Histology, Ovary, Reproductive Cycle, Heteropneustes fossilis (Bloch)*

INTRODUCTION

The present study has been planned to observe the seasonal changes in the ovary of *Heteropneustes fossilis* (Bloch) during its reproductive cycle. Important work on the structure of the fish ovary alongwith its seasonal changes in the annual reproductive cycle have been described by various workers. Aggrawal N. *et al.*[1] have reported armotase activity in brain and ovary: seasonal variations correlated with circannual gonadal cycle in the catfish, *Heteropneustes fossilis*. Belsare[3] has shown the seasonal changes in the ovary of Ophiocephalus punctatus. Bentivegna and Benedetto [4] have studies the Gonochorism and seasonal variations in the gonads of the Labrid Symphodus (Crenilabrus) Ocellatus ocellatus (Forsskal). Buxton[5] has reported the reproductive biology of Chrysoblephus laticeps, Cristiceps cristiceps Teleostei: Sparidae. Chandrasoma and De Silva[6] have studied the reproductive biology of Puntius sarana: an indigenous species and Tilapia rendalli (Melanopleura) and an exotic fish in an ancient man made lake in Sri Lanka. Coetzee[7] has shown seasonal, histological and macroscopic changes in the gonads of Cheimerius nufar. Merwe *Vander et al.*[8] have studied the cyclic histomorphological changes in the ovary of Mud fish, Labeo capensis.

Cite this article: Singh, K., Seasonal Changes in the Ovary of *Heteropneustes fossilis* (Bloch) in Correlation with its Reproductive Cycle, *Int. J. Pure App. Biosci.* **6(3):** 658-665 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6447

Milton james *et al.*[9] have reported ovarian devolvement and histological observations of threatened dwarf snakehead fish, Channa gachua. Mahmod abdelghaffar emam and Badia abughrien10 have reported seasonal histological changes in gonads of the catfish. Prabhu[11] has reported on Maturation of intraovarian eggs and spawning periodicity of some fishes. Raizada[12] has also studied the reproductive system and the reproductive cycle in some teleosts Rasbora daniconius (Ham). Ranjan prabhat has reported[13] ovarian cycle and spawning season of ophiocephalus punctatus inhabiting chapra water bhihar. Saksena [14] has studies on Hypothalamo-Hypophysial Neurosecretory system in fresh water fish Glassogobius giuris (Ham.) in relation to reproduction. Subhedar and Prasada Rao 16have studied the seasonal changes in the corpscles of stannius and gonads of the catfish, Heteropneustes fossilis (Bloch). Tricas and Hiramato[18] have shown the sexual differentiation, gonad development and spawning seasonality of the Hawaiian butterfly fish, Chaetodon multicinctus. Urashani T.J. *et al.*[19] have reported histological changes in pituitary gonadotrophic hormone cells in relation to seasonal changes in ovaries of the fish Orieochromis mossambicus. Yamamoto[20] has studies on the formation of fish egg[1]. Annual cycle in the development of ovarian eggs in the flounder, Liopsetta obscura.

MATERIAL AND METHODS

The fish Heteropneustes fossilis (Bloch) were obtained from local Sagar lake, Sagar, M.P. Twenty four adult fishes were collected during the first week of every month for one complete reproductive cycle i.e.; for continuous 12 months.

The eyes as well as the surface bones of skull were removed and an incision was given in the abdomen so as to ensure efficient and optimum fixation. The mature female fish was weighted (25-30gm) and ranging between 12 to 17 cm in length and the gonads were removed, then immediately fixed the bouin's solution for 24hours, dehydrated through ascending grads of ethanol clear xylem, softening of yolk in ovary embedded in paraffin wax and section cutting at 5µm. The sections were stained with haematoxylin and Eosin.

RESULTS

MORPHOLOGY AND HISTOLOGY OF THE OVARY:

The ovary in the Heteropneustes fossilis (Bloch) is an elongated and spindle shaped structure suspended in the coelom by a peritonium fold known as mesovarian in between the kidney and alimentary canal. The anterior portion of the ovary is conelike and tapering, the middle is wider and posterior is narrower. The peritoneal folds of the posterior side of each ovary show an extension of a hollow tube like structure forming the oviduct which ultimately joins posteriorly with the fellow of the opposite side to form a common oviduct and opens to the exterior through the genital pore.

The ovaries are enclosed in thin peritoneal covering. The ovarian wall is composed of an outer layer of fibrous connective tissue traversed by the blood capillaries and an inner layer of germinal epithelium. The ovigerous lamellae containing oocytes in various stages of development projected from the ovarian wall towards the centre of the ovary. The early stages of oocytes are found on the periphery of the lamellae. They migrate towards the ovocoel centre during their development. The ovigerous lamellae are covered by a thin layer of epithelial cells.

OOGENETIC STAGES

Different oogenetic stages in Heteropneustes fossilis (Bloch) can be devided into ten developmental stages on the basis of both nuclear and cytoplasmic changes with special reference to vitellogenesis. The different

developmental stages as described here are based on the work of Yamamoto [20] which provides enough scope to study the various stages of the ovary as follows: Early chromatin –nucleolus stage, late chromatin-nucleolus stage, early peri-nucleolus stage, late peri-nucleolus stage, early yolk-vesicle stage, late yolk-vesicle stage, early yolk stage, late yolk stage, Pre-maturation stage, Maturation stage.

CORPORA ATRETICA & POST OVULATORY FOLLICLES:

Corpora atretica:

The immature oocytes which fail to attain maturity and the mature oocytes which fail to spawn ultimately undergo resorption or atresia and are called corpora atretica or atretic follicles. In the process of resorption, granulosa layer or follicular epithelium of the ovarian follicle plays an important role. Bhargava[2] has used the term corpora atretica. They produce the enzymes which digest the yolk, the cytoplasm and the nucleus. The process of atresia of mature oocytes have been distinguished into four different stages such as Stage I, Stage II, Stage III, Stage IV (Fig.8)

Post-ovulatory follicles:

The post-ovulatory follicles (empty or ruptured follicles) are formed after the extrusion of mature oocyte from the ovary. The frequency of the number of post-ovulatory follicles in the ovary is an useful measure to estimate the spawning periodicity of the fish.

Post-ovulatory follicles of *Heteropneustes fossilis* are convoluted structure containing an irregularly shaped structure. (Fig.7)

Post-spawning period (September to December):

During this period the ovarian wall is thick and distinct. Ovigerous lamellae are present in the month of September and few ripe ovas are also present. The interfollicular space is now quite distinct, corpora atretica and post ovulatory follicle are present. Early oocyte stage though few in number shows the chromatin nucleolus stage. In the month of October and November few blood vessels are found in the ovarian wall as well as within the ovigerous lamellae. The Chromatin-nucleolus stage and peri-nucleolus stage are abundantly seen in the stroma of the ovary. (Fig.1) during month of December, the ovigerous lamellae contain numerous oogonia. Interfollicular space is still distinct. Late peri-nucleolus and yolk vesicle stage were also observed. Corpora atretica and post-ovulatory follicle are totally absent. (Fig.2)

Pre-spawning period (January to April):

During this period the ovarian wall is thick and highly vascular. In the month of January and February the vacuoles started to appear in the oocyte. A large number of early yolk vesicle stages were also observed. A few late chromatin nucleolus stages are seen. Corpora atretica and post ovulatory follicle are not seen (Fig.3.). In the month of March and April, the ovarian wall becomes thinner as compared to that of preceding month. The late yolk vesicles stage showed vaculation while yolk formation also started in the cytoplasm. Early yolk stage lies towards the border of the ovigerous lamellae. A few corpora atretica are present. Post-ovulatory follicles are completely absent. (Fig.4 &5).

Spawning period (May to August):

During the spawning period, the ovarian wall is very thin a large number of ripe oocytes are present alongwith few immature oocytes. Their number is fewer than in the preceding month. In month of May, interfollicular space is further reduced. The yolk granules appear in the oocyte. Corpora atretica and some post ovulatory follicles are also evident. In June and July, majority of the ova in the ovary become fully ripened and are closely packed together. A very few immature oocyte are also present in between the ripe ones. The corpora atretica and post ovulatory follicles are also seen during this period. (Fig.6&7). In the month of August corpora atretica and post ovulatory follicles are again noticed but their number has increased. (Fig.8)

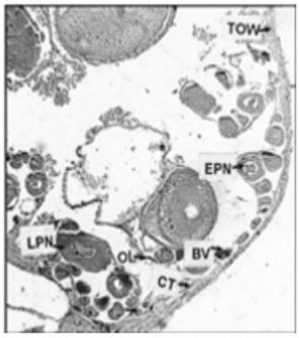

Fig. 1 Photomicrograph of Transverse Section of Ovary of *Heteropneustes fossilis* (Bloch) Showing seasonal variation during post-spawning period (October)
H & E 60x

Fig. 2 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing seasonal variations during post-spawning period (December) late Peri-nucleolus and early yolk vescle stage.
H & E 60x

Fig. 3 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing seasonal variations during pre-spawning period (February)
H & E 60x

Fig. 4 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing seasonal variations during pre-spawning period (March)
H & E 60x

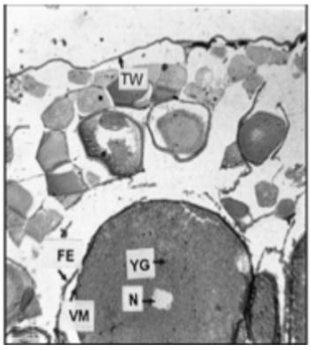

Fig. 5 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) showing seasonal variations during pre-spawnin period (April)
H & E 60x

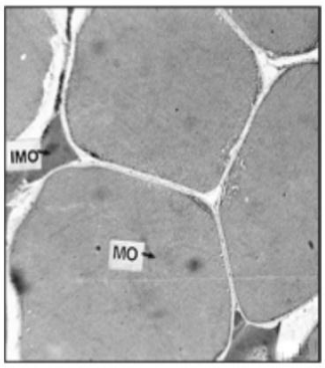

Fig. 6 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing seasonal variations during spawning period (June)
H & E 60x

C.T. : Connective,E.PN. : Early Peri-nucleolus,E.YN. : Early Yolk vesicle stage, L.PN : Late Peri-nucleolus stage, O.L. : Ovigerous Lamellae T.O.W. : Thick Ovarian wall, C.A. : Corpora atretica F.E. : Follicular epithelium, L.PN. : Late Peri-nucleolus stage, T.W. : Thin Ovrarian wall V.M. :Vetelline membrane,Y.G. : Yolk granules,P.OF : Post-ovulatory follicle, N.M. : Nuclear membrane, N : Nucleus,

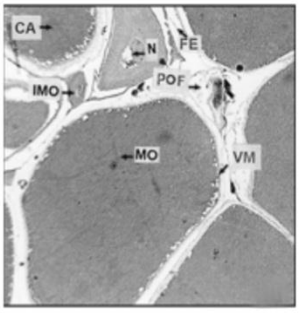

Fig. 7 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing seasonal variations during spawning period (July) H & E 60x

Fig. 8 Photomicrograph of T.S. of the Ovary of *Heteropneustes fossilis* (Bloch) Showing seasonal variations during spawning period (August) H & E 60x

C.T. : Connective,E.PN. : Early Peri-nucleolus,E.YN. : Early Yolk vesicle stage, L.PN : Late Peri-nucleolus stage, O.L. : Ovigerous Lamellae T.O.W. : Thick Ovarian wall, C.A. : Corpora atretica F.E. : Follicular epithelium, L.PN. : Late Peri-nucleolus stage, T.W. : Thin Ovrarian wall V.M. :Vetelline membrane,Y.G. : Yolk granules,P.OF : Post-ovulatory follicle, N.M. : Nuclear membrane, N : Nucleus,

DISCUSSION

The origin of the new crop of oocytes in faced with divergent opinions and the observations in this direction are variable. Yamamoto 20 state that the new crop of germ cells originates from the follicles. Cells contained in the empty follicle are left behind after the extrusion of the mature oocytes. Raizada[12] has observed that the new oocyte are formed as a result of proliferation of germinal epithelium while Belsare[3] has noticed the origin of new crop of the oocytes take place from the pre existing oogonia. Saxena[14] has suggested that the new crop of oocytes is derived from the germinal epithelium. In *Heteropneustes fossilis* it has been observed that the new oogonia appear from the germinal epithelium and transformed into oocyte during a short rest period i.e. the early chromatin-nucleolus stage is always found associated with the germinal layer.

The process of the yolk formation is also described by various workers. Yamamoto [20] has studied the formation of the fish egg with reference to vitellogenesis and histochemistry of yolk granules. Aggrwal *et al.*[1] have reported aromatase activity was detectable in the hypothalamus throughout the year where as in ovary only during active vitellogenesis. Saxena[14] has been noticed that the yolk granules start deposition in between the yolk vesicles. The yolk granules or globules are at first lightly basophilic but soon become light acidophilic. Tricas and Hiramoto[18] have suggested that the yolk uptake rapidly accelerated oocyte enlargement. In early vitellogenesis, the vitelline membrane appeared as a thin eosinophilic band around the oocyte. Yolk spheres first appeared near ooplasm periphery and migrate towards the cell interior. As cell enlarged, the vitelline membrane thickened. In later stages of vitellogenesis, enlarged yolk spheres filled the oocyte interior and lipid dropletes aggregated near the nucleus. Coetzee [7] and Buxton [5] have reported the acidophilic secondary yolk globules which are next to appear first in the region of the primary yolk vesicles but later as extra vesicular yolk throughout the cytoplasm. In the tertiary yolk vericle stage the zona radiata is clearly visible and cytoplasm become entirely filled with yolk vesicles. At the end of development the nucleus migrates towards the periphery of the cell which is followed by a degeneration of nuclear membrane, formation of oil droplet and the coalescence of yolk will follow accordingly.

In *Heteropneustes fossilis* (Bloch) the yolk formation starts at the primary yolk stage of oocyte as small granules of yolk inside each visible. These small granules are of first lightly acidophilic in nature. Later on they fused to form large yolk granules which are strongly acidophilic. The yolk granules or globules are of different shapes and size and therefore show differential staining property to acidic dyes.

In *Heteropneustes fossilis* (Bloch) the oocytes are covered with a layer of follicle cells, below which lies a vitelline membrane as also observed in Ophiocephalus punctatus, Belsare 3 Rasbora daniconius, Raizada[12] and Glossogobius giuris, Saxena[14]. The present study showed no other layer comparable to the theca layer. Certain follicle cells i.e., granulosa cells take parts in the absorption of yolk. The above observation is confirmed in the present study with *Heteropneustes fossilis* (Bloch) where the hypertrophied cells, by their phagocytic activity, first destroy the vitelline membrane and then invade the yolk through it. The disintegrating ferments Buxton [5] has reported the two distinct atretic events which are recognized in the ovaries of Chrysoblephyus laticeps and Cristiceps cristiceps. The first is a resorption of yolk oocytes during normal ovarian cycle in the breeding season but is most common immediately after spawning. The second is a condition associated with sex reversal which includes a degeneration of all oocyte stages and the progressive absorption of the entire ovary. Merwe *et al.*[8] have suggested that in the Labeo capensis the atretic oocytes are present during primary oocyte development (summer and autumn). The degeneration first occurs in the oocyte membrane where after ooplasm and nucleoplasm material extrudes in to the interstitial tissue. Raizada[12] Saxena[14] have used the term corpora atretica for the degeneration of unspawned ripe oocytes or the immature oocytes undergoing oolysis. This term has been also followed in the present work in *Heteropneustes fossilis* (Bloch), the follicles undergoing resorption after the discharge of the ripe ova have been formed as the post-ovulatory follicles or ruptured follicles. Corpora atretica and post-ovulatory follicle are present in the ovaries of Heteropneustes fossilis (Bloch) during spawning phase. Their number is largest during this period. The post-ovulatory follicle cells undergo hypertrophy and proliferation but the whole mass later on merged into the ovarian stoma. The significance of the presence of the corpora atretica and post-ovulatory follicles in the determination of the reproductive cycle of the fish has been suggested by Saxena [14] Seasonal changes in the ovary have been studied by many workers.

Bantivegna and Benedetto,[4] Belsare,[3] Buxton,[5] Coetzee,[7] Merwe *et al*.,[8] Prabhu, [11] Raizada, [12] Subhedar and Prasada Rao,[16] sundararaj[17] prabhat ranjan[13] Saxena,[14] Tricas and Hiramoto,[18] in several fishes. Similar observations have also been found to occur in Heteropneustes fossilis (Bloch). Bantivegna and Beneditto [4] have noticed the study of the seasonal changes in gonads of Symphodus ocellatus and revealed four phases is pre-reproductive (March to April), reproductive (May to August), post-reproduction (September to October) and quienscence (November to February). Female gonads showed maximum activity during the reproductive period. Milton *et al*.[9] were observe that the fish (channa gachua) spawned once in a year with peak spawning from December to February. Merwe *et al.*[8] have reported that the reproductive cycle of Labeo capensis can therefore be divided into four phases, namely, the primary oocyte developing phase (summer), yolk production phase (autumn), maturation phase (winter) and the final maturation and spawning phase (spring). Urshani *et al.*[19] have categorized in three phase of ovarian development[1]. Recrudesces (December to April) 2. Spawning May to July,3. Quiescent (September to November). The GIS in Orieocromis mossambicus was minimum during resting phase of ovaries. It progressively increased during the preparatory and prespawning phase and reached to its peak at the spawning phase. Prabhu[11] has studied the spawning periodicities of several species and found variations in the spawning periods in different species. He distinguished four types of spawning viz., spawning once in a year with a short period, spawning once in a year with longer durations, spawning twice a year and spawning throughout the year.

In Heteropneustes fossilis (Bloch) the reproductive cycle has also been divided into three phases. The post-spawning (September to December), the pre-spawning (January to April) and the spawning period (May to August). During post-spawning period, the thick ovarian wall, ovigerous lamellae, chromatin nucleolus stage, peri-nucleolus stage and yolk vesicle stage were observed, whereas corpora atretica and post-ovulatory follicles were totally absent in late post-spawning period (December).

During pre-spawning and spawning period, thinner ovarian wall containing follicular epithelium, vitelline membrane as important membrane are noted while yolk granules and corpora atretica were also seen. The post-ovulatory follicles are present in late spawning period (August month). The fish breeds only once in a year while the spawning period extend from May to August. The conclusion from my research work help in understanding the pattern of reproduction in fish *Heteropneustes fossilis* in sagar district, which will be fruit full in aquaculture foe sustainable development of catfish.

Acknowlegement

We are thankful to Head, Department of Zoology, Dr, H.S. Gour University Sagar for providing us the space and other lab facilitates.

REFERENCES

1. Aggrwal N. Goswami sv. Khandelwal p. Sehgal N. armotase activity in brain and ovary: seasonal variations correlated with circannual gonadal cycle in the catfish, Heteropneustes fossilis. *Ind J Exp Biol*. **52(5):** 672 (2014).
2. Bhargava, H.N: The pineal organ and the hypothalamus pituitary relationship in the minnow phoxinus phoxinus with special reference to the control of the reproductive cycle and colour change. Ph.D. *Thesis, University of London* (1966).
3. Belsare, D.K., Seasonal changes in the ovary of Ophiocephalus punctatus. Bloch. *Ind. J. Fisheries,* **IX:** 140-154 (1962).
4. Bentivegna, F. and Benedetto, F., Gonochorism and seasonal variations in the gonads of the labrid Symphodus (Crenilabrus) Ocellatus ocellatus (Forsskal), *J. Fish Biol*., **34:** 343-348 (1989).
5. Buxton, C.D., The reproductive biology of Chrysoblephus laticeps and Cristiceps

cristiceps (Teleostei: Sparidae), *J. Zool. London*, **220:** 497-511 (1990).

6. Chandrasoma, J. and De Silva, S.S., Reproductive biology of Puntius sarana: an indigenous species and Tilapia rendalli (Melanopleura) an exotic in an ancient man made lake in Sri Lanka. *Fish Mgmt*. **12 (1):** 17-28 (1981).
7. Coetzee, P.S., Seasonal, histological and macroscopic changes in the gonads of Cheimerius nufar (Ehrenberg. 1820 Sparidae: Pisces). *S-Afr. Tydskr. Dierk* **18(2):** 75-88(1983).
8. Merwe, W.V.D.; Vuren, J.H.J.V. and Vermaak, J.F.,Cyclic Histomorphological Changes in the ovary of Mudfish, Labeo capensis, Aquaculture, **72:** 349-358(1988).
9. Miton james. Ajaz a bhat. M.A. haniffa. Shaik althaf hussain. Irfan A. Rather. Waleed A.Q. Hailan. And Mohammad Abul Farah, ovarian devolvement and histological observations of threatened dwarf snakehead fish, *Channa gachua Saudi j Biol* **25(1):** 149-153(2018).
10. Mahmod abdelghaffar emam and Badia abughrien, seasonal histological changes in gonads of the catfish Fish aquac j **5:** 087 (2014).
11. Prabhu, M.S., "Maturation of intraovarian eggs and spawning periodicity of some fishes. Indian J. Fish., **3:** 59-90(1956).
12. Raizada, A.K., A study on the ovary and the ovarian cycle of a percoid teleost, Nandus nandus (Ham.*). Zool. Jab. Anat. Bd*. **98s:** 53-75 (1977).
13. Ranjan prabhat, ovarian cycle and spawning season of ophiocephalus punctatus inhabiting chapra water Bihar Biomed pharmacol j4(1- 13) (2011).
14. Saksena, D.N., HYpothalamo-Hypophysial Neuro secretory system in fresh water fish Glassogobius giuris (Ham.) in relation to reproduction, Ph.D. Thesis, University of Sagar (1974).
15. Singh, K., Ph.D. Thesis, Dr. H.S. Gour University, Sagar (Unpublished Data) (2002).
16. Subhedar, N. and P.D. Prasada Rao, The seasonal changes in the corpuscles of stannius and the gonads of the catfish, Heteropneustes fossilis (Bloch). Z. Mikrosk.anat. *Forsch., Leipzig* **93(1):** S: 74-90 (1978).
17. sundararaj A study on the correlation between the structure of the pituitary gland of the Indian catfish Heteropneustes and the seasonal change in the ovary. Acta anat **(37):** 17 – 80 (1959).
18. Tricas, T.C. & Hiramoto, J.T: Sexual differentiation, gonad development, and spawning seasonality of the Hawaiian butterflyfish, Chaetodon multicinctus, Environmental Biology of Fishes, **25 (1-3):** 111-124 (1989).
19. Urashani T.J. S.A. shaikh. M.H. soomro. And S. Malik : histological changes in pituitary gonadotrophic hormone cells in relation to seasonal changes in ovaries of the fish *Orieochromis mossambicus* (2012).
20. Yamamoto, K.: 'Studies on the formation of fish egg. 1. Annual cycle in the development of ovarian eggs in the flounder, Liopsetta obscura, J. Fac. Sci. Hokkaido University, **12 (V):** 362-373(1956).