RESEARCH PAPER | Zoology | Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

Histological structure of CS and serum calcium level during different stages of reproductive cycle in the freshwater fish *Notopterus notopterus*

KEYWORDS: corpuscles of Stannius, serum calcium, reproductive cycle

Pruthvi raj C.Bedjargi
Fish Endocrinology Research Laboratory, Department of Studies in Zoology, Gulbarga University Gulbarga, Karnataka INDIA-585 106. * corresponding author

R.S.Kulkarni
Fish Endocrinology Research Laboratory, Department of Studies in Zoology, Gulbarga University Gulbarga, Karnataka INDIA-585 106.

ABSTRACT *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 histology of gonads (ovariyan cycle and testicular cycle) during different months of one year period has been studied and correlated with CS. The observation on the serum calcium level during four phases of the reproductive cycle in the fish, Notopterus notopterus indicates that the serum calcium level increases on approach to gonadal growth i.e., during preparatory and prespawning phases and decreases after spawning i.e., during post-spawning. This study indicates that the increase in serum calcium during breeding phase may be because of necessity of calcium for gonadal growth particularly for vitellogenesis. The increase in serum calcium during breeding phase mainly a result of the high protein bond calcium content in the fish, N. notopterus as reported in other studies on fishes.*

INTRODUCTION:
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 and in addition to a role in calcium regulation, the CS may have a special function during gonadal maturation, (Subhedar and Rao, 1979). In some fishes, it has been reported that the CS become activated during gonadal maturation, specially in female fish (Lopez, 1969; Hiroi, 1970). Subhader and Rao (1979) have reported seasonal changes in the CS and the gonads of the Indian catfish *Heteropheustes fossilis* showing that the high CS activity during reproduction has been connected with the changes in calcium metabolism that may accompany gonadal maturations. Plasma calcium levels are generally raised considerably during gonadal growth in females (Martin Blomberg Jensen *et al* , 2011 Oguri and Takada, 1967; Pang, 1973; Bohemen Van and Lambert, 1982) 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 cichild teleost fish, *Oreochromis mossambicus* (Urasa and Wendelaar Banga, 1985).

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 2007-2008 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 cms 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 sex 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.

Seasonal Studies:
Collection and sacrifice of the fish:
More than 200 individuals *Notopterus notopterus* were netted from Bheema, river during the period from January to December, 2006-2008. Single day collections were made in the second week of every month at 10:00 AM, at which time the water temperature was also recorded. Netted fishes were brought to the laboratory and kept in plastic tanks (size 90 cms in diameter and 60 cms in height). Moisture was blotted from the body of each specimen which was then measured for total and standard length. Fish of similar size were sacrificed by decapitation and sexed after dissecting them, since the sexes are not distinguishable externally. Ten fish of each sex provided the materials for further processing. After sacrifice the gonads and corpuscles 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. The fixative was removed by washing through running tap water for overnight. Then the tissues were processed for dehydration. Ethyl alcohol was used as the dehydrating agent, as it is the most suitable and economical besides its less hardening effect. The alcoholic transfer schedules were so arranged as to utilize both dehydration and hardening effects. The tissues were passed through successive series containing 50%, 70%, 90% and absolute alcohols. Then the tissues were cleared in toluene, and embedded in paraffin wax (58^0-60^0C). Sections of five microns thickness were cut using WESWOX microtome. The sections were stained with Ehrlich's Hametoxylin and counter stained with eosin dissolved in 95% alcohol. After dehydration and cleaning the sections were mounted in dibutylpthalate plasticizer xylen (DPX). Photomicrographs of the section preparations were taken using, OLYMPUS DP-12, Olympus BX 51, Model-ULH 100HG, Olympus Optical Co Ltd. Made in Japan.

OBSERVATIONS:
The CS cells of prespawning phase have become small and more concentrated (Fig.1). They do contain small valuoles in their cytoplasm. The cells appeared less granular oval to spherical in shape. The nucleus of these cells intensely stained and cytoplasm was also conspecously eosinophilic. In the testis during prespawning phase, the gematogenic 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 (Fig. 2). The histology of the ovary during this phase showed the presence of all the stages of oocytes (Fig. 3) with large number of oocytes belonging to vitellogenic group. The transformation of oocytes from primary yolk globule stage to secondary yolk globule stage and

Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

to tertiary yolk globule stage might be during later months (April to June).

The CS cells of spawning phase exhibited less vacuolated and the cells have attained irregular shape (Fig. 4). The cytoplasm is homogenous, granular and stained bluish red in colour. 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. 5). 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 (Fig. 6). This phase ovary can be classified as matured ovary.

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 The total serum calcium is low during post spawning, with values increases markedly during growth of gonads i.e., during preparatory and prespawning phase. The values 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*.

The observation made in the present study on the levels of serum calcium (Table-1 Fig-7) 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*.

Similarly Swaup (1992) reported that there exists corresponding changes in the activity of corpuscles 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 oocyte, apart from association of calcium with vitellogenin it has been found to have regulatory function in steriodogenesis in pre-ovulatory follicles.

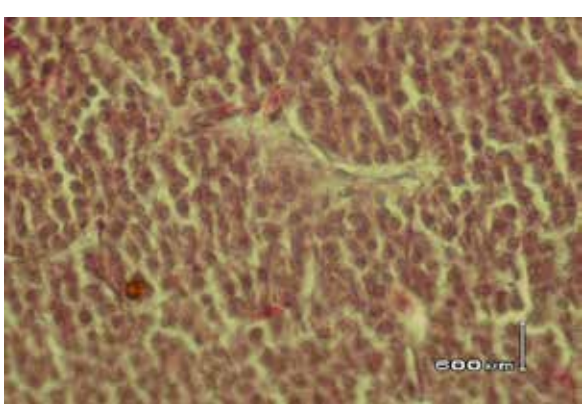


Fig. 1: Section of corpuscles of Stannius showing active cells in the fish, *Notopterus notopterus* **during prespawning phase. H & E × 1200.**

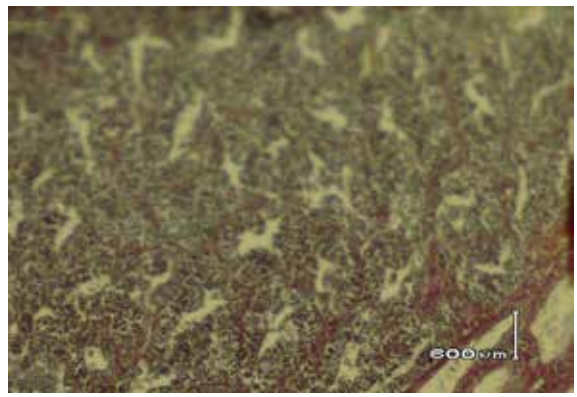


Fig. 2: Section of testis showing dividing spermatogenic elements during prespawning phase, H & E × 1200.

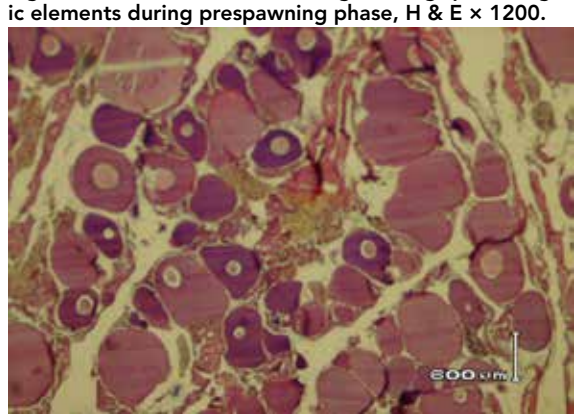


Fig. 3: Section of ovary showing vitellogenic oocytes during prespawning phase. H & E × 1200.

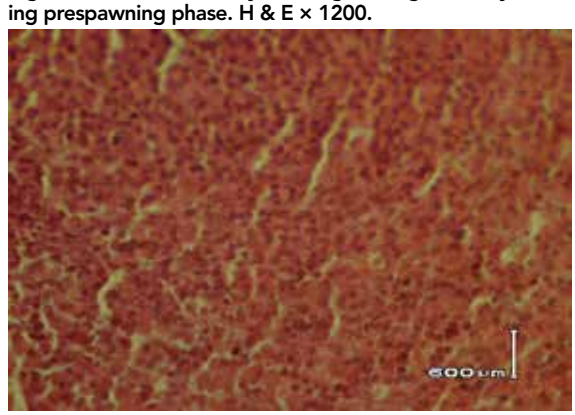


Fig. 4: Section of corpuscles of Stannius during spawning phase of the fish, *Notopterus notopterus.* **H & E × 1200.**

Fig. 5: Section of testis showing empty spermatogenic lobules during spawning phase H & E × 1200.

Volume : 4 | Issue : 4 | Apr 2014 | ISSN - 2249-555X

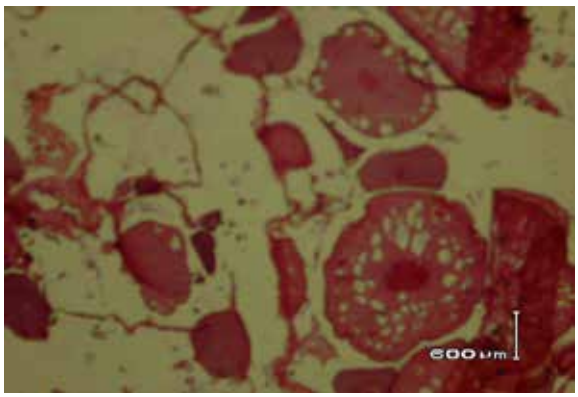
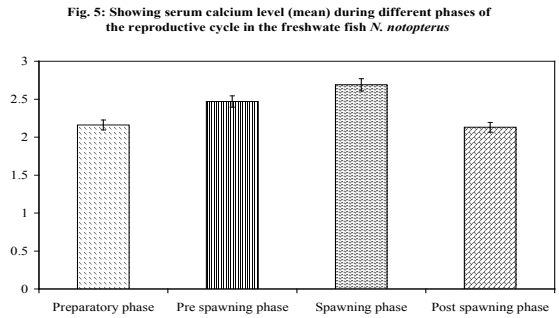


Fig. 6: Section of ovary showing reminent oocytes during spawning phase H & E × 1200.

Table-1: Showing serum calcium level (mean) during different phases of the reproductive cycle in the freshwater fish *N. notopterus*

Preparatory phase	Pre spawning phase	Spawning phase	Post spawning phase
2.16 ± 2.82	2.47 ± 0.21*	2.69 ± 0.20*	2.13 ± 3.82

Fig.7: Showing serum calcium level (mean) during different phases of reproductive cycle in the freshwater fish notopterus notopterus



REFERENCE 1. Balbontin, F., Espinosa, X. and Pang, P.K.T. (1978). Gonadal maturation and serum calcium level in two teleosts, the hake and the killifish. Comparative Biochemistry and Physiology, 61A: 617-621. | 2. Bohemen G. van and Lambert J.G.D. (1982). Estrogen synthesis in rela¬tion to estrone, estradiol and vitellogenin plasma levels during the reproductive cycle of the female rainbow trout, Salmo gairdneri. Gen Comp Endocrinol., 45: 105-114. | 3. Fontaine, M. (1964) [Stannius' corpuscles and ionic (CA, K, NA) of the interior environment of the eel (Anguilla Anguilla L.)]. C R. Hebd. Seances Acad. Sd. 259, 875-878. | 4. Hiroi, O. (1970). Morphological changes in the corpuscles of Stan¬nius of the Chum salmon Oncorhynchus keta, during the migra¬tion and maturation. Bull Fac Fish Hokkaido Univ., 21:179-192 | 5. Lopez, E. (1969) Etude histophysiologique des corpuseules de Stannius de Salmo salar L. au cours des diverses étapes de son cycle vital. Gen. Comp. Endocrinol., 12: 338-349. | 6. Martin Blomberg Jensen , Poul J. Bjerrum, Torben E. Jessen, John E. Nielsen,Ulla N. Joensen, Inge A. Olesen, Jørgen H. Petersen, Anders Juul,Steen Dissing and Niels Jørgensen (2011) Vitamin D is positively associated with sperm motility and increases intracellular calcium in human spermatozoa. Oxford Journals of Medicine Human Reproduction .V 26, Issue 6 Pp. 1307-1317. | 7. Oguri, M. and Takada, N. (1967). Serum calcium and magnesium levels of goldfish, with special reference to the gonadal maturation. Bull Jpn Soc Scient Fish., 33: 161-166 | 8. Pang, P.K.T. (1973). Endocrine control of calcium metabolism in teleosts. American Zoologist, 13: 775-792. | 9. Pang, P.K.T. and Pang, R.K. (1974). Environmental calcium and hypocalcin activity in the Stannius corpuscles of the Channel catfish Ictalurus punctatus (Rafinesque). Gen. Comp. Endocrinol factor from corpuscles of Stannius of the teleost Heteropneustes, 23: 230-241. | 10. Subhedar, N. and Rao, P.D.P. (1979). Seasonal changes in the corpuscles of Stannius and the gonads of the catfish, Heteropneustes fossi¬lis (Bloch). Z mikroskanat Forsch (Leipzig) 93:74-90 | 11. Swarup, K., Srivastav, S.P. and Srivastav Ajai, K. (1986). Seasonal changes in the structure and behaviour of Stannius corpuscles and Serum Calcium level of Clarias batrachus in relation to the reproductive cycle. | 12. Urasa, F.M. and Wendelaar Bonga, S.E. (1985). Stannius corpuscles and plasma calcium levels during the reproductive cycle in the cichlid teleost fish Oreochromis mossambicus. Cell. Tiss. Res., 241: 219-227. |