Corpuscles of Stannius and serum calcium during reproductive cycle in the fish, *Notopterus notopterus*

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Abstract

Corpuscles of Stannius (CS) and serum calcium during different phases of the reproductive cycle has been studied in the fish, *Notopterus notopterus*. The CS is paired organ embedded in the anterior portion of the posterior kidney in the fish, *N. notopterus*. The CS cells exhibit cellular changes identified by the nuclear contents and cytoplasmic stain ability during four phases such as preparatory, prespawning, spawning and post spawning phases of the reproductive cycle. The comparison made between CS and gonadal (testis and ovary) during different phases indicates that the cellular activity increases during preparatory phase and prespawning phase may be because of high metabolic activity and involvement of CS and its hormone for gonadal growth.

The serum calcium level estimated during different phases of reproductive cycle in the fish *N. notopterus* indicates positive correlation between serum level and gonad growth. The total serum calcium increases gradually from prespawning to spawning phase which decreases in the post spawning phase, suggesting serum calcium requirement for gonad growth in the fish, *N. notopterus*.

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Key words: reproductive cycle, seasonal, morphological, histology, ultrastructure, *Notopterus notopterus*.

INTRODUCTION

The freshwater fish, *N. notopterus* is available in good numbers in the freshwater ponds, tanks and rivers around Gulbarga. The fish for the present study were collected from Bheema River. The fish collections were made with the help of a fisherman using cast net. The fishes were collected monthly for 2 year from (2006-07 and 2007-08). More than 160 fishes of similar sizes were used for the study. The fishes were brought to the laboratory and kept in plastic pool tanks having size 90 cm in diameter and 60 cm in height. The fish were measured for total length as well as total weight of the body. Fish of similar sizes were sacrificed by decapitation and sexed after dissecting them because this fish don’t exhibit sexual dimorphism.

The histology of the ovary, testis and CS of the fish during different stages of the reproductive cycle was studied using microtomy technique and a comparison is made. The tissue sections were stained in hematoxylin and eosin. The serum calcium level has been also determined during different phases.

The corpuscles of Stannius (CS) have been shown to be involved in the endocrine control of calcium metabolism, (Fontaine, 1964; Pang and Pang, 1974). Although the function of the CS has not been fully elucidated, its role in calcium regulation has been reported. In some fishes, it is reported that the CS become activated during gonadal maturation, especially in female fish (Lopez, 1969; Hiroi, 1970). Subhedar and Rao (1979) have reported seasonal changes in the CS and the gonads of the Indian catfish *Heterophyes fossilis* showing that the high CS activity during reproduction has been connected with the changes in calcium metabolism that may accompany gonadal maturation. Plasma calcium levels are generally raised considerably during gonadal growth in females (Oguri and Takada, 1967; Pang, 1973) and also in males (Balbontin et al., 1978). The correlative changes concerning CS structure and plasma calcium levels during gonadal maturation have been reported with a substantial experimental study in the cichlid teleost fish, *Oreochromis mossambicus* (Urasa and Wendelaar Banga, 1985).
In this report the histological structure of CS and serum calcium level during different stages of reproductive cycle in the freshwater fish *Notopterus notopterus* is being investigated and presented. In addition the changes in the gonads during one year period has been studied and correlated with CS.

**Material and Methods**

**Fish Collection and Maintenance:**

About 200 fish *Notopterus notopterus* were collected during each period of January-February, April-May, August-September and November-December (preparatory, pre-spawning, spawning and post-spawning) during the year 2006-07 and 2007-08 with the help of fisherman. The live fish were brought to the laboratory and were kept in large plastic pool tanks having size of 90 cm diameter and 60 cms height. Each tank accommodated 10-15 fishes. About 8-10 days were needed for the fishes to acclimatize. During acclimatization antibiotic tablets (Chlromphenol 80 mg in one gallon of water) has been given to prevent from infections. Fishes of both sexes were fed with live earthworms; boiled eggs and small fishes (*Gambusia*). Sex of the fish cannot be identified based on the morphological characteristics. However, they were differentiated after observing their gonads as females and males.

Fish of similar size were sacrificed by decapitation. Ten fish of each sex provided the materials for further processing. After sacrifice the gonads and corporcles of Stannius were removed and weighed with the help of electronic balance. They were fixed in Bouin’s fluid (75 ml saturated aqueous picric acid, 25 ml of 40% formaldehyde and 5 ml of glacial acetic acid) for 24 hours. Ethyl alcohol was used as the dehydrating agent,,after clearing in toluene, embedded in paraffin wax (58°-60°C). Sections of five microns thickness were cut using WESWOX microtome. The sections were stained with Ehrlich’s haematoxylin and counter stained with eosin dissolved in 95% alcohol. Photomicrographs of the section were taken using, OLYMPUS DP-12, Olympus BX 51, Model-ULH 100HG, Olympus Optical Co. Ltd. Made in Japan.

The ultrastructural studies of CS was performed using ultra thin sections observed under electron microscope

Following formula was used for determining gonadosomatic index (GSI)

\[
GSI = \frac{\text{Weight of gonads}}{\text{Weight of fish}} \times 100
\]

**Estimation of serum Calcium (Clark and Collip, 1925):**

Serum calcium is precipitated as calcium Oxalate on the addition of ammonium oxalate, on treatment with Sulphoric acid, liberates equivalent amount of free oxalic acid which is then titrated against a standard potassium permanganate solution in presence of H₂SO₄. The oxalic acid is oxidized to CO₂ and H₂O.

\[
2\text{K MnO}_4 + 3\text{H}_2\text{SO}_4 + 5[\text{COOH}]_2 \rightarrow \text{K}_2\text{SO}_4 + 2\text{MnSO}_4 + 10\text{CO}_2 + 8\text{H}_2\text{O}
\]

Statistical analysis was performed using the data and expressed as arithmetic means with their standard deviation, standard error N=6, the student’s test was applied.

**OBSERVATIONS:**

**Morphology and histology of the gonads:**

The gonadal activity was assessed on the basis of gross morphology based on the external appearance (size, shape and color etc.) of the gonads (Table-1). Seven morphological stages of their maturity were identified in accordance with the classification recommended by International Council for the Exploration of the sea. These stages were further grouped into a sequence of four phases. Meopta optical light microscope (*Czechoslovakia*) offering up to 1500x magnification was used for visualising the testicular cells.

**Morphology of the gonads:**

The study on the gonads is based on the morphological examination in both female and male fish during four reproductive phases (Table-1). The morphological stages of maturity are presented in the Table-1 for all the four phases. The four phases of the one year period in which gonadal condition observed are; preparatory phase (January and February), prespawning...
The cells in the CS are active cycle of all concentration of spermatozoa in the phase conspicuously seen in the histological preparation stained centrally placed nucleus and a nucleolus which is arranged closely the fish, embedded in the anterior portion of the posterior kidney in correlated. The CS of have been studied and relations with gonadal events are Stannius (CS) during four phases of the reproductive cycle: Histology of corpuscles of Stannius in relation to spawning in the post spawning phase. Weight during prespawning phase and depletion after phase. This indicates that the gonads undergo increase increases from preparatory phase to prespawning phase and and female fish presented in the Table Gonadosomatic index: (Table September) and post spawning (November and December). Table-2: Showing gonadosomatic index in the female and male fish, Notopterus notopterus during different phase. Table-3: Showing serum calcium level (mean) during different phases of the reproductive cycle in the freshwater fish N. notopterus.

<table>
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<th>Reproductive stage</th>
<th>Ovaries</th>
<th>Testes</th>
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<td>I. Preparatory phase</td>
<td>Pale yellow in colour yolky oocyte are transparent and visible occupying space in the visceral cavity.</td>
<td>Whitish, translucent uneven in size broader at one end.</td>
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<td>Yellow in colour occupying more space in the abdominal cavity, vitellogenic oocytes clearly visible</td>
<td>Increases in size appear turgid, opaque and pink in colour vascularity increases</td>
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Values are expressed as mean ± standard error N=6
* P < 0.05; ** P < 0.01; *** P < 0.001.

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Table-1 Gross characteristics of gonads during different phases of the reproductive cycle in the fish, Notopterus notopterus (Pallas).

Table-2: Showing gonadosomatic index in the female and male fish, Notopterus notopterus during different phase.

Table-3: Showing serum calcium level (mean) during different phases of the reproductive cycle in the freshwater fish N. notopterus.

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Phase (April to May), up to July, spawning phase (August-September) and post spawning (November and December).

Gonadosomatic index: (Table-2)
The gonadosomatic index determined in both male and female fish presented in the Table-1 showing that it increases from preparatory phase to prespawning phase and further decreases after spawning in the post spawning phase. This indicates that the gonads undergo increase is weight during prespawning phase and depletion after spawning in the post spawning phase.

Histology of corpuscles of Stannius in relation to reproductive cycle:
The histological changes in the corpuscles of Stannius (CS) during four phases of the reproductive cycle have been studied and relations with gonadal events are correlated. The CS of N. notopterus is paired organ embedded in the anterior portion of the posterior kidney in the fish, Notopterus notopterus. The cells in the CS are arranged closely packed. They are polygonal in shape with centrally placed nucleus and a nucleolus which is conspicuously seen in the histological preparation stained in hematoxylin and eosin. The CS cells of preparatory phase (Fig.2) are polygonal in shape with intensely stained eosinophilic cytoplasm. The histological section of the testis consisting of lobules are having primary and secondary spermatogonial cells and actively dividing primary and secondary spermatocytes (Fig.3). In the ovary (Fig.4) during this phase the histological section shows large number of growing oocytes belonging to the early and late per nucleolus stage.

The CS cells of prespawning phase (Fig.5) have become small and more concentrated. They do contain small vacuoles in their cytoplasm. The cells appeared less granular oval to spherical in shape. The nucleus of these cells intensely stained and cytoplasm was also conspicuously eosinophilic. In the testis during prespawning phase (Fig.6), the gametogenic activity increases results in the concentration of spermatozoa in the lobular lumen. In the later stages of testis during prespawning, the testicular inter tubular connective septa shows thickening and disorganised . The histology of the ovary (Fig.7) during this phase showed the presence of all the stages of oocyes with large number of oocytes belonging to vitellogenic group. The transformation of oocytes from primary yolk globule stage to secondary yolk globule stage and to tertiary yolk globule stage might be
Fig. 2: Section of corpuscles of Stannius during preparatory phase. H & E × 1200.

Fig. 3: Section of testis showing dividing spermatogonial cells during preparatory phase. H & E × 1200.

Fig. 4: Section of ovary showing growing oocytes during preparatory phase. H & E × 1200.

Fig. 5: Section of corpuscles of Stannius showing active cells in the fish, *Notopterus notopterus* during prespawning phase. H & E × 1200.

Fig. 6: Section of testis showing dividing spermatogenic elements during prespawning phase, H & E × 1200.

Fig. 7: Section of ovary showing vitellogenic oocytes during prespawning phase. H & E × 1200.

during later months (April to June).

The CS cells of spawning phase exhibited vacuolated and the cells have attained irregular shape (Fig.8). The cytoplasm is homogenous, granular and stained bluish red in colour. The ultrastructural studies have during this phase also indicates that the corpuscles of Stannius cells are vacuolated (Fig.14). The histology of testis in this phase shows the lobular lumen contains primary and secondary spermatogonia and the lobular lumen found to be empty (Fig.9). The histology of the ovary in the earlier phase shows the presence of oocytes belonging to earlier stages (late perinucleolus) and oocytes belonging to advanced stages of oocytes. This phase ovary can be classified as matured ovary (Fig.10).

The cells of post spawning corpuscles of Stannius have smaller nucleus with inconspicuous nucleoli, the cytoplasm is homogenous and eosinophilic (Fig.11). In the post spawning phase the testis is characterised by the presence of spermatogonia on the lining of lobules which are not in the process of division (Fig.12). The lobular lumen is small and empty. The ovary in the histological

Fig. 8: Section of corpuscles of Stannius during spawning phase of the fish, *Notopterus notopterus*. H & E × 1200.

Fig. 9: Section of testis showing empty spermatogenic lobules during spawning phase H & E × 1200.

Fig. 10: Section of ovary showing reminent oocytes during spawning phase H & E × 1200.

Fig. 11: Section of corpuscles of Stannius during post spawning phase of the fish *Notopterus notopterus* H & E × 1200.

Fig. 12: Section of testis showing spent condition during post spawning phase H & E × 1200.

Fig. 13: Section of ovary showing spent condition during post spawning phase H & E × 1200.

sections consists of the stages belonging to oogonium and some unspawned oocytes which are undergoing atresia (Fig.13).
The comparison made between CS and ovarian histology during different phases indicates that the cellular activity increases during preparatory phase and prespawning phase may be because of high metabolic activity and involvement of CS and its hormones for gonadal growth.

**Serum calcium level during reproductive cycle:** The serum calcium level estimated during different phases of reproductive cycle in the fish *N. notopterus* indicates that their exists a correlation between serum level and gonadal growth. During the reproductive cycle in both female and male fish, the GSI increases from preparatory phase to prespawning phase, reduces after spawning (Table-1 Fig.1). The total serum calcium is low during post spawning, w increases markedly during growth of gonads i.e., during preparatory and prespawning phase. The which alues further reaches peak shortly before spawning. However, after spawning during September the values further decrease indicating that, there exists a clear correlation between serum calcium and gonadal growth in the fish, *N. notopterus*.

**DISCUSSION**

**Corpuscles of Stannius and reproductive cycle:**

The corpuscles of Stannius of *Notopterus notopterus* are considerably larger in females than in males. In the fish *O. mossambicus*, the CS is larger in females than in males and this structural difference probably reflects a difference in secretory activity between the sexes (Urasa and Wendelaar Bonga, 1985). A sex difference similar to that of *O. mossambicus* has been described in the mullet, *Mugil cephalus* (Johnson, 1972). The size difference in the CS observed in female and male fish *N. notopterus* may also reflect the difference in the secretory activity between the sexes. (Urasa and Wendelaar Bonga 1985) have reported that in the cichlid fish, *O. mossambicus* during ovarian cycle, the size of the CS increases in parallel with the growth of ovaries and this increase after sexual maturation is because of an increase in the number of type-I cells of CS. The seasonal changes in the corpuscles of Stannius and the gonads of the catfish, *Heteropreustes fossilis* was studied by Subhedar and Rao (1979), showing that the annual sex cycle of the fish has been divided into 4 phases on the basis of the variation in the gonadosomatic index and histocytological features displayed by the testes and ovaries. There is a rise in the percentage of AF positive cells in the CS and an increase in the nuclear diameter, at the beginning of preparatory period (February). In the prespawning period (May-June), the AF positive cells undergo degranulation. A slight regranulation and rise in the percentage of AF positive cells occurs during early spawning period (July). During post spawning phase (September-January), The CS remain predominated by AF negative cells and show histological changes, the nuclear indices are reduced. Further, it has been suggested that in view of the concomitant changes occurring in the CS and the gonads the possibility of some direct or indirect relationship between the two has been reported. In the present study the seasonal changes in the CS of *N. notopterus*, is similar to that of *H. fossilis* reported by Subhedar and Rao (1979) that during prespawning period the CS are found to be enlarged, oval or elongated in shape, lobes are clear and show cellular and nuclear hypertrophy while spawning phase shows less vacuolation and the cells have attained irregular shape. During post spawning period CS cells have smaller nucleus with inconspicuous nucleoli. This study shows that CS is more active during late prespawning and spawning period during such period fish are sexually mature. Thus, this observation suggests a correlation with the gonadal maturation and structural changes in the CS during seasonal variations. There are some reports showing that the CS cells are activated during gonad maturation such as chilean clingfish, *Sicyases sanguineus* (Galli-Gallardo et al., 1977) and in the Atlantic salmon, *Salmo solar* as the CS cells are stimulated in both male and females. In the salmon *Omorhynchus keta* caught in sea during the spawning migration the CS cells are enlarged in fish with maturing gonads (Heyl, 1970). *Mugil cephalus* (Johnson, 1972) the CS cells are progressively activated during ovarian maturation and show regression after spawning. In *O. mossambicus*, the CS are enlarged in all stages of varian cycle when compared to the CS of males and further observed that changes in CS size that are positively correlated with the ovarian somatic index, thus it is suggested that secretory activity of the CS in females *O. mossambicus* is related to ovarian maturation (Urasa and Wendelaar Bonga, 1985). Similarly, such correlative changes between CS cells and gonadal development in the fish *N. notopterus* has been observed in the present investigation.

**Serum calcium level and reproductive cycle:**

The observation made in the present study on the levels of serum calcium in relation to different phases of reproductive cycle indicates that the serum calcium level increases on approach to gonadal growth i.e., during preparatory and prespawning phase the serum calcium increases and further decreases during post spawning phase. This study indicates that CS activation during reproduction in fish is connected with elevation in serum calcium levels that accompanies gonadal growth (maturation) in *N. notopterus*. As per (Urasa and Wendelaar Bonga 1985) high total plasma calcium levels during ovarian maturation are typical of many fish species. Most of the calcium is bound to the calcium phospholipoproteins (vitellogenins) that are synthesised in the liver, transported via the blood to the ovaries, and incorporated in growing oocytes (Pang, 1973; Van Bohemen et al., 1982). This process is under the control of estrogens secreted by the ovaries and can be induced experimentally, in males as well as in female by estrogen administration (Oguri and Takada, 1967; Mugiya, 1982; Urasa and Wendelaar Bonga 1985) have observed that the activation of CS during the reproductive period in female *O. mossambicus* is connected with the calcium status of the fish is based on the following: (1) Activation of the CS occurs in female fish only, show elevated plasma calcium levels during sexual maturation cycle, (2) changes in size of the CS are closely correlated with the changes in plasma total calcium levels during the ovarian cycle, (3) The reduction of total plasma calcium level that follows overectomy is accompanied by involution of the CS, (4)
The activation of the CS in sexually mature female fish is due to stimulation of the type-I cells. These cells are also stimulated when plasma calcium rises during the exposure of fish to increased calcium level in the tank (Wendelaar Bonga, et al., 1980). These cells probably represent the source of the hypocalcemic factor, hypocalcin (Pang et al., 1973) of the CS. Although identification of different type of cells during four stages of reproductive cycle under histological observation is quite difficult in the present study on the fish N. notopterus. However, it is possible that the similar response may be expected of CS which may be activated and results in the activation of CS during gonadal growth (maturation) during preparatory a presupawning phase.

A marked seasonal variation in serum calcium level was observed in female Channa punctatus associated with ovarian maturation i.e., during different phases of vitellogenesis (Srivastava and Srivastava, 1998). Their results were reported to be in agreement with earlier observations made in other fishes (Fleming et al., 1964; Woodhead and Woodhead, 1964; Oguri and Tikada, 1967; Woodhead, 1968; Balbotin et al., 1978; Bjornsson and Haux, 1985) reported in the fish rainbow trout on the distribution of calcium, magnesium and inorganic phosphate in plasma of estradiol 17 β treated rainbow trout indicating that free calcium levels are not affected, but the increase in total plasma calcium is due to the appearance of the calcium containing yolk protein precursor vitellogenin in plasma. It is also reported that the enhanced secretion of estrogen during the sexual maturation of females increases the serum calcium level (Ho and Vanstone, 1961; Pang, 1973). In both male and female fishes serum calcium level increased after the administration of estradiol (Swarp et al., 1986; Pang, 1973; Bailey, 1957; Ho and Vanstone, 1961; Fleming et al., 1964; Chan and Chester, 1968; Woodhead, 1969; Pang and Balbotin, 1978; Singh and Srivastav, 1990).

In Channa punctatus, Srivastava and Srivastava (1998) reported that the lowest serum calcium levels were recorded during the resting phase (March and October), with values increasing there after and peaking in June and early January where the end of the prespawning phase occurs both reproductive peaks. During the spawning and post spawning phases, the serum calcium levels showed a progressive fall. In the present study also serum calcium levels exhibited a progressive increase with gonadal maturation i.e., on approval to spawning phase may be due to the appearance of the calcium containing yolk protein precursor vitellogenin in plasma in the fish N. notopterus.

Similarly Swarp et al., (1986) reported that there exists corresponding changes in the activity of corpuses of Stannius and seasonal changes in the serum calcium during reproductive cycle of the fish Cyprinus carpio and suggested that functionally, the binding of calcium to vitellogenin appears necessary to keep protein in solution, calcium bound vitellogenin may provide vital source of calcium for the embryogeny after its sequestration into oocytes, apart from association of calcium with vitellogenin, it has been found to have regulatory function in steroidogenesis in pre-ovulatory follicles.

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Reference

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