

Ovarian Cycle and Spawning Season of *Ophiocephalus punctatus*, Inhabiting Jammu Waters, India

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Abstract The ovarian cycle and spawning season of *Ophiocephalus punctatus* from Jammu, India, a subtropical area, is described. The oocyte shows six well differentiated stages of maturation and the fish has a prolonged spawning season extending from May to August. The spawning tends to be bimodal in character.

The stages in the formation of the yolk nucleus of Balbiani are described and the probable origin of this cytoplasmic inclusion is discussed.

Ophiocephalus punctatus Bloch is a widely distributed and economically important freshwater fish capable of adapting itself to diverse climatic conditions over nearly half of the world (Nelson, 1976). Successful exploitation of any fish solicits a thorough knowledge of its reproductive biology. In view of the considerable variations reported in gonadal cycle, spawning time and spatial behaviour of this fish (Sunderraj, 1916; Khan, 1924; Mukherjee, 1945; Swarup, 1954; Belsare, 1962), a study was undertaken to find out the exact pattern of ovarian cycle and spawning season of *O. punctatus* in Jammu, a sub-tropical province of J & K state, India. Jammu is located at an altitude of 300 meters between 32°15' to 33°30'N latitude and 74° to 76°15'E longitude.

Material and methods

Weekly collections of *Ophiocephalus punctatus* were made from freshwater ponds in the area, their ovaries removed and fixed in dichromate-formalin-acetic acid fixative within two hours of the catch. After routine paraffin embedding, 5~8 μ thick sections were cut and stained with Mallory's triple stain for microscopical studies. Stages of maturity of ova were fixed after the method of Srivastava and Rathi (1970). The dimetrical determinations were made of every 10th section and average worked out from 50 such measurements. The different stages of maturing eggs were translated into percental values. Weight of fish and ovaries were determined and gonado-somatic index (GSI) calculated by the formula: weight of ovary \div weight of fish \times 100 and the results thus obtained were

recorded in Table 1.

Observations

General morphology and histology. The ovaries are paired and when young are translucent structures which turn yellow upon maturing. These lie addressed to the dorsal body wall closely flanking the air-bladder within their inner margins. Posteriorly each ovary is continuous with its own oviduct. The oviducts from two sides meet near termination to open to the outside by a common aperture.

Each ovary remains ensheathed by a tough covering, the tunica albuginea which is lined by a single layered germinal epithelium. The tunica albuginea is made up of muscles and connective tissue. The tunic together with the germinal

Table 1. Seasonal variations in the average diameters of large oocytes and their nuclei and the gonado-somatic index of the fish. N=50.

Month	Average diameter of oocyte (μ)	Average diameter of nucleus (μ)	Gonado-somatic index of fish
January	240	112	0.306
February	288	112	0.414
March	480	160	1.200
April	720	128	4.333
May	560	144	2.247
June	950	160	4.604
July	720	144	3.352
August	690	128	2.445
September	480	112	1.108
October	160	80	0.411
November	192	80	0.421
December	208	96	0.280

epithelium projects at places into ovocoel to form the characteristic ovigerous lamellae (Fig. 5). Embedded in the ovigerous lamellae are observed the oogonia and oocytes at various stages of development which do not conform to any regular arrangement. A discontinuous mass of filling tissue, the stroma (Fig. 3) is seen to lie between oocytes and within the ovocoel of the ovigerous lamellae. The germinal epithelium lining the ovigerous lamellae shows extensive prolificity in the production of oocytes from October to March with peak activity observed in February and March (Figs. 2, 5). This activity involves the transformation of some of the germinal cells initially into oogonia from which eventually arise the future oocytes. Supplements to this crop of oocytes are also made from oogonial nests (Fig. 1) and the resting oogonia from previous year's crop (Figs. 7, 11).

Interstitial tissue appears as isolated patches of irregular cells during the period extending from October to early February (Fig. 6). These cells often stain differentially and may appear as yellow or brown in Mallory's triple stain. Soon after February they become scarce almost to the point of disappearance in the ovaries.

Changing morphology of growing oocytes. The entire sequence of events that a growing oocyte in this fish passes through before getting transformed into an ovum can be conveniently arranged in the following six stages:

Stage 1: Chromatin-nucleolar stage (Fig. 2). The first recognizably differentiated oocytes are placed in this stage and vary in size from 16 to 98 μ . Their nuclei range in diameter from 12 to 50 μ . The nuclei are centrally placed and each bears an eccentric primary nucleolus which measures from 4 to 12 μ in diameter (Fig. 3). However, a few oocytes were seen to contain several deeply staining nucleoli, much smaller in size. A thin follicle layer is seen to surround the oocytes.

Stage 2: Peri-nucleolar stage. The oocytes in this stage are surrounded by a flattened follicular layer. The size of the oocytes, however, varies from 100 to 200 μ with nuclei varying from 50 to 100 μ . A number of morphological changes are characteristic of these oocytes: (i) the well sized primary nucleolus characteristic of stage 1 oocytes becomes indistinguishable from a number of small fuchsinophilic nucleoli arranged peri-

pherally in the nuclei of nearly 98% of oocytes at this stage; (ii) a well differentiated darkly staining body, the yolk nucleus of Balbiani (Fig. 4), about 12 μ in diameter makes its appearance in the ooplasm close to the nucleus; (iii) cytoplasm shows a regional differentiation into a lightly staining peripheral and a darkly staining peri-nuclear zone (Fig. 5); and (iv) a number of oocytes, mostly of higher diameters, show an altered position of yolk nucleus of Balbiani from the neighbourhood of the nucleus to close proximity of the periphery of the oocytes (Fig. 5).

Stage 3: Yolk vesicle stage. The oocytes included in this stage measure from 200 to 300 μ in diameter with nuclei measuring 100~160 μ in diameter. In oocytes of this stage, a distinct vacuolated zone nearly 8 μ deep is observed in the ooplasm all along the periphery (Fig. 7). These vacuoles are the yolk vesicles. The yolk nucleus of Balbiani persists (Fig. 8). The nuclear membrane assumes an irregular outline and several nucleoli are seen pushed into its granulations. A few nucleoli were observed to have even extruded out into the ooplasm but not very far from the nucleus. The follicular epithelium thickens and a thin vitelline membrane forms around the ooplasm of the oocyte.

Stage 4: Vitellogenetic stage (Fig. 9). During this stage, oocytes varying in diameter from 300 to 650 μ with apparently no increase in the size of nuclei are included. The yolk globules are seen to deposit in the yolk vesicles whose number increases with the increase in the size of the oocytes. The yolk globules were also seen to be deposited outside the vesicles. The extrusion of nucleoli from the nucleus into the ooplasm is generally speeded up. The vitelline membrane is prominent. A clear and distinct theca is observed around a prominent follicular epithelium (Fig. 10).

Stage 5: Maturation stage (Fig. 11). The maturing oocytes measure from 650 to 950 μ in diameter and are characterized by the absence of a distinguishable nuclear membrane. Yolk deposition is apace. The typical three layers of a mature ovum are distinct and prominent.

Stage 6: Ripe egg stage. The eggs at 950 μ diameter, regarded as ripe eggs, are opaque, spherical and bear comparatively thin egg membranes which do not show a specific adhesive

nature.

Annual cycle of the oocytes. Fig. 13 represents the seasonal growth pattern of an oocyte together with its percental contribution in the general make up of the ovary. A perusal of the above figure reveals that stage 1 and 2 oocytes are found in the ovary throughout the year although in varying numbers. These oocytes are maximally found in the winter months i.e., from October to January reaching a peak in their count in November and December when the average water temperature of the pond is around 14°C. Stage 3 oocytes remain comparatively low until their number increases over the period and a maximal count (35%) attained in February. The numerical increase in stage 3 oocytes corresponds well with progressive increase in the water temperature from January onward. Their peak occurrence (stage 3) is followed by the appearance of stage 4 oocytes, the latter showing two peaks, one in March contributing 41% and other in May contributing 24% of the total ovular crop in the ovary. The stage 4 oocytes in turn alternate with the peaks of stage 5 and 6 oocytes in April (69%) and June (72%) during which months the stage 4 oocytes are least available in the ovary.

Discussion

Ophiocephalus punctatus has a cystovarian ovary and the ova are led out of the body through a short oviduct.

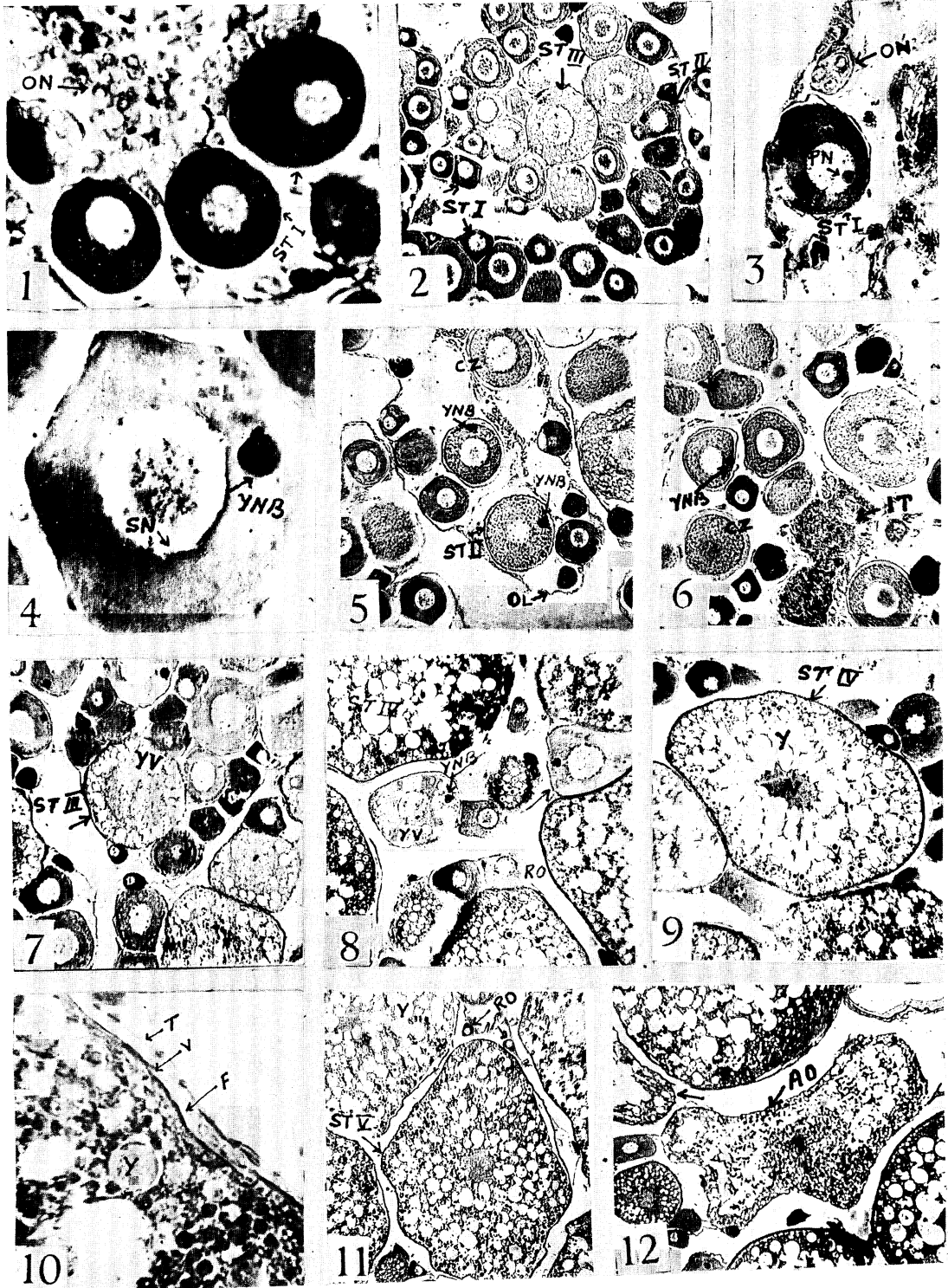
Histologically, a spent maturing ovary contains large number of germ cells at different stages of maturation. The germ cells get refurbished from the lamellar epithelium, residual oogonia and oogonial nests (Figs. 1, 11), as is

true of the species from more tropical areas (Belsare, 1962). This mode of germ cell formation, thus, appears to be a genetically fixed trait for the species with apparently no impact on its ecological diversities. However, in the involvement of oogonial nests for ova production, the species shows similarity with *Mugil cephalus* (Strenger, 1959), and *Plecoglossus altivelis* (Honma, 1961).

A lot of confusion exists in the recognition of different stages of a growing fish oocyte as labelled by various workers (Wood, 1930 for Scottish herring shoals; James, 1946 for *Lepomis macrochirus* and *Huro salmoides*; Yamamoto, 1956 for *Liopsetta obscura*; Lal, 1963 for *Cirrhina mrigala*). In the presently investigated fish, *Ophiocephalus punctatus*, the stages given for *Heteropneustus fossilis* by Srivastava and Rathi (1970) are readily identifiable except for an overlap of yolk granule stage and yolk globule stage (Srivastava and Rathi, 1970) which have, therefore, been merged into one and designated here as the vitellogenetic stage.

During its early growth phase, a young oocyte in *Ophiocephalus punctatus* shows some changes such as (i) the appearance of primary nucleolus in the nucleus of freshly formed oocyte (Fig. 3), (ii) concomitant increase in the size of nucleus and the attendant oocyte, and (iii) almost concurrent disappearance of primary nucleolus and appearance of yolk nucleus of Balbiani in the ooplasm (Fig. 4). Considerable doubt exists about the exact origin of yolk nucleus of Balbiani in fishes. Some workers assign its origin to nuclear activity (Hubbard, 1894; Wheeler, 1924; Subramaniam and Aiyar 1935; Sathyanasen, 1959) and others believe it to be cytoplasmic in

Fig. 1. T. S. of ovary in November, showing oogonial nests and stage 1 oocytes. $\times 320$. Fig. 2. T. S. of ovary in October showing stage 1, 2 and 3 oocytes. $\times 80$. Fig. 3. T. S. of ovary in October showing primary nucleolus inside the nucleus of oocyte. $\times 320$. Fig. 4. Cross section of an oocyte in October showing yolk nucleus of Balbiani. $\times 320$. Fig. 5. T. S. of ovary in December showing ovigerous lamellae, zonation of cytoplasm and peripheral position of yolk nucleus of Balbiani. $\times 80$. Fig. 6. T. S. of ovary in December showing a patch of interstitial tissue. $\times 80$. Fig. 7. T. S. of ovary in February showing stage 3 oocyte. $\times 80$. Fig. 8. T. S. of ovary in May showing yolk nucleus of Balbiani in stage 3 oocytes $\times 80$. Fig. 9. T. S. of ovary in May showing stage 4 oocytes. $\times 80$. Fig. 10. Cross section of an oocyte showing egg membranes. $\times 480$. Fig. 11. T. S. of ovary in June showing stage 5 oocytes. $\times 32$. Fig. 12. T. S. of ovary in July showing atretic oocytes. $\times 80$. AO, atretic oocyte; CZ, cytoplasmic zonation; F, follicular epithelium; IT, interstitial tissue; N, nucleus; OL, ovigerous lamella; ON, oogonial nests; PN, primary nucleolus; RO, residual oogonia; SN, secondary nucleoli; T, theca; V, vitelline; Y, yolk; YNB, yolk nucleus of Balbiani; YV, yolk vesicles.



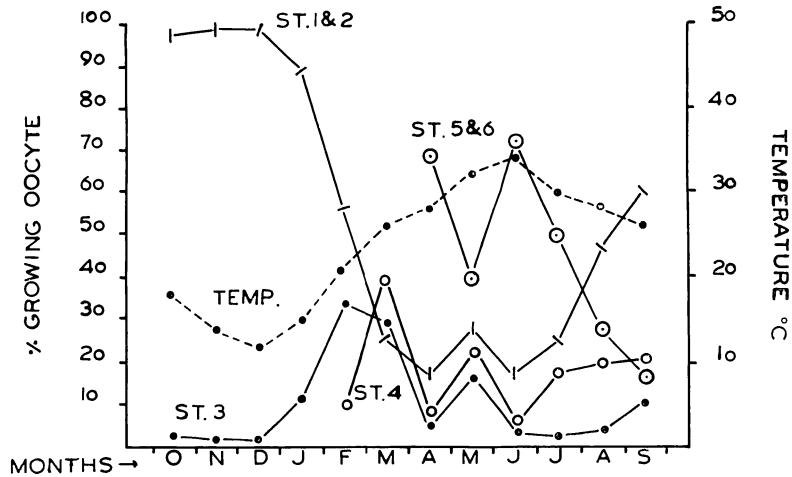


Fig. 13. Seasonal fluctuations in the percent occurrence of different stages of oocytes. Temp, temperature; ST 1~6, stages 1~6.

origin (Narain, 1951; Chaudhry, 1952; Bara 1960; Nayyar, 1964).

Appearance of yolk nucleus of Balbiani in *Ophiocephalus punctatus* oocyte closely following the disappearance of primary nucleolus is fairly suggestive of its nuclear origin and not cytoplasmic. Indirect evidence to this presumption also comes from such facts as (i) similarity in the size of newly appeared yolk nucleus of Balbiani and last observed primary nucleolus in the nucleus and (ii) similarity in the tinctorial behaviour of the two structures (both being fuchsinophilic). The appearance of yolk nucleus of Balbiani followed immediately by cytoplasmic zonation in the oocytes, however, indicates the role the former possibly plays in the maturation of the oocytes in *O. punctatus*. This view is more or less consistent with that of Belsare (1962) and Bhargava (1971).

Ovarian activity of *O. punctatus* follows a cyclic pattern which can be divided broadly into four phases viz., resting phase (from November to January), pre-spawning phase (from February to April), spawning phase (from May to August) and post-spawning phase (from September to October). During the resting phase the oocytes are immature and do not show any yolk deposition (Fig. 1). During the pre-spawning phase there is active vitellogenesis and some of the oocytes are seen on way to maturation (Fig. 7). These precociously mature ova constitute the crop for the first act of spawning. Remaining advanced

oocytes mature and are subsequently extruded during the second spawning act being separated from the first by a short interval. During the post-spawning phase any mature ova left unspawned get finally resorbed (Fig. 12).

Variability in the spawning period of this fish from place to place is known. It spawns twice a year at Madras, once in January/February and second time in July/August (Sunderraj, 1916). In Panjab it spawns from late April to the end of July (Khan, 1924). In Bengal the spawning period extends from June to August (Mukherjee, 1945) and in Madhya Pradesh from May to the end of September (Swarup, 1954). Our investigations on the spawning and breeding in *Ophiocephalus punctatus* from a sub-tropical area to some extent agrees with the reports of Swarup (1954) and Khan (1924). A look at the graphic representation of stages (Fig. 13) and gonado-somatic index (Table 1) reveals that in Jammu waters this fish has a prolonged spawning period extending from May to the end of August. The spawning tends to assume a bimodal character with stage 5 and 6 laden ovaries appearing in late April and again in June. A dip in the ovular number in between these two months is suggestive of an act of spawning but stands in sharp contrast to observations of Nair (1958) on *Hilisa ilisha*, where the fish gonads attain two peaks in a year of which the first peak results in atresia and subsequent resorption of the follicles rather than culminating in a spawning act. How-

ever, as the temperature keeps on rising to a maximum of 34°C in June the ovular number (stage 5 & 6) correspondingly rises whereafter not only the temperature of the surrounding water but the stage 5 & 6 ova also show a gradual decline. The progressive decrease in number of these ova from July onward in fact represents the normal act of spawning.

Seemingly this bimodal spawning tendency could be an adaptation to a subtropical habitat of this species in a bid to accomplish a temperate nature by increasing the number of its spawning acts, a feature which is already reported in an allied species from China (Chen and Lin, 1935). It may thus be concluded that the two spawning seasons known for *Ophiocephalus punctatus* from more tropical areas are merged into a single prolonged spawning season with multiplicity of spawning acts, which characterizes this fish, as it moves to more temperate climate.

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Ophiocephalus punctatus の卵巣周期と産卵期

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Ophiocephalus punctatus を年間に亘り毎月採集し、卵巣周期と産卵期の関係を調べた。産卵期は 5 月から 8 月までである。比卵巣重量は 4 月と 6 月に極大を示

した。卵母細胞は組織学的に 6 ステージに分けられ、各ステージの特徴を記載した。成熟卵細胞の直径は 6 月に最大であった。月別の、各ステージの成育しつつある、あるいは成熟した卵細胞数は二峯性を示した。これらの結果から卵巣周期と産卵期との関係について考察し、また、Balbiani の卵黄核の起原について論じた。