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CYCLIC VARIATIONS IN THE OVARIAN ACTIVITY OF A FRESH WATER PERCH **COLISA FASCIATUS** (BL. & SCHN.)

S. C. RAI

Department of Zoology.
Tribhuwan University.
IAAS, Central Campus
Rampur, Chitwan, Nepal.
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RESUMO - A morfologia geral, histologia e mudanças cíclicas no ovário de **Colisa fasciatus** foram descritas. O ovário apresenta um tipo assincrônico de desenvolvimento ovocitário,com um só pico de desova que se estende de junho a agosto. O ooplasma, núcleo e constituintes da parede celular exibem distintas mudanças morfológicas e histológicas durante seu ciclo anual O aparecimento de núcleos de vitelo, extrusões nucleolares e deposição de vitelo foram discutidas.

ABSTRACT - The general morphology, histology and cyclic changes in the ovary of **Colisa fasciatus** have been described. The ovary shows an asynchronous type of oocyte development with only one spawning peak which extends from June to August. The ooplasm, nucleus and constituents of cell wall exhibit distinct morphological & histological changes during its annual cycle. The appearence of yolk nucleus, nucleolar extrusions and deposition of yolk have also been discussed.

INTRODUCTION

A great deal has been written on the development of gonads and their cyclic variations in the teleosts. Important contribution on the ovarian cycle have been made by Ball (1960) Dodd (1960), Ingram (1962), Barr (1968), Hoar(1969), De Vlaming (1974), Guraya **et al.** (1979) and Wallace and Kellyselman (1980) in fishes inhabiting in different ecological conditions. From the foregoing accounts it is evident that more work has been done on the ovarian activity of the temperate fishes than those of tropical and subtropical zones. The present study, therefore, includes the cyclic variations in the histophysiology of the ovary of **Colisa fasciatus**.

MATERIAL AND METHODS

Living specimens of female **C. fasciatus** were collected throughout a calendar year from Ramgarh lake Gorakhpur. Observations were confirmed in nest calendar year as well. In all cases the weight (g) of fish and its ovary were recorded to assess the variation on their gonosomatic index (GSI)

$$GSI \ \frac{\text{Weight of the ovary}}{\text{Weight of the fish}} \times 100$$

Small pieces of ovaries were fixed in Bouin's (both aqueous and alcoholic) fixative. Sections were cut at 5-6 µm and stained with Heidenhain's haematoxylin and Haematoxylin/eosin. To test the chemical nature of yolk vesicles and yolk granules, sections were treated with periodic acid schiff (PAS) and sudan black B.

For determining the mean diameter of ova, about 500 oocytes were selected randomly from each fish sample.

RESULTS

Morphology

The ovary of adult **C. fasciatus** is a paired sac like elongated structure with their free anterior ends and fused posterior ends which form a thin walled sac like oviduct. The oviduct opens to the exterior through an urinogenital aperture. During breeding season, the urinogenital aperture gets swollen and is pink in colour The length of ovary does not show marked change during the annual cycle, however, mature ovary measures about 1.0 to 1.2 cm where as regressed ovary measures about 0.6 to 1.0 cm.

Histology

C. fasciatus possess typical teleostean type of ovaries with an ovarian wall and an ovocoel. Three layers comprise the ovarian wall (i) the outermost thin layer of peritoneum, (ii) the tunica albuginea which is made up of connective tissue cells, muscle fibres and blood capillaries. The tunica albuginea is relatively thick during the post-spawning period but becomes thin and vascularized during spawning season (Fig. 1, 2) and (iii) the innermost layer of germinal epithelium. The germinal epithelium projects into the ovocoel at several places to form ovigerous lamellae from which new crop of oogonia is derived. Oogonia and the oocytes of various stages are arranged on either side of these lamellae (Fig. 3). Oogonia undergo successive maturation division and form new generation of oocytes. This involves complex changes in the cytology of the nucleus and the cytoplasm.

Ovarian Cycle

On the basis of morphological appearance of ovary, gonosomatic indices, nature of ovarian wall, state of developing oocytes and the average diameter of the ova, the ovarian cycle of **C. fasciatus** has been divided into six successive phases.

I. Resting phase (November - December)

The ovaries are small, thin, opaque and creamy to flesh colour with poor vascularization. The gonosomatic index recorded during this phase ranges from 0.720-0.797

With thick tunica albuginea the ovary shows prominent ovigerous folds enclosing nests of oogonia and numerous immature oocytes. The oogonia, ranging between 8-9 µm, are seen in the form of small groups enclosed by stromal elements (Fig. 4) Each oogonium has a large conspicuous nucleus with a spherical nucleolus and a thin layer of ooplasm. The chromatin threads are seen radiating from them. The oogonia which acquire increased amount of cytoplasm become oocyte. The oocytes are seen in early perinucleolus stage and ranges 79.73 ± 1.98 - 82.88 ± 2.25 µm in diameter

II. Preparatory phase (January - March)

The ovary is slightly thick and creamy to light yellow in colour The vascularization at this stage is relatively more. The gonosomatic index ranges from 0.816-0.858.

As the oocyte grows, the nucleoli increase in number and become arranged along the inner surface of the nuclear membrane. At this stage, a spherical yolk nucleus is seen adjacent to the nuclear membrane. This structure is well organised and has great affinity for haematoxylin (Fig. 5).The nucleolar extrusions are commonly visible in this phase (Fig. 5) The oocytes of the chromatin nucleolus and perinucleolus stage are fairly common, while a very few oocytes in early yolk vesicle stages are also encountered. The oocytes measure during this phase ranges 90.01 ± 2.25 - 101.92 ± 1.51 µm in diameter

III. Early maturation phase (April - Early May)

The ovaries are thick, solid, yellow in colour having increased in volume. Distinct vascularization is seen on the outer surface of the ovaries. The gonosomatic index shows a gradual increase which ranges from 1.241-6.449.

The Ovigerous lamellae are thin and ovarian lumen is greatly obliterated due to the presence of various stages of developing ova. Early and late yolk vesicle stages of the oocytes are common (Fig. 6). A few oocytes in early yolk granule stage are also seen. The oocytes at this phase show a

Fig. 1 - T.S. of ovary showing three layers in the wall: Peritoneum (P), tunica albuginea (TA) with blood vessels (BV) and ovarian epithelium (OE) during the post-spawning phase X 1000.

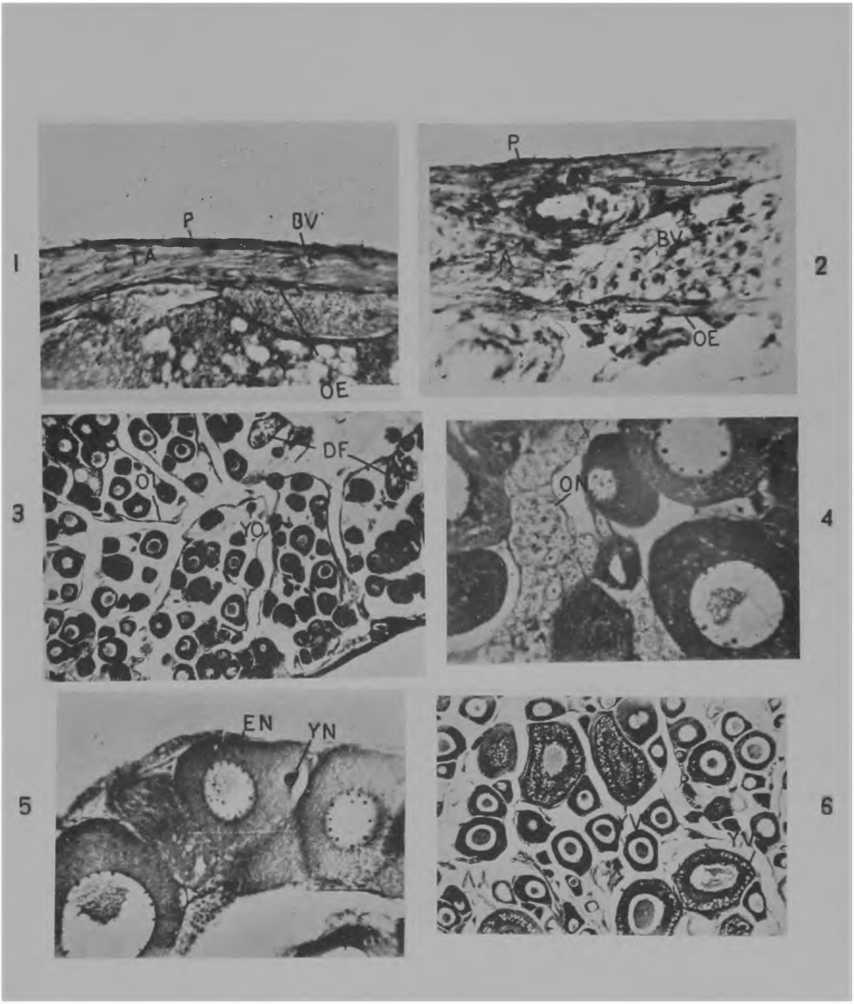
Fig. 2 - T.S. of ovary showing three layers in the wall: Peritoneum (P) tunica albuginea (TA) with blood vessels (BV) and ovarian epithelium (OE) during the spawning phase X 1000.

Fig. 3 - T.S. of ovary showing ovigerous lamellae (OL) young oocytes (YO) and discharged follicles (DF) during nonbreeding season X 200.

Fig. 4 - T.S. of ovary showing oogonial nests X 450.

Fig. 5 - T.S. of ovary showing yolk nucleus (YN) and extruded nucleoli (EN) X 450.

Fig. 6 - T.S. of ovary showing early and late yolk vesicle (YV) stage of the oocytes X 100.


P
BV
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1
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YV
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Fig. 7 - T.S. of ovary showing yolk granule (YG) stage of the oocyte X 200.

Fig. 8 - T.S. of ovary showing eccentric position of the nucleus in the mature oocyte (→) X 200.

Fig. 9 - Egg envelop showing different layers, theca (T) Zona granulosa (ZG) and Zona rediata (ZR) X 1000.

Fig.10 - T.S. of ovary showing discharged follicles (→) X 50.

Fig.11 - T.S. of ovary showing atretic follicles (AF) in various stages of resorption X 200.

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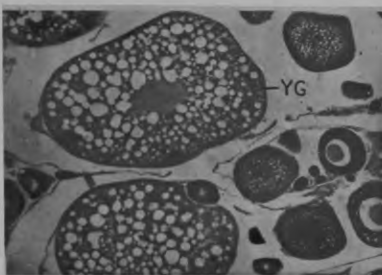

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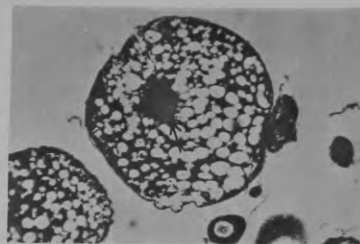

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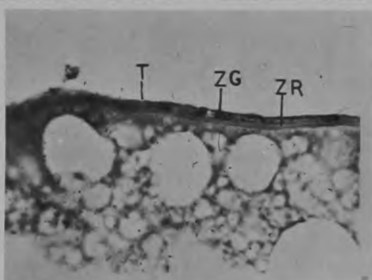

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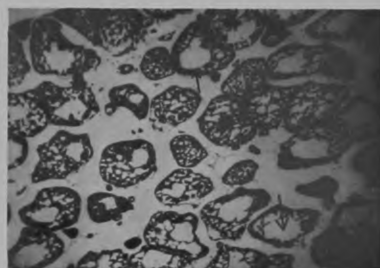

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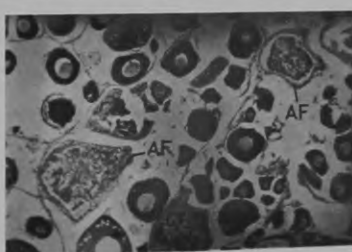

striking increase in diameter (159.04 ± 3.55 - 388.60 ± 6.25 μm) and thus reducing the lumen of the ovary. The oocytes are enclosed by a distinct follicular layer which comprises two layers: i) an outer zona granulosa (follicular layer) and ii) inner zona radiata (vitelline membrane)

IV Advance maturation phase (Late May - June)

The ovary is greatly enlarged thick with rough surface and occupy more than three-fourth of the abdominal cavity. The ovarian wall becomes so thin that ova are seen bulging on the surface of the ovary. Vascularization is prominent. The ovary is yellow in colour The gonosomatic index recorded during this month is 12.96.

The tunica albuginea becomes extremely thin and inter-follicular space is highly reduced. The appearance of extra-vesicular yolk granules in the cortical region of the maturing oocyte is the characteristic features of this phase. The yolk granules extends centripetally and ultimately occupy the entire ooplasm (Fig. 7) and oocytes measure 446.56±8.55 μm in diameter.

Among oocytes early and late yolk granule stages are dominant. A thin layer of fibroblast are known as 'theca' is seen differentiating outside to the zona granulosa.

V. Spawning phase (Late June to early August)

From June to August the fish spawns profusely. The ovaries are fully gravid due to the presence of heavily yolk laden oocytes. At the beginning of spawning the ovary attains maximum weight and later on due to the partial discharge of eggs lose its turgidity The gonosomatic index has been recorded during this phase ranges from 15.338 - 8.521.

The tunica albuginea becomes extremely thin and the ovigerous lamellae become indistinct. Some unripe eggs are also visible along with the mature eggs in the ovary, they probably replenish the ovary after spawning. The oocytes become heavily impregnated with yolk granules resulting in an eccentric position and irregular outline of the nucleus(Fig. 8) Discharged follicles are also encoutered indicating the start of spawning act. The oocytes at this phase measure from 511.37 ± 5.55 - 405.10 ± 3.55 μm in diameter The mature egg is enveloped by three layers: Zona radiata, Zona granulosa and theca (Fig. 9)

VI. Post-spawning phase (September - October)

The ovary is thin, translucent and less vascular. The weight is considerably reduced and colour becomes fleshy. The gonosomatic index has been recorded to be lowest (0.567).

The ovigerous lamellae with oogonial nests and abun -

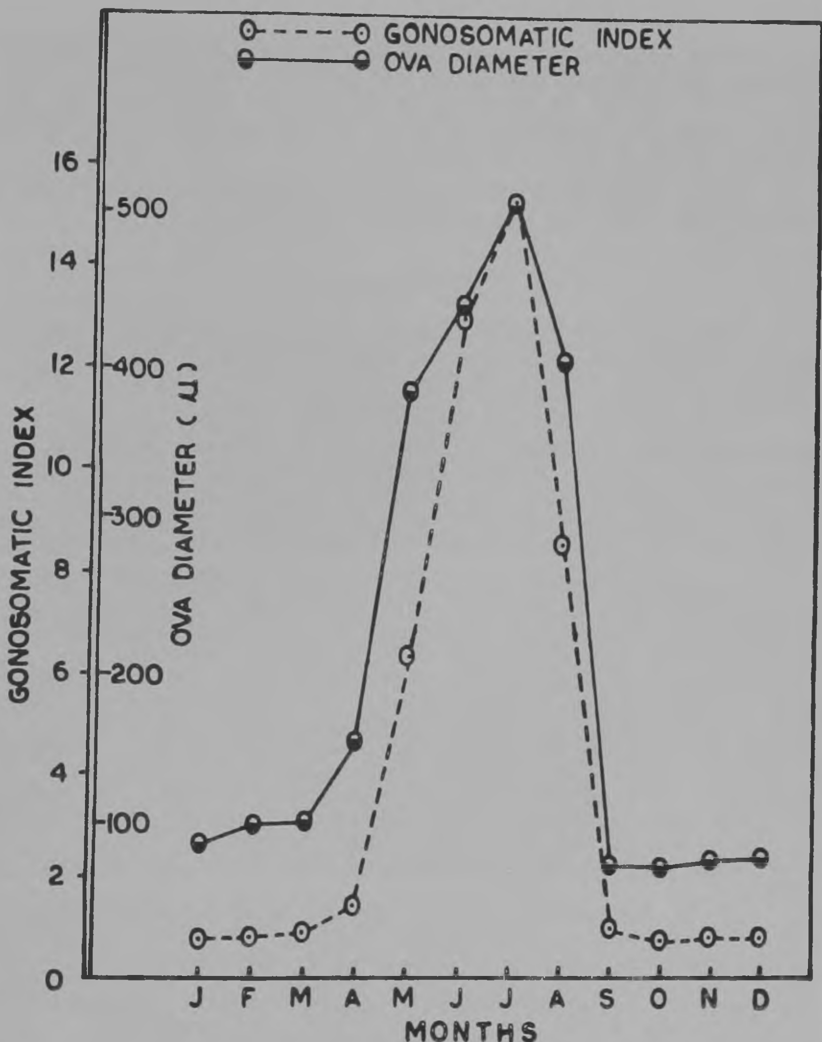

FIG. 12

Fig. 12 - Graph showing the ovarian cycle in **C. fasciatus** (Dotted line-Gonosomatic index, solid line - ova diameter)

dant young oocytes are seen alongwith the increased somatic tissues. Discharged follicles are present abundantly (Fig. 10) Large number of atretic follicles are also seen in various stage of resorption (Fig. 11). The tunica albuginea becomes thick and oocytes measure 77.94 ± 2.25 - 76.80 ± 3.51 µm in diameter.

In **C. fasciatus** the values of gonosomatic index show a correlation with the ova diameter (Fig. 12)

DISCUSSION

The study of cyclic changes in the teleostean ovaries has attracted the attention of many workers in the past Bara (1960) Belsare (1962), Guraya (1965), Joshi (1975), Raina (1976), Fishelson (1978) Saxena **et al.** (1979).

Contrary to the two peak periods of ovarian cycle in **Ophiocephalus striatus**, (Willey, 1910), **Channa striatus** , (Srivastava, 1977), **Colisa fasciatus** displays only one peak period of ovarian activity (June to August) and thus is an annual breeder All of them have made their observations considering only the ova-diameter frequencies and gonosomatic index.

According to Marza (1938) the development of animal oocytes has been classified into three main types: (i) total synchronism, (ii) group synchronism, and (iii) asynchronism. Prabhu (1956) has described four types of spawning, (i) spawning once a year with a short duration, (ii) spawning once a year with a long duration, (iii) spawning more than once a year, and (iv) spawning throughout the year intermittently. **Colisa fasciatus** falls under the third category of Marza (1938) and to the second category of spawning as mentioned by Prabhu (1956) In the present study. the oocytes have been observed in the various stages of development in the ovary at the same time. This process is similar to that seen in **Fundulus heteroclitus** (Wallace & Kellyselman 1980)

In **C. fasciatus**, during the course of previtellogenesis and vitellogenesis, remarkable changes have been observed in the germinal vesicle of the oocyte. Chaudhory (1951), Raven (1961), and Rai (1967) have reported that the nucleoli are descrete structure and are formed through fragmentation of single nucleolus. In present study. the gradually increasing number and simultaneous reduction in the size of the nucleoli confirm the possibility of their division by fragmentation. No such fragmentation of the nucleolus has been observed by Yamamoto (1956b) Bara (1960) Chouinard (1963), Mac Gregor (1972). but they have shown that nucleoli arise from nucleolar organizers. In **C. fasciatus** some previtellogenic oocytes exhibit nucleolar extrusion and nucleolar fragments extruded in the cytoplasm which may probably be involved in the yolk formation. Chaudhry (1951) and Guraya (1965) have reported that the nucleolar extrusions do not play any direct role in vitellogenesis. According to Mac Gregor (1972), nucleolar extrusions in the growing oocytes contribute ribosomes to the ooplasm.

As regard the origin of yolk nucleus, there are two schools of thought one school states its origin from the cytoplasm (Chaudhry, 1952; and Nayyar, 1964), while second school describes its origin from the nucleus (Wheeler, 1924, Sathyanesan, 1959) In **C. fasciatus** the yolk nucleus appears adjacent to the nuclear membrane in young oocytes which gradually becomes distinct and finally moves to the peripheral zone of the ooplasm where it disintegrates and disappears. According to Wheeler (1924), yolk nucleus does not participates in the formation of yolk. Chaudhry (1952) treats it as a possible catalytic agent in the formation of yolk. Bara (1969) observed an association between mitochondria and yolk nucleus. According to Guraya (1968), the yolk nucleus performs the function of synthesizing and distributing its material during previtellogenesis and as such forming probably the basic cytoplasmic machinery for various other synthetic activities of oocyte. In the present study no visible role can be assigned to the yolk nucleus in the process of vitellogenesis. However, no trace of this structure has been observed in any oogenetic stage after yolk vesicle stage . Thus it is quite possible that the components of the yolk nucleus after its dispersion in the cytoplasm play some role in the completion of the process of vitellogenesis.

The pattern of yolk deposition have been reported in fish eggs. During vitellogenesis, three types of yolk material is deposited in the ooplasm of fishes viz; (i) intra-vesicular yolk (carbohydrate yolk, cortical alveoli, yolk vesicle), (ii) extra-vesicular yolk (protein yolk, yolk granules), (iii) fatty yolk. In **C. fasciatus** intravesicular yolk in the form of numerous vesicles appear along the periphery of the oocytes and extends towards the centre. The vesicles appear empty and are nonstainable with basic dyes. Similar observations has also been reported by Shrestha & Khanna (1979). As the oocyte grows and differentiates, the formation of yolk vesicles continue and proceed inward towards the nucleus and fill the entire cytoplasm. Controversy arises regarding the origin of intravesicular yolk. The literature reveals only speculative suggestions, viz; (i) that it originates in the precortex (Aketa, 1954), (ii) that it arises from vacuolar bodies or ground substance of ooplasm (Yamamoto, 1955), (iii) that it is produced by the Golgi complex (Anderson, 1968). Regarding its function, Yamamoto (1961) suggests that it plays an important role during fertilization.

In the present study the yolk arise in the cortical region of the oocyte in the form of granules in the extravesicular ooplasm and later the whole cytoplasm becomes heavily impregnated with yolk granules. Similar observations,have been made by Yamamoto (1958), Rai (1967), Khanna & Sanwal (1971), Dixit & Agarwala (1974). Different views have been expressed regarding the formation of extravesicular yolk in fishes viz; (i) according to Wallace (1903) this type of yolk is formed in the cytoplasm without any relationship with formed elements, (ii) Raven (1961) has reported that the nucleolar materials gets directly transformed into yolk, (iii) while Yamamoto (1956) Chopra (1958) and Guraya(1956) considered that the yolk granules often arise in close contact with mitochondria and it might be related with yolk formation. Saxena **et al.** (1979) have reported that mitochondria are RNA (-)ve and does not participates in the formation of the yolk.

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