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Studies on the Seasonal Changes in the Ovary of *Schizothorax niger* Heckel from Dal Lake in Kashmir

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Abstract Seasonal changes with special reference to histological changes in the oocytes of a Kashmir marinka fish *Schizothorax niger* Heckel have been described. Germinal epithelium from May to August is prolific in production of young oocytes. Best development of tunica albuginea from June to August provides mechanical protection to young oocytes in stage I. Sub-germinal tunica albuginea is present below the germinal epithelium. Stage I oocytes are observed during the months of June and July; vitellogenesis within the oocytes is completed from August to December (stages II and III) while during severe winter from January to March this fish carries histologically mature oocytes (stage III) in a dormant phase; these oocytes get ovulated only on return of favourable exteroceptive factors in April. The spawning season as evidenced by histology of oocytes and confirmed by diameters of oocytes and gonadosomatic index extends from mid April to mid June. The fish spawns only once a year.

Introduction

The Kashmir marinka *Schizothorax niger* Heckel is a prized food fish of Kashmir, but it is facing tough competition for its survival in Kashmir waters against the exotic carp, *Cyprinus carpio*. A study of the gonadial cycles and breeding season of this fish will help fishery adminstration to formulate laws and define closed fisning periods for this fish. Moreover, histological studies of the maturity stages of gonads form an initial stage in the attempt to make a fish breed in captivity and thus boost the production of the desired species.

Previous investigations relating to seasonal changes in the oocytes of fishes are many, among which more recent and relevant to the present studies are those of James (1946), Cooper (1952), Ghosh and Kar (1952), Beach (1959), Nair (1958), Sathyanesan (1961 and 1962), Belsare (1962), Khanna and Pant (1967) and Ochiai and Umeda (1969). The present account is a pioneer investigation relating to ovarian cycles for any Kashmir fish. In this fish cyclic changes undergone by gonads are different from fishes of rest of India because of prolonged Kashmir winter during which surface freezing of lakes take place and physiological activities within the gonads are considerably arrested.

Material and method

Fishes of the same size group (18–21 cm) were caught every fortnight. The weight of the fish were recorded. The fish was dissected and the ovary weighed. The ovaries were then fixed in specially prepared Smith's fixative (Potassium dichromate 5 g, 40% formalin 25 cc, glacial acetic acid 13 cc, and distilled water 500 cc). The ovary was kept in the fixative for 48 hours in dark, after which the material was taken out and washed in 2% formalin till it stopped giving colour. Bouin's fluid and Zenker's-formol

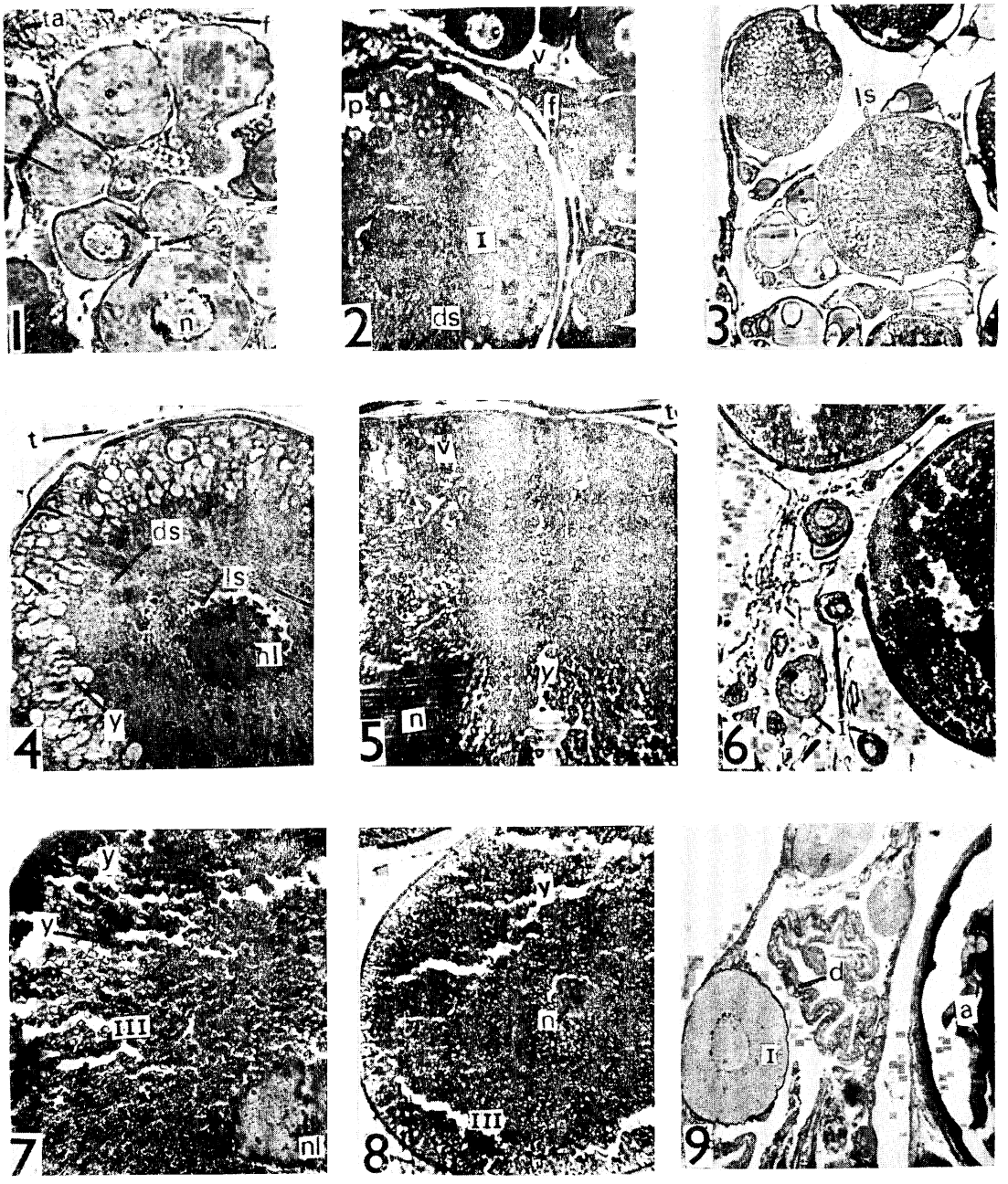

Fig. 1. Photomicrographs of sections of ovary of *Schizothorax niger* representing conditions observed in different months. 1, May and June, ×90; 2, July, ×90; 3, August, ×54; 4, September, ×90; 5, October, ×90; 6, November, ×54; 7, December, ×90; 8, January to March, ×54; 9, April and May, spent ovary, ×90. a, astretic follicles; d, discharged follicles; ds, dark staining area of cytoplasm; f, follicular epithelium; l, light staining cytoplasma around nucleus of oocyte; n, necleus; nl, nucleolus; p, peripheral vacuoles; t, theca; ta, tunica albuginea; v, vitelline membrane; y, yolk platelets; I, stage I oocyte; II, stage II oocyte; III, stage III oocyte.

were also used as fixatives. Sections were cut ranging from 5–10 μ, and stained in Mallory's triple stain and Delafield's haematoxylin. The diameters of the oocytes were measured by the oculometer standardized against a stage micrometer. Gonado-somatic index has been calculated according to the formula: weight of ovary $\times$ 100/weight of body. Only calculated gonado-somatic indices have been incorporated in this account, while the weights of ovary and fish recorded have not been given for paucity of space.

Observations

The ovaries are two elongated structures attached to dorsal body wall and occupy the length of the body cavity. In May the ovaries are flat ribbon-like in which oocytes are not visible to naked eye. During August and September oocytes are visible in an enlarged ovary. From November onwards the ovary is greatly enlarged occupying greater part of body cavity and has distinct yellow oocytes.

Histological studies of the ovaries reveal a distinct germinal epithelium with rows of cells during May, June and July; it is during these months that bulk of oocytes are produced from germinal epithelium. In the following months from August to November the activity of germinal epithelium decreases and it becomes a passive layer from December to March.

Underlying the germinal epithelium is a variable extent of connective tissue called as sub-germinal tunica albuginea. The tunica is well developed during June, July and August, when the oocytes are mostly young, it decreases in its extent from September to November and may be absent from December to March.

There is no definite response of the extent of stroma and the so-called interstitial cells in ovary to different seasons of the year. Sometimes connective tissue stroma is present in the interfollicular space in an ovary, and may be seen arising from the sub-germinal tunica albuginea.

The majority of oocytes towards the end of May and in the month of June are in stage I of maturity (Fig. 1, 1). Each oocyte has a cell membrane within which is present granular cytoplasm surrounding a distinct nucleus. The nuclear membrane is smooth in an early stage I oocyte, but it gets wrinkled when the oocytes are more advanced within this stage. The nucleoplasm is uniformly distributed in the nucleus or may be concentrated at a spot. The oocyte is surrounded by a uniform cellular layer, the follicular epithelium or membrana granulosa; in more advanced oocytes a thin vitelline membrane is added internal to the follicular epithelium. Simultaneously peripheral vacuoles appear in the cytoplasm of oocyte demarcating a cortical layer of cytoplasm (Rindenschiht des His) from the medulary perinuclear cytoplasm.

During July (Fig. 1, 2) a light staining irregular area appears in the cytoplasm of the oocytes in the vicinity of nucleus. A few of the oocytes forming the vanguard enter stage II during which a theca layer appears around the follicular epithelium and a distinct membrana propria separates these two layers. The vitelline membrane is now radially striated, and these radial striations are definite canaliculae when observed under oil-immersion lens. The peripheral vaculoes are now extended to two or three layers and the yolk platelets appear in some of these vacuoles.

During August definite canaliculae can be recognized in the radial striations of vitelline memberane. Peripheral vacuoles increase in number and yolk plates appear in them. A light staining perinuclear zone of cytoplasm is present. Some of the nucleoli are observed to extrude out of the wrinkled nuclear membrane (Fig. 1, 3). During September theca layer on outside of follicular epithelium is differentiated into a theca externa and a theca interna; the perinuclear cytoplasm of oocytes shows definite cytoplasmic stratification into light staining and dark staining areas in addition to peripheral vacuoles and 'Rin-

denschiht des His' (Fig. 1, 4).

During October (Fig. 1, 5) most of the oocytes pass into stage III of maturity but some persist in different sizes of stage I; the noteworthy point about these stage I oocytes is that none of these is either passing into stage II or showing any preparatory change towards the next stage. A full complement of egg membranes consisting of vitelline membrane, follicular epithelium, membrana propria, theca interna and theca externa are present. The yolk in the perinuclear area is arranged in two zones: (i) the peripheral zone consisting of larger yolk plates which colour blue with Mallory's triple stain, and (ii) inner zone of smaller yolk plates which take a pink stain. The cytoplasm, if any is scattered between the yolk plates. The nucleoli are centrally arranged and the nuclear membrane is smooth.

During November (Fig. 1, 6) advanced stage III oocytes are present, although I and II stage oocytes are also observed, these II stage oocytes forming the rear in the process of vitellogenesis. The perinuclear area in advanced oocytes is completely filled with yolk plates. During December (Fig. 1, 7) yolk plates completely fill the oocytes and no trace of cytoplasm is observed around the nucleus or between the yolk plates. Nucleus is in centre of oocyte with the nucleoli in its own centre. Some stage I oocytes are also present, but there are no stage II oocytes. During January, February and March (Fig. 1, 8) the oocyte does not show any change over the condition obtained in the month of December. During these winter months the present author has not observed any spent or partly spent fish, and the fishes dissected towards the end of March also show a ripe ovary with oocytes similar to those observed first during November.

The ovary during the month of April has oocytes in which vitellogenesis is complete. The nucleus becomes excentric in more advanced oocytes but there is no indication of the nuclear sap merging into the cytoplasm while the oocytes are still within the ovary. During the months of April and May fishes with spent ovaries are also characterstic. In a spent ovary most of the oocytes are of the new crop and are in different sizes in stage I. Mature oocytes if present are few in number and are those which have failed to get ovulated during the spawning act of the fish, these oocytes are now atretic being in the process of atresia. Discharged follicles (Fig. 1, 9) are characterstic for this stage and consist of all the membranes of intra-ovarian oocytes except the vitelline membrane, these membranes undergo hypertrophy to fill in the space left vacant by the ovulated oocyte and form 'corpus luteum D.'

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

Month	Average diameter of oocyte (in micra)	Average diameter of nucleus (in micra)	Gonado-somatic index of fish
June	233.33	89.59	2.13
July	575.00	162.50	1.53
August	637.49	150.00	1.77
September	735.42	166.66	3.85
October	1228.17	183.26	5.24
November	1066.66	220.83	8.20
December	1380.20	184.80	10.87
January	1358.33	229.60	7.85
February	1400.00	230.00	9.00
March	1582.70	178.43	10.55
April	1600.00	230.00	8.80
May	417.09	145.83	7.26

Discussion

Yamamoto (1956) has stated that the new crop of oocytes is produced by the follicular epithelial cells, while Tromp-Blom (1959) and Khanna and Pant (1967) suggest the origin of oocytes from germinal epithelium. In *Schizothorax niger* the oocytes in the adult are developed from the germinal epithelium of the ovigerous lamellae. The germinal epithelium is thus the living potential of the ovary into which primordial germ cells migrate at an early stage. In *S. niger* germinal epithelium is prolific from May to September, because this period is the postspawning season of fish during which oocytes are being actively formed for the next year's crop.

James (1946) and Cooper (1952) have suggested the projections of ovigerous lamellae from the tunica albuginea of connective tissue; James (1946) also suggested the presence of this tunica within the ovigerous lamellae. In *S. niger* it has been observed that the tunica albuginea forms a definite layer under the peritoneal epithelium of the ovary. It continues as an internal layer to the germinal epithelium in the ovigerous lamellae, where the present author has named it as subgerminal tunica albuginea. Brambell (1956) has stated that definitive tunica albuginea like the primary tunica albuginea is formed by outgrowths of mesenchymatous elements, which in adult ovary take the form of connective tissue. In *S. niger* the tunica albuginea is formed by the transformation of the cells of outer epithelium. In this fish the tunica responds to the impact of different seasons and is best developed from June to August when young oocytes are being proliferated from the germinal epithelium; it is for the mechanical protection of these delicate young oocytes that the tunica appears to be best developed during this part of the year.

The oocytes of *S. niger* show a period of growth from May to December although this growth is steady during May, June and July (Table 1), as the oocytes during these months are in primary growth phase during which only cytoplasmic growth takes place; this cytoplasmic growth thus does not result in much increase in diameters of oocytes or in gonado-somatic index of the ovary of fish. Some times large mature oocytes may be retained in the spent ovary which cause an increase in the average value of the diameter of oocytes and the gonadosomatic index; their higher values recorded in June (Table 1) have therefore no significance beyond this explanation (the present studies having been made on monthly basis and not according to stages of oocytes). A sharp increase in diameters of oocytes is first observed during August (when only peripheral vacuoles appear), but the gonado-somatic index does not show an increase till September (Table 1), when actually considerable quantity of yolk is added within the oocytes (Fig. 1. 4).

The histological changes that take place within the oocytes mainly involve the deposition of yolk plates in the perinuclear area of the oocytes. Present observations on *S. niger* confirm histologically the previous observations on the same fish made by macroscopic studies (Malhotra, 1965) regarding their maturity and breeding season. From the study of histology it has been observed that the vitellogenesis is completed by the end of December, but ovulation does not take place till May. Gonado-somatic index (Table 1) also reaches its maximum range (8–10) from November to March; it is also during this period that the diameters of oocytes also attain their maximum. All these factors confirm that completely mature oocytes are carried from November to March without any ovulation taking place. This is winter dormancy observed in mature oocytes of *S. niger*.

Nucleoli in oocytes of *S. niger* are few and show a peripheral arrangement within the nucleus when the oocytes are in early stage I (May to July). In this regard the present observations are in line with those of Khanna and Pant (1967). Nucleolar extrusions in

the oocytes of this fish take place towards the end of stage I (in July or August) and have been already described by the present author (Malhotra, 1963).

Histological studies of the oocytes, the study of the diameters of oocytes and the gonado-somatic index of fish point out that *S. niger* spawns only once in a year any time from middle of April to middle of June. This spawning season depends upon the prevalence of optimum exteroceptive factors like food, temperature and light, both for the parent as well as the offspring. In this fish breeding after a prolonged winter dormancy of the ovary stands in sharp contrast to observations made on other Indian fishes, where either the fish spawns more than once in a year (Khan, 1939 on *Barbus tor* and Khanna and Pant, 1967 on *Glyptosternum pectinopterium*); or the fishes spawn immediately after attaining histological maturity of gonads (Sathyanesan, 1961 on *Barbus stigma*, and Belsare, 1962 on *Ophicephalus punctatus*). The present observation of the fish carrying oocytes in a dormant phase also stands in sharp contrast to the observations made by Nair (1958) on *Hilsa ilisha*, wherein fish gonad attains its peak twice in a year, but the first peak attained during February and March does not culminate in spawning, but result in atresia and subsequent resorption of follicles.

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カシミール，ダル湖の *Schizothorax niger* の卵巣の季節的変化 **Y. R. Malhotra**

コイ科に属する本種の卵巣組織の観察と卵形，体重卵巣比の測定を季節的に行ない，その結果，卵母細胞は 5 月から 12 月にわたって成長することがわかった．若い卵母細胞は生殖上皮より発生し，それらは組織性白膜によって保護される．生殖上皮の下層には，亜生殖性の組

織性白膜がある．卵黄形成は8月から9月にかけて開始され，12月まで続く．産卵は4月以前には行なわれず，成熟した卵母細胞は冬期の休眠中の魚体内に保持されている．本種は4月中旬より6月中旬の間に1回だけの産卵を行なう．

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