



Annual ovarian cycle of fresh water catfish *Ompok bimaculatus* (Bloch, 1794) from Gomati river, India

ABHA MISHRA¹ and ANURAG RAWAT²

Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh 226 025 India

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ABSTRACT

The present study examined the gonadosomatic index (GSI), oocyte diameter (OD), ovarian morphology and histology of freshwater butter catfish *Ompok bimaculatus* from Gomati river, India. For the annual cycle study, monthly samples were collected from the Gomati river. The GSI increased with the progressively maturation of gonad. It increased significantly in July (4.98 ± 0.047) and it reached its peak point in the month of August (5.37 ± 0.037) and lowest value was observed in October month (0.64 ± 0.019). The OD varied from 0.052 ± 0.005 to 0.84 ± 0.024 ; the maximum OD was observed in August month and lowest was observed in September month. It showed a significant relationship with GSI. Histological studies of ovary of *O. bimaculatus* revealed eight stages in oocyte development (oogonia, chromatin nucleolus, early perinucleolus, late perinucleolus, yolk vesicle, vitellogenesis, vitellogenic and post ovulatory follicles). The month wise studies of different parameters helped in confirming annual cycle as; resting phase (November-February), preparatory phase (March-April), pre-spawning phase (May-June), spawning phase (July-August) and post-spawning phase (September-October). These observations are important for a better understanding of reproductive biology of this fish in northern region to adopt breeding practices of female *Ompok bimaculatus*.

Key words: Gomati river, Gonadosomatic index, *Ompok bimaculatus*, Oogenesis, Oocyte diameter, Ovary histology

The butter catfish, *Ompok bimaculatus*, is assessed as near threatened and is relatively abundant throughout its distribution. As an alternate food source, this fish is hugely harvested. Therefore its culture is recommended. Very few studies compiled about the reproductive physiology of *O. bimaculatus* (Chakrabarti *et al.* 2007). From that limited resources, very scarce or nil are from Northern region. Similar to other catfish species, its culture is also facing problem of not responding to gonadotropin hormones as major carp fish (Banik *et al.* 2011). This may be due to lack of exact information regarding annual reproductive cycle. The study describing annual changes in gonad of any species would help in understanding their breeding behaviour specially (Vazzoler 1996). In fish, reproduction is a periodic event that depends on gonadal changes. Majority of fishes are annual breeder, their breeding directly depends on the photoperiod, temperature and rainfall (Davis *et al.* 1999, Bhattacharyya and Maitra 2006).

The reproductive cycle studies are essential for optimization of appropriate brood stock management strategies, successful fish culture and conservation programme (Doha and Hey 1970). It is extremely important to identify gonad development and reproductive performance in order to improve culture techniques. These

studies are based on quantitative indices, viz. oogenesis, ova size or diameter and gonadosomatic index which help to define reproductive cycles and physiological condition of species during the course of its life span (Kreiner *et al.* 2011, Braga 2005).

The oogenesis of teleost's has been studied extensively (Coward and Bromage 1998, Cek *et al.* 2001). The description of the various stages is based on distinct morphological, histological and physiological characteristics (GSI, oocyte size and shape) (Casadevall *et al.* 1993, Tyler and Sumpter 1996, Rinchard *et al.* 1998, Sivakumaran *et al.* 2003, Santos *et al.* 2010).

In aquaculture, the freshwater butter catfish, *O. bimaculatus* (Bloch, 1794), has not been studied well as other catfish due to insufficiency of gravid stock for experimentation. Because of shortage of information regarding its breeding potential and larval rearing, in spite of its economic importance as valuable food fish, its culture practices are still not well developed (Banik *et al.* 2011). This fish has unique lipo-protein combination with soft bones, good taste and higher nutritional value (Banik *et al.* 2011). Lack of definite updated information on reproductive biology of *O. bimaculatus* of the North riverine system has hampered the planning and implementation of its conservation and management strategy. Accordingly, the purpose of this study was to determine the annual gonadosomatic index and determination of the ovarian cycle

Present address: ¹Assistant Professor (drabhamishra@gmail.com), ²(anurag11684@gmail.com), Department of Animal Applied Sciences.

by oocyte development stage study of *O. bimaculatus* from Gomati river of Northern region (Lucknow, UP). These studies would provide useful information on its ovary maturation pattern which is very important for its conservation and culture programme in this state. It is the first complete description of the ovarian anatomy and oogenesis of *O. bimaculatus*.

MATERIALS AND METHODS

Chemicals: All the chemicals used in study were of analytical grade, and purchased locally from scientific suppliers.

Fish sampling sites: The fish were handled in accordance with local/national guidelines for experimentation on animals and care was taken to prevent cruelty of any kind. Around ten live adult female fish *O. bimaculatus* of average length (25.5±7 cm) were brought to the laboratory during the first week of each month (for one year from October 2014 to September 2015). They were kept in laboratory under natural photoperiod and temperature. During acclimatization process fish were fed *ad lib*.

Study parameters

Gonadosomatic Index (GSI): The fish were weighed using digital balance (Shimadzu-AY220) and sacrificed to remove ovary. Ovary weights were recorded and the GSI was calculated for every month and each individual through the following formula:

$$GSI = \text{Ovary weight} \times 100 / \text{Body weight}$$

Histological analysis: For histological studies, ovary of each month was fixed in the Bouin's solution for 24 h, the tissues were later transferred to 70% ethanol and further preceded the routine histological method. Tissue sections were cut at 7 µm using a rotary microtome (Weswox). This cut section were mounted at glass slide and stain Erlich hematoxyline and eosin stain and examine at 20 × magnification using a Bright Field microscope (Olympus). The images were captured by Olympus CX41 camera for anatomical study.

Oocyte diameter: For this study, 20 ova from majority of ovum were taken randomly from the mixed sample of eggs from each fish (ten fish each month). The diameter of the oocytes was measured at 10× magnification using stage and eye-ocular micrometer. Measurements of ova diameter were taken along the longest axis of the ova.

Oocyte stage counting: The different oocyte stage counting was done in histological sections of ovary with the help of Image analysis software (Magnus Pro). For this, 4–5 random area were selected from each slide. Data are a mean of ten fish in duplicate slides for each month. Month wise percentage frequencies of different stages of ova were calculated using the formula: number of ova of particular stage × 100/Total ova counted. The microscopic developmental stages of oocyte were categorized according to Janssen *et al.* (1995) and the atretic oocytes (degenerating follicle before maturity) and post ovulatory follicles histological classification was followed (Hunter *et al.* 1986).

Statistical analysis: The data were expressed as mean±SEM. The one way analysis of variance (ANOVA) was used for overall significance (P<0.001) and Newmann keul test (P<0.05) was done to get intergroup variation.

RESULTS AND DISCUSSION

Gonadosomatic index and oocyte diameter: The GSI is a most reliable reproductive measure when associated with other indicators as macroscopic, oocyte diameter and microscopic observations to study reproductive cycle (DeMartini and Lau 1999). The monthly variations in the gonadosomatic index of *O. bimaculatus* ranged from 0.64±0.019 to 5.37±0.037 respectively to represent an annual cycle for ovary maturation (Fig. 1). The gonadosomatic index of months were significantly different during the annual reproductive cycle of *O. bimaculatus* (F=1175.45; P<0.01). The GSI registered a low value during October and November month (0.64±0.019, 0.76±0.017 respectively). During December and January, it increased significantly (1.05±0.014 to 1.17±0.016) from the previous months. The GSI further recorded significant increase during February, March and April months (1.84±0.034, 2.02±0.032, 2.23±0.015 respectively) from earlier months but not significant different with each other. After this increase, GSI showed a successive but significant increase during each month, viz. May, June, July (3.2±0.01, 3.93±0.015, 4.32±0.047 respectively) with peak in August (5.37±0.037). The spawning period was the month of August for this fish species. During this period, fish attained maximum ovary weight, and ovary was full of mature vitellogenic follicles. GSI exhibit increasing trend from February onwards (preparatory phase) and highest in August (spawning phase), which is the indication of large quantity of yolk accumulation in mature ova (Encina and Lorencio 1997, Roy and Hossain 2006, Alam and Pathak 2010, Gupta *et al.* 2014). Highest GSI value support final maturity level

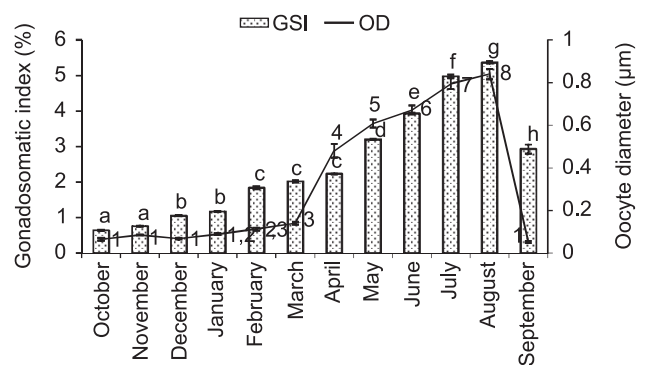


Fig. 1. Monthly changes in gonadosomatic index (GSI) (%) and mean oocyte diameter (OD) (µm) of majority in ovary of *O. bimaculatus*. Ten fish were examined in each month. Value expressed as Mean±SEM. Data were analysed by one way ANOVA and intergroup difference were analysed by Neuman Keul's test (P<0.05). Same alphabets and number represents non-significant different but different alphabets and number represents significant difference among groups in GSI and OD data respectively.

of gamete. Same fish species in North-East region (Tripura) registered GSI peak in June to August as spawning period (Mishra *et al.* 2013, Malla and banik 2015). This may be due to difference in the climatic conditions of two states but roughly it coincides with the current study. During September we recorded significant sharp drop of GSI (2.93 ± 0.126). The oocyte diameter also registered sharp fall in September to October due to atretic follicles followed by generation of new oogonia. The lower value of GSI is a result of intense spawning activity (Adamassu 1996). The GSI has a positive relationship with the developmental stages of ovary (Stoumbondi *et al.* 1993, Manna *et al.* 2010). It reached its maximum at the peak of maturity and at minimum during resting phase of ovary (Shengde and Mane 2006, Rao and Krishna 2009, Ghanbahadur and Ghanbahadur 2012, Mishra and Saksena 2012).

The oocyte diameter (OD) showed the same pattern in size difference as like of GSI in different months. The mean OD ranged from $0.085 \pm 0.002 \mu\text{m}$ (immature oocyte) to $0.84 \pm 0.024 \mu\text{m}$ (mature stage) (Fig. 1). The oocyte diameter was significantly different during the sampled months ($F=432.035$; $P<0.001$). The OD was minimum during September to January months (0.052 ± 0.005 to $0.089 \pm 0.004 \mu\text{m}$ respectively). Thereafter a slight but non-significant increase was noticed during February when compared with January (0.1125 ± 0.007). A significant increase in OD was seen during March month ($0.48 \pm 0.032 \mu\text{m}$; $P<0.01$). That maintained in sharp significant increase from previous months to April onwards ($P<0.01$) with peak value in August ($0.84 \pm 0.024 \mu\text{m}$). The oocytes diameter was also significantly increased during spawning phase (Foucher and Beamish 1980). This fish show a small spawning phase different to sister fish *O. pabda* (Siddiqua *et al.* 2000). At the end of this phase, the ovary decreases in weight not only due to ovulation or discharge of the eggs, but also due to degeneration of oocytes which is referred to as atresia during September month. This post-spawning phase supported by low GSI and OD. Similar observations were reported in different fish species (Sivakumaran *et al.* 2003, Chakraborty *et al.* 2007).

Annual macro- and micro-scopic features of the ovary with oocyte stages study: The ovary was two-lobed, elongated structure (Fig. 2). It was attached to abdomen by dorsal mesenteric and extended posterior in to oviduct. Intact ovary over graph paper showed a clear cut changes in its size, shape and colour (Fig. 2). The anatomical changes of ovary suggested oocyte arrangement and their development stages in an annual study (Figs 3,4). The relative abundances or monthly changes of percentage frequency of different maturity stages of oocytes within the ovary is clearly seen (Fig. 4). The histological study of adult female *O. bimaculatus* at Northern region exhibit a distinct annual ovarian cycle with distinct five reproductive phases, viz. resting (November, December, January), preparatory (February, March, April), pre-spawning (May, June, July), spawning (August) and post-spawning (September, October) phase (Fig. 3) on the basis of its

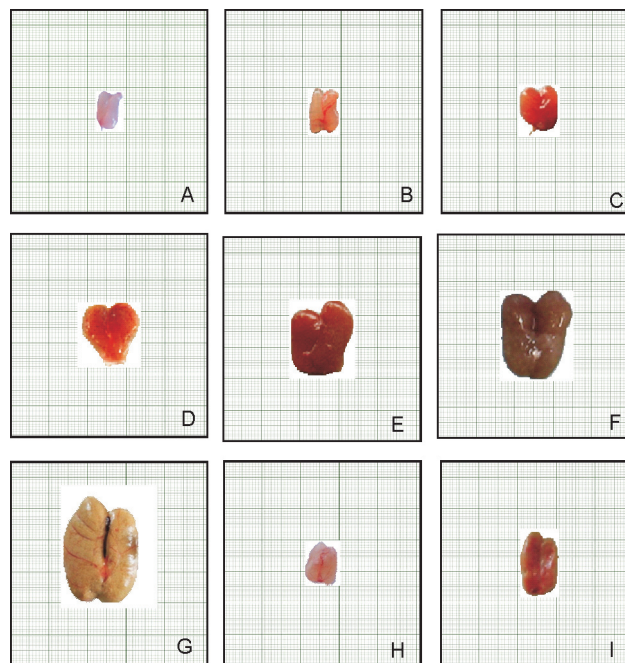


Fig. 2. Morphological appearance of ovary of *Ompok bimaculatus* during different months. A, January; B, February; C, March; D, April; E, May; F, June; G, July; H, August; I, September; J, October; K, November; L, December.

macroscopic and microscopic study and percent frequency of oocyte development stages present in each month ovary of an annual study. In teleosts, the annual ovarian development process may be divided into four to eight maturity stages or phases (West 1990, Fishelson *et al.* 1996, Unal *et al.* 1999, Dey 2004, Verma 2013). These ovarian changes were coinciding with environmental clues. Increase in photoperiod and temperature promote gonadal maturation and so the breeding response (Miranda *et al.* 2009). The reproductive events like recrudescence of oogonia, oogenesis and maturation were related with the environmental parameters (Dey 2004).

Resting phase: Immature ovary was found from the month of November to January. The ovary was small, semi-transparent, light pinkish in colour. The oocytes cannot be seen by naked eyes (Fig. 2K,L,A). The major event in this phase was proliferation of oogonia and recruitment of oocytes (Fig. 3K,L,A). During this phase, ovary has majority of oogonia stage (Htun-Han 1978). Oogonia were arranged in cluster stages. The oogonia were very small round cells with a clear cytoplasm and a single nucleolus in an oval nucleus (Fig. 3K,L,A). Ovary of the resting phase containing maximum of oogonia with increasing chromatin nucleolus and early perinucleolus stage oocytes in progressing resting phase (Ebisawa 1990) (Fig. 4). This phase represents arrival of new crop.

Preparatory phase: This phase of ovary was extend to February, March and April (Figs 2,3; B,C,D) as reported by Raghuvver and Senthilkumaran (2010) in other catfish of Northern region. During this development phase, ovary was elongated and reddish in colour. During this phase,

ovary occupy one-fourth of the ventral cavity. This registered increase in overall size with months. Oocytes were not distinctly visible by naked eye (Fig. 2B,C,D). The oocyte arrangement shows slightly loosening than in resting phase. The oocytes were of primary growth phase, spherical and small (Fig. 3B,C,D). In preparatory phase ovary, oogonia, chromatin nucleolus and early perinucleolus stage oocytes still present, late perinucleolus and yolk vesicle or cortical alveoli oocytes seen in increasing percentage frequency with the development of reproductive phase (Fig. 4). In this phase, ovary was dominated by perinucleolar oocyte with large nuclei and many various sized nucleoli. The nucleoli play an important role in vitellogenesis (Malhotra 1963).

Pre-spawning phase: The pre-spawning phase of *O. bimaculatus* ovarian cycle was found during May, June and July (Figs 2,3; E,F,G). In this period, ovary was enlarged in size as compared to earlier months, reddish-green in colour and had opaque and translucent oocytes which were visible by naked eye. The vascularization of ovary was increasingly started. Ovaries occupy less than one-third of ventral cavity (Fig. 2E,F,G). The oocytes showed low affinity to hematoxyline. Numerous rounded nucleoli were found in

the periphery of the nucleolus and the cytoplasm was dense. A flattened follicular layer surrounding oocytes could be distinguished at the end of this stage (Fig. 3E,F,G). Yolk granule oocytes predominate, late perinucleolus and vitellogenic oocyte can also be observed (Fig. 4). The growth during pre-spawning phase is mainly due to formation of yolk vesicles and deposition of yolk. Yolk vesicle or cortical alveoli were the characteristics feature of vitellogenic oocytes (Guraya 1993). Yolk vesicles contained endogenously synthesized lipids and glycoprotein and increased the space for incorporation of exogenously synthesized yolk protein (Selman *et al.* 1986). The vitelline membrane appears commonly at the yolk vesicle stage and sometimes at the end of primary oocyte (West 1990, Unal *et al.* 1999).

Spawning phase: This stage was observed in August month. Ovary was highly vascularised, greenish in colour and full of mature oocytes. Ovaries occupy most of the ventral cavity, distinctly and lobular in appearance and eggs extruded with slight abdominal pressure. The oocyte size was big due to hydration and vitellogenesis (Fig. 2H). The granular cells of follicle layer appear stretched and become

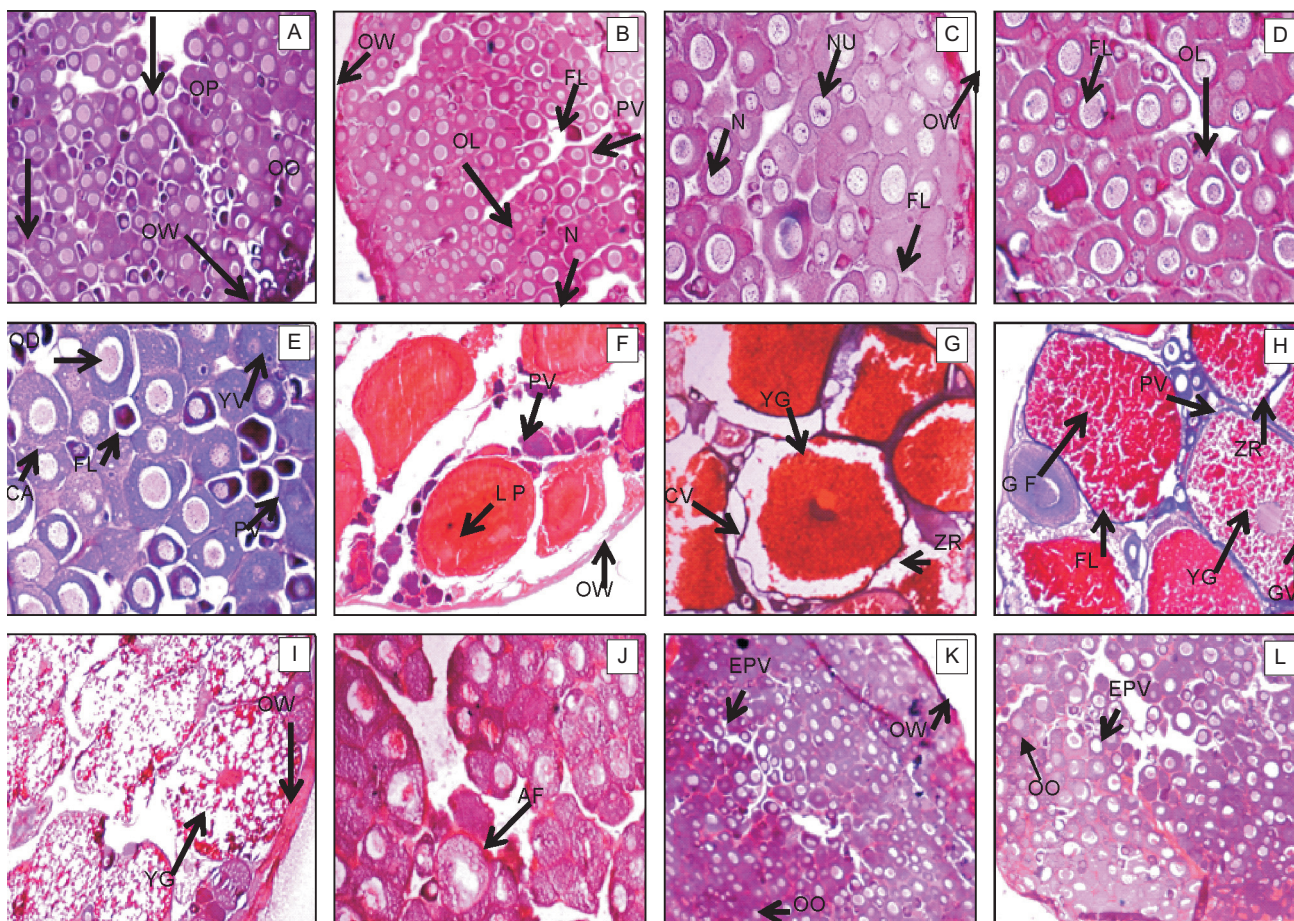


Fig. 3. Histological appearance of ovary of *Ompok bimaculatus* during different months. A, January; B, February; C, March; D, April; E, May; F, June; G, July; H, August; I, September; J, October; K, November; L, December. OL, Ovarian lumen; OO, Oogonia; OW, Ovarian wall; N, Nucleus; OP, Ovarian peritoneum; FL, Follicle layer; PV, Previtellogenic oocyte; GF, Graffian follicle; AF, Atretic follicle; NU, Nucleolus; YV, Yolk vesicle; LP, Late perinucleolus; CA, Corticle alveoli; EPV, Early perinucleolus vesicle; YG, Yolk globule; ZR, Zona radiata; GV, Germinal vesicle. Images were captured with 10× magnification.

thin. The nucleus was smaller as compared to the earlier stages and chromatin threads still occurred in the nucleus, and the nuclear membrane began to degenerate. The zona radiate layer was very evident and follicle epithelium was more developed. Vitellogenic oocyte seen with predominance of germinal vesicle migratory oocytes (Fig. 3H). Ovary of August month mainly had postvitellogenic follicle but late perinucleolus follicles were also seen (Fig. 4).

Post-spawning phase: The ovary of the post-spawning stage was occurred in September and October month. Ovary were slightly brown in colour, shrunken, full of red dots due to residual follicle layers, containing a few atretic oocytes or fully empty (Fig. 2I,J). This phase ovaries were flaccid, with hyaline oocyte. In micrograph scattered residual vitellogenic and atretic oocytes were dominating. The October month ovary was rich in remnant of follicle layers (Fig. 3I,J). Abundant post-ovulatory follicle, perinucleolar oocytes and atretic oocytes were present (Fig. 4). Same changes have been reported in the ovaries of several teleostean species (Jadhav and Bapat 1983, Burton and Idler 1984).

The present findings provides clear evidence of annual reproductive cycle in form of GSI, oocyte diameter, macro-microscopic study and the percentage of frequency of oocyte maturity stage in monthly ovary of *O. bimaculatus* (Bloch, 1974) of Gomati river, tributary of Ganga basin, India for which data remain scarce in the literature. This study would contribute knowledge to the research on the oogenesis process of near threatened spp. The observed parameters may beneficial for species conservation strategies, sustainable fishery management and aquaculture programs of *O. bimaculatus*.

The present study examined the gonadosomatic index (GSI), oocyte diameter (OD), ovarian morphology and

histology of freshwater butter catfish *Ompok bimaculatus* from Gomati River, Lucknow, India. The month wise studies of different parameters helped in confirm annual cycle as; resting phase (November-February), preparatory phase (March-April), pre-spawning phase (May-June), spawning phase (July-August) and post-spawning phase (September-October).

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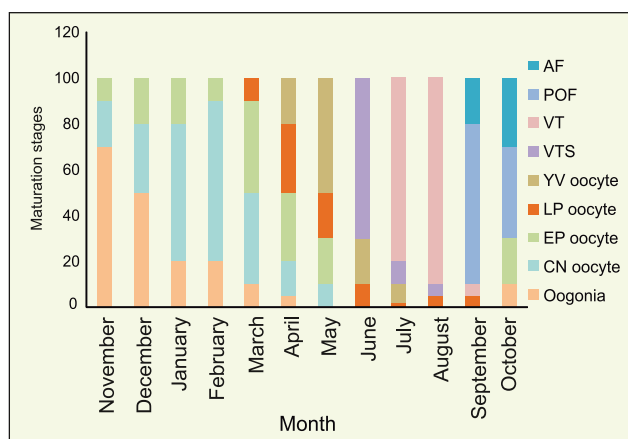


Fig. 4. Monthly changes in frequency (%) of oocyte maturation stages in microscopic sections of *O. bimaculatus*. The oocyte maturation stage frequency calculation was done with the help of Olympus Magna software. AF, Atretic follicles; POF, Post ovulatory follicles; VT, Vitellogenic oocyte; VTS, Vitellogenesis; YV, Yolk vesicle oocyte; LP, Late peri-nucleolus oocyte; EP, Early peri-nucleolus oocyte; CN, Chromatin nucleolus oocyte; Oogonia.

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