Research Paper

Volume : 3 | Issue : 3 | March 2014 • ISSN No 2277 - 8179

Ultrastructure of the Corpuscles of Stannius in a Freshwater and Sea Water Fish

Zoology

KEYWORDS : Marine, freshwater, hormone, kidney,sea water.

Pruthvi raj C.Bedjargi	Fish Endocrinology Research Laboratory, Department of Studies in Zoology, Gulbarga University Gulbarga, Karnataka INDIA-585 106
Nagaveni P.Bedjargi	Post-Graduate Department of Studies in English, Karnatak University, Dharwad
R.S.Kulkarni	Fish Endocrinology Research Laboratory, Department of Studies in Zoology, Gulbarga University Gulbarga, Karnataka INDIA-585 106

ABSTRACT

The corpuscles of Stannius (CS) of two species of fish having different habitat has been compared under electron microscopic observation. The two species of fish are; a freshwater fish, Notopterus notopterus and Indian marine mackerel fish Rostragellar kanagurta. The ultrastructural study of corpuscles of Stannius in two representative fishes collected from freshwater and sea water indicates that although three types of cells noticed in freshwater fish, N. notopterus predominantly one type of cell (type-I) is active having abundant secretary granules along with all characteristic of cytoplasmic hyper condition including large number of vacuoles. Similarly in the marine fish, R. kanagurta although single type of cell identified similar to type-I cells of freshwater fish exhibiting all characteristic of active cell, the other type of cells are not a abundance. However, in both the fishes a single type of cell (type-I cell) is active and probably involved in calcium homeostersis. The studies indicates that the corpuscles of Stannius in the freshwater fish, Notopterus notopterus has three types of cells and amongst them one type (type-I) is predominent a found to be active.

Introduction

The corpuscles of Stannius from various fishes have been extensively examined morphologically both at the light and electron microscopy level (Olivereau and Fontaine, 1965; Krishnamurthy and Bern, 1969; Wendalaar Bonga and Greven, 1975; Wendelaar Bong *at al.,* 1980; Battacharya *et al.,* 1982) and in many reviews (Krishnamurthy, 1976; Wendeliaar Bonga and Pang, 1986, 1991; Hirano, 1989; Wagner, 1994).

The ratio between osmotic pressure of body fluids and of the external environment is completely different in various groups of marine and freshwater fishes. Consequently, the osmoregulatory organs have to perform two opposite functions: in freshwater fishes not only to retain but also to collect the missing ions from the environment, whereas in marine teleosts to excrete the excessive ions (Cykowska, 1979).

Sea water is high in calcium compared to freshwater and histological observations on the corpuscles of Stannius indicate that the glands are more active in sea water than freshwater. This supports the idea that the corpuscles of Stannius promote hypercalcemia and removal of corpuscles of sea water adopted eels and killfish results in a greater hypercalcemic response than in freshwater adopted animals (Matty, 1985). Amongst the endocrine glands studied, the corpuscles of Stannius have been most consistently implicated in the control of plasma calcium metabolism (Wendelaar Bonga and Pang, 1991). Histological examination of the CS has revealed that the glands are consistently more active in sea water adopted fish than fish in freshwater fish (Hanke *et al.,* 1967; Wendelaar Bonga *et al.,* 1976; Meats *et al.,* 1978).

Hanssen *et al.,* (1992) have studied influence of ambient calcium levels on stanniocalcin secretion in the European eel, *Anguilla anguilla* showing that the cells of the corpuscles of Stannius appeared to be more active in eels acclimated to sea water than in eels acclimated to freshwater.

In view of calcium in the environment as a regulatory factor for functioning and structural differences of corpuscles of Stannius. Hence, in the present investigation the histological and ultrastructural studies on the corpuscles of Stannius in two representative examples one selected from freshwater and the other selected from sea water is made. This study provide information on the difference in the cytoarchitecture of CS in fishes having different habitat i.e., freshwater and sea water.

Materials and Methods

Fish Collection

The corpuscles of Stannius (CS) of two species of fish having different habitat has been studied under light and electron microscopic observation. The two species of fish are; a freshwater fish, *Notopterus notopterus* and *Rastrelliger kanagurta* [Indian mackerel].

The fish, *Notopterus notopterus*, (fresh water fish)were collected from Khaja Kotanoor reservoir 15 km away from Gulbarga University campus. The live fish were brought to the laboratory and kept in large plastic tanks. The sea water fish, *Rostregellar kanagurta* (Indian mackrel) collected from Karwar coast.

Collection of corpuscles of Stannius and fixation for Electron Microscopic studies

Fish were killed with a blow to the head. Then a longitudinal incision was made through the ventral body wall to expose the kidneys. The CS or white bodies were clearly visible along most of the length of the kidney. each CS together with the surrounding kidney was flooded with ice-cold glutaraldehyde (ICG) buffered to pH 7.3 with phosphate and fixed for 5 min. CS were excised from the adjacent kidney tissue and placed individually, in separate 2 ml glass vials containing additional ice-cold fixative for 2 h. In the fish, *N. notpterus,* there are two white oval corpuscles, approximately 0.5 to 2.00 mm in diameter, which are embedded in the ventral surface of the anterior position of the posterior functional kidney at the point where the right post cardinal vein leaves it. Both CS were transferred to the Ice cold Glutaraldehyde (ICG) fixative for 2 min then removed, cut into about four pieces, and placed in a vial of ICG for 2 hrs.

For transmission electron microscopic study, two fishes were used. Small samples from different parts of the corpuscles Stannius were taken. The samples were fixed in a mixture of paraformaldehyde 2.5 % and glutaraldehydehyde 2.5 % solution in 0.1 M phosphate buffer for 4 hours at 4^0C. After washing in the same buffer, the specimens were post-fixed in osmiumtetroxide 1% in phosphate buffer for two hours followed by washing in the same buffer. The samples were then dehydrated in ascending grades of ethanol followed by critical point drying in carbon dioxide, then sputter-coated with gold and examined with transmission electron microscope.

Semithin sections for light microscopy

Before proceeding to ultrathin sectioning 1µ thick section are cut for scanning the tissue under the light microscope. The semithin sections floated on the water are lifted with a thin glass rod on a clean glass slide. The slide is placed on a hot plate at about 80^0C and dried. The sections are stained using 1% toludine blue for 1 minute. Washed in running water, dried and mounted with DPX. The slides were observed under light microscope.

Observation
The corpuscles of Stannius (CS) of two species of fish collected one from freshwater and another from sea water fish has been studied under light and electron microscope. The two species of fish are 1) *Notopterus notopterus* from freshwater and 2) *Rostregellar kanagurta* from sea water.

In this study the cellular activity of corpuscles of Stannius (CS) has been determined based on the cytoplasmic state, nuclear structure and presence of prominent secretary granules.

The corpuscles of Stannius (CS) in both the fish are two in number located in the anterior portion of the posterior kidney. The CS is embedded dorsally on either side of the posterior kidney. Each CS is invested by connective tissue capsule from which many septa extend in between the cells. The cells composed of compact cords arranged in a single layer of cells along the connective tissue septum. The gland is richly vascularised and thin blood capillaries extend along the connective septa. All the cells of CS uniformly stained with eosin (Fig.1). The cytoplasm looks homogenous with oval or round nucleus and contains nuclei.

The semi thin section prepared for ultrastructural studies shows that the cells are arranged under lobules in the freshwater fish *Notopterus notopterus* (Fig.2). Where as, in the sea water fish, *R.kanagurta* the cells are large in number and compactly arranged (Fig.3).

The CS of freshwater fish, *N. notopterus* though small compared to larger size in marine fish, *R. kanagurta*. In freshwater fish, *N. notopterus* three types of cells are identified and the cells are named as type-I, II, and III, (Fig. 4,5&6). Amongst the three types, type-I cells are more in number and have large number of secretary granules, mitochondria, Golgi and rough endoplasmic reticulum (RER) (Fig.6). The other two types of cells are less in number and exhibit variation in the cytoplasmic components. The marine Indian mackerel fish, *R. kanagurta* has major one type of cell distributed in large number throughout the CS (Fig.7) and this cell type exhibit ultrastructural characteristic of type-I cells of a freshwater fish. Although other types of cells are present but less in number. The predominant cell type of CS in this fish (*R. kanagurta*) shows oval nucleus, strands of granular endoplasmic reticulum a few large mitochondria, Golgi zones and large electron dense secretary granules. The cell type of this is referred to as type-I cells of freshwater fish, *N. notopterus*. These cells have larger secretary granules of varying sizes. In between these cells, another type of cell is present (as type-II), it is less in number and structurally different from type-I cells. The cell body is long and slender with cytoplasmic penetrating in between the type-I cells. The nucleolus is cylindrical the cytoplasm contains strands of granular endoplasmic reticulum, many small mitochondria, an occasional Golgi zone and small very few secretary granules.

The comparison made between CS cytology of freshwater fish *N. notopterus* and a marine fish, *R. kanagurta* under ultrastructural studies indicate that although three types of cells found in freshwater fish, predominantly one type (Type-I) cells are exhibiting cellular activity whereas amongst the cells of marine fish *R. kanagurta*, a single type of cell similar to the type-I cells of freshwater fish exhibiting all ultrastructural characteristic of active cell which is in large number. Hence, one type of cell (Type-I) is more active in both freshwater and sea water fish. The secretary granule size of CS cells in marine fish, *R. kanagurta* were larger than the secretary granules of freshwater fish, *N. notopterus* indicating secretion of large amount of hormone.

The calcium concentration in the water samples collected from sea near Karwar beach and water from local aquatic body has been estimated and presented in the Table-6.2 shows increase in the calcium concentration of sea water.

Discussion
As per survey of literature on the corpuscles of Stannius, their number and location in different species of fish has some significance. In the present study the fish collected from freshwater and sea water, the number and location of the CS has been observed and found that a pair of CS embedded in the posterior portion of the kidney. The number of CS in several teleostean fishes have been studied. In *Onchorhynchus gorbuscha*-2 pairs (Ford, 1959), *Oncorhynclus tshawylscha* and *Oncorhynchus kisutch* – 5-6 CS (Nadkarni and Gorbman, 1966). In *Salmo airdnerii* 4-6 CS *Oncohynchus kisutch* 4-5, *Atheronopsisi californiensis,* -2,3 or 4 and *Sepastodes auriasbiva* -3 CS (Krishnamurthy and Bern, 1969). The CS may vary from 4-10 in *Salmo solar* (Heyl, 1970) and 6-8 in *Salmo trutta* (Bauchot, 1953). In catfish *Heteroprenstes fossilis*, the CS are as many as 4 corpuscles (Subhedar and Rao, 1976). In the bow fin, *Amia calva*, there are very many corpuscles, numbering fifty or more; it is thought that in the evolution of bony fish there has been a general contraction in numbers and an increase in relative size of the corpuscles of Stannius. The CS of the fish studied in the present investigation a pair of CS is present, these fishes are present day fishes.

The location of CS in the teleostean kidney presents several variations and it has been suggested by Bauchot (1953) that it is related to the taxonomic position. In fishes such as *Carassius auratus* (Ogawa, 1967) the CS were present in mesonephros and embedded in the dorsal and dorsolateral parts of the kidney, similar location has been observed in two species of *Oncorhynchus* (Nadkarni and Gorbman, 1966). In salmonid fish *A. californiensis* the CS are situated near the middle of Mesonephros (Krishnamurthy and Bern, 1969). In majority of fish they are located at the posterior end. In *H. fossilis* they are found in the posterior third of the mesonephrous. It was suggested that the CS move progressively backwards during the evolution as a result of actual shortening of body cavity rather than a migration of CS (Garrett, 1942). In view of localization of the CS in the kidney of the fish studied in the present investigation that freshwater fish*N.Notopterus* has the CS situated at the posterior portation of the posterior kidney and this is also true with the location of CS in marine fish.

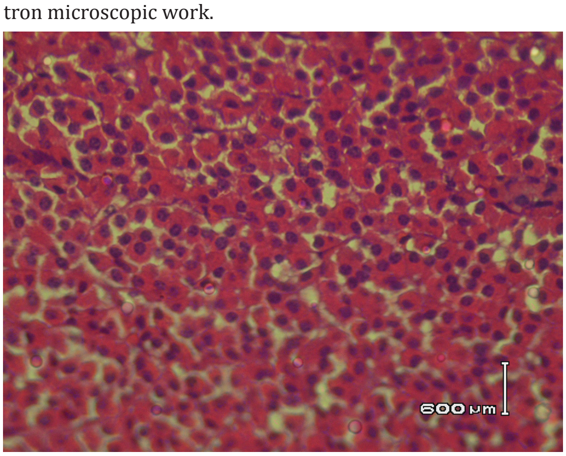
The ultrastructural studies undertaken on the CS of fish from freshwater (*Notopterus notopterus*) and sea water (*Rosregillar Kanagurta*) for comparison shows that fish, *N. notopterus* from freshwater consists of three different cell types. The first type of cells are type-I cells are large in number having electron transient cytoplasm with vacuolation, large secretary granules and abundant mitochondria and also ribosomal endoplasmic reticulum. The second type of cell known as type-II are less in number characterised by electron dense cytoplasm, few membrane bound small secretary granules. These cells have cytoplasmic extensions amongst the type-I cells. The third type of cells also have been observed lying small in size having broader nucleus, well marked nuclear membrane as uniform cytoplasm with very less secretary granules. The most conspicuous feature of these cells particularly type-I cell is the presence of large number of vacuoles of irregular shape which gives exhausted appearance. The secretary granules are large in type-II cells whereas they are smaller in type-I cells. The Golgi areas are seen, the mitochandria are large and more in number in every cell particularly in the type-I cells. The nuclei of all the three type of cells are clear with prominent nucleous. The chromatin patches are seen in the type-II and type-III cells, which are not seen in the type-I cells. Hence, based on the structure of nucleus and cytoplasm, the three types of cells can be distinguished.

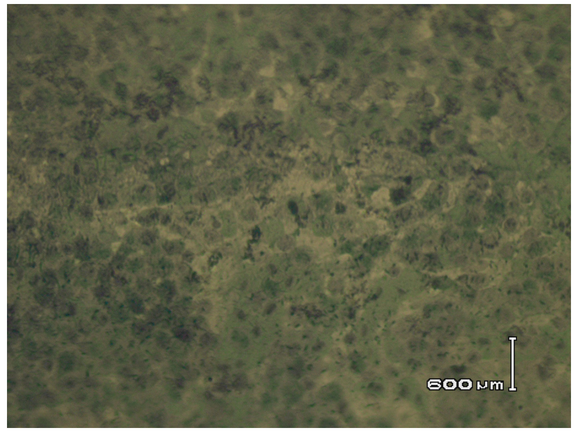
In the sea water fish, *R. kanagurta* the ultrastructural observation of CS cells shows that predominantly two types of cells based on the cytoplasmic components. The predominant type in the CS of *R. kanagurta* shows an oval nucleus, strand of granular endoplasmic reticulum, a few large mitochondria, some Golgi zones and large electron dense secretary grannules of varying sizes. In between these cells, another cell type is present (type-II), it is less numerous and structurally different from type-I cell. The cell body is long and slender with cytoplasmic projections penetrating in between the type-I cell. The nucleus is cylindrical the cytoplasm contains strands of granular endoplasmic reticulum, many small mitochandria, an occassional Golgizone and small very few secretary granules.

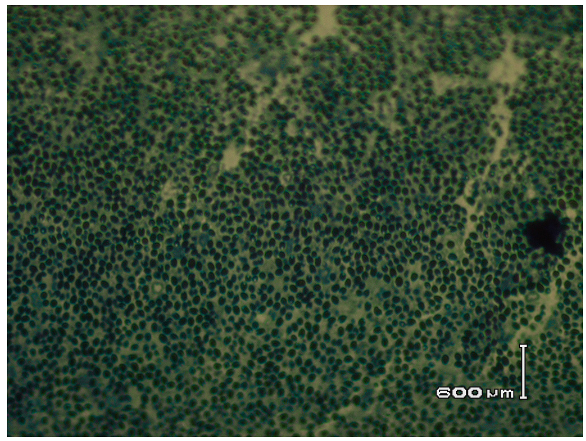
The comparison made on the CS of freshwater fish *N. notopterus* and sea water fish *R. kanagurta* indicate that three types of cells are present in *N. notopterus* while only one predominant type of cell i.e., type-I cells form the major component of CS in *R. kanagurta*. The type-I cells of freshwater fish *N. notopterus* has small and many secretary granules while type-I cells of marine fish *R. kanagurta* has large secretary granules indicating that these type of cells are the major cell types in both species of fish involved in calcium homeostasis.

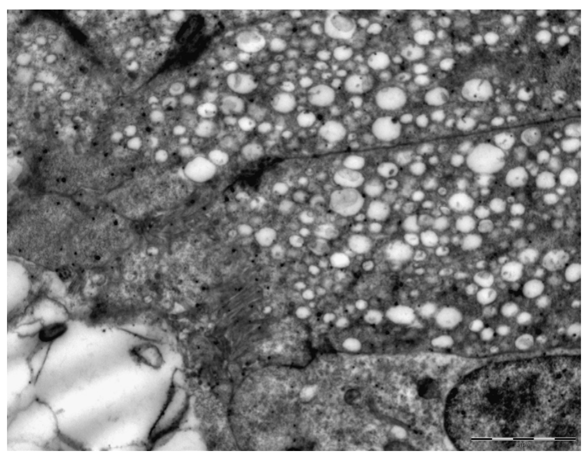
The CS of teleost fishes residing entirely in freshwater or euryhaline species, spending part of their life cycle in freshwater, have been shown to contain heterogeneous populations of gland cells, unlike the marine forms where the CS contain cells of only one type (Feroz Ahmed *et al.,* 2002). In some of the fishes such as *Onchorhynchus mytis* (Krishnamurthy and Bern, 1969; Meats *et al.,* 1978). *Fundulus heteroclitus* and *Carassius auratus* (Wendelaar Bonga *et al.,* 1977) and *Onchorhynchus kisutch* (Aida *et al.,* 1980) have been reported to contain two structurally different cells namely type-I and type-II. The type-I cells numerically predominate and possess characteristically large abundant electron dense secretary granules, prominent RER and Golgi area. On the contrary, type-II cells possess relatively few small secretary granules, scarce Golgi and RER but long slender processes extending between types-I cells, such observation of two type cells noticed in both freshwater fish and marine fish studied under ultra structurally. However, the CS cells of few sea water fishes studied so far, *Gadus marhua* and *Pleuronectes plutessa* (Wendelaar Boga and Greven, 1975) and *Opsanus tau* (Bhattacharya and Butter, 1978) referred by (Feroz Ahmed *et al.,* 2002) confirm the presence of only type-I cells with their characteristic features. In the present study on the ultrastructure of CS of sea water fish, *R. kanagurta*, shows only one type of cell predominantly distributed with abundant larger secretary granules. The type-II cells although located without secretary granules needs to be confirmed for the presence of type-II cells in the CS of *R. kanagurta*. The type-I cells predominantly seen seems to be involved in the calcium homeostasis as reported in other marine fishes.

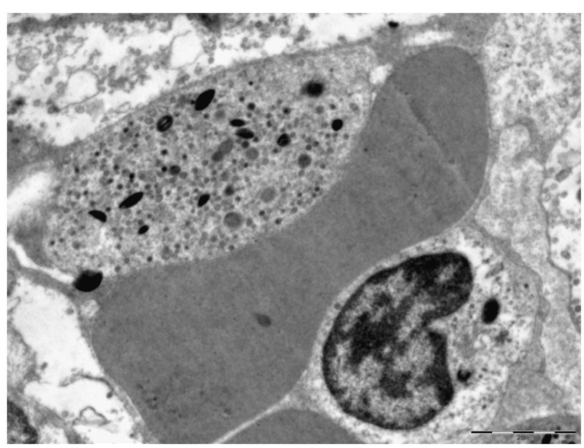
Acknowledgments
First author (P.C.B.) is grateful to University Grants Commission, New Delhi (India)No.F.16-30/2006 [SA-II] for financial assistance. And Director NIMHNS Bangalore for permission. and Dr. Sagar. Professor for technical assistance for undertaking electron microscopic work.

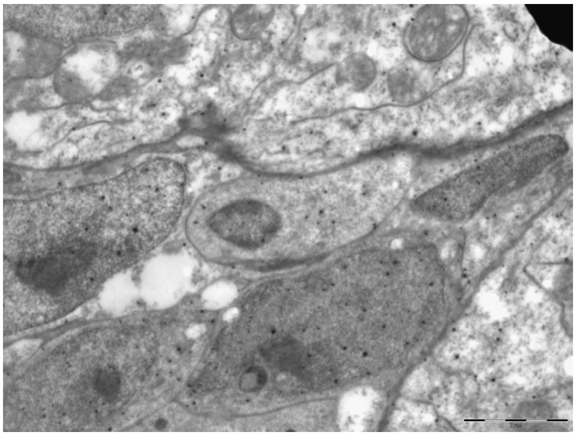












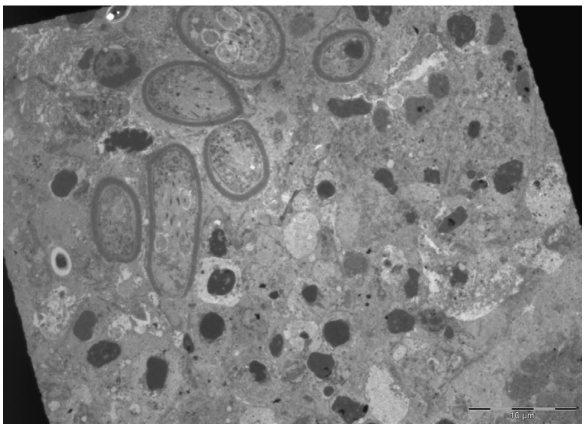


Fig. 1: Section of corpuscles of Stannius in the freshwater fish, *Notopterus notopterus* H & E × 1200.

Fig. 2: Semithin section of corpuscles of Stannius in the freshwater fish, *Notopterus notopterus* × 1200.

Fig. 3: Semithin section of corpuscles of Stannius in the sea water fish, *Rastrelliger kanagurta* × 1200.

Fig. 4: Ultra structure of corpuscles of Stannius showing vaculation in the type-I cell of fresh water fish, *Notopterus notopterus* **× 9300.**

Fig. 5: Ultra structure of corpuscles of Stannius in the fresh water fish, *Notopterus notopterus* showing type-II cell near the blood vessel × 9300.

Fig. 6: Ultra structure of corpuscles of Stannius showing vaculation in the type-III cell of fresh water fish, *Notopterus notopterus × 9300.*

Fig. 7: Ultra structure of corpuscles of Stannius in the sea water fish, *Rastrelliger kanagurte* showing big secretory granules in type-I cells × 2900.

REFERENCE

Aida, K., Nishioka, R, S. and Bern, H.A. (1980). Degranulation of the Stannius corpuscles of Coho salmon (Oncorhynchus kisutch) in response to ionic changes in vitro. Gen. comp. Enclocr. 41: 305-313. | Bauchot, R. (1953). Anatomie Comparee des corpuscles de Stannius chez les Teleosteens. Arch. Zool. Exp. Gen., 89: 147-149. | Bhattacharya, T.K. and Butler D.G. (1978). Fine structure of the corpuscles of Stannius in the toadfish. Journal of Morphology., 155: 271-286. | Bhattacharyya, T.K., Butler, D.G. and You Son J.H. (1982). Ultra structure of the corpuscles of Stannius in the garpike (Lepisosteus platyrhynchus). Gen. Comp. Endocrinol., 46: 29-41. | Cykowska (1979). Effect of environmental osmotic pressure of kidney Histology in Rainbow trout, Salmo gairdnere. Richardson. Acta. Icht. E.T. Discat., Vol. IX, Fasc-I. | Firoz Ahmad, M., Alim, N.S., Sen, Lakra, G.K.D., Mishra, Bushraraza, B. Chakarborty, Rao and Wendelaar Bonga, S.E. (2002). J. Biosci., Vol. 27, No. 5, 509-513. | Ford, P. (1959). Some observations on the corpuscles of Stannius. In comprative Endocrinology, Ed. Gorbman, A., New York, Wiley, 728-734. | Garrett, F.D. (1942). The development and phylogeny of the corpuscles of Stannius in ganoid and teleostean fishes- Journal of Morphology 70 41-67. | Hanke, W., Bergerhoff, K. and Chan, D.K.O. (1967). Histological obsevations on pituitary ACTH-Cells, adrenal cortex and the corpuscles of Stannius of the European Cel (Anguilla anguilla L.), Gen. Comp. Endocrinol., 9: 64-75. | Hanssen, Mayer-Goston, Fiik, G. and Wendelaar Bonga, S.E. (1992). Influence of ambient calcium levels on stanniocalcin secretion in the European eel (Anguilla anguilla), J. Exp. Biol., 162: 197-208. | Heyl, H.L. (1970). Changes in the corpuscles of Stannius during the spawning journey of Atlantic salmon (Salmo salar). Gen Comp Endocrinol 14:43-52. | Hirano, T. (1989). The corpuscles of Stannius. In Vertebrate Endocrinology: Fundamentals and Biomedical Implications, vol. 3 (ed. P. K. T. Pang andM. P. Schreibman), pp. 139-170. New York: | Krishnamurthy, V.G. (1976) Cytophysiology of corpuscles of Stan¬nius. Int. Rev. Cytol., 46: 177-249 | Krishnamurthy, V.G. and Bern, H.A. (1969) Correlative histologic study of the corpuscles of Stannius and the juxtaglomerular cells of teleost fishes. Gen. Comp. Endocrinol., 13:313-335 | Matty, A.J. (1985). Fish Endocrinology Timber Press, Portland, USA, pp. 174-197. | Meats, M., Ingleton, P.M., Chester Jones, I., Garland, H.C. and Kenyon, C.J. (1978) Fine structure of the corpuscles of Stannius of the trout, Salmo gairdneri: structural changes in response to in¬creased environmental salinity and calcium ions. Gen Comp Endocrinol, 36:451461. | Nadkarni, V.B. and Gorbman, A. (1966). Structure of the corpuscles of Stannius in normal and radiothyroidectomized chinook finger lings and spawning pacific salmon. Acta. Zool. Stockholm, 47: 61-66. | Ogawa, M. (1967). Fine structure of the corpuscles of Stannius and the interrenal tissue in gold fish, Carassius auratus Z. Zellforsch., 81: 174-189. | Olivereav, M. and Fontaine, M. (1965). Effect de' hypophysectomie sur les CS de, languille C.R. Hebd. Seances. Acad. Sci. Ser. D261, 2003-2008. | Subhedar, N. and Rao (1976). Z. on the cytoarchitechive of corpuscles of Stannius of the cat fish, H.fossilis cbi Mikrosk. Anat. Forsch., Leipzig, 90: 4. S. 737-748. | Wagner, G.F. (1994) Fish Physiology, eds. Sherwood, N. & Hew, C. (Academic, New York), 13: pp. 273-306. | Wendelaar Bonga S.E. Vander Meij, J.C.A. and Pang, P.K.T. (1980). Evidence for two secretary cell types in the Stannius bodies of the teleost Fundulus heteroclitus and Carassius auratus. Cell Tis¬sue Res., 212: 295-306. | Wendelaar Bonga, S.E. and Pang, P.K.T. (1986). Stannius corpuscles. In Vertebrate Endocrinology, Fundamentals and Biomedical Implications (ed. P. K. T. Pang and M. P Schreibman), pp. 439-464. New York: Academic Press. | Wendelaar Bonga, S.E. and Pang, P.K.T. (1991). Control of calcium regulating hormones in the vertebrates: Parathyroid hormone Calcitonin. Prolactin and Stanniocalcin; Int. Rev. Cytol., 128: 138-213. | Wendelaar Bonga, S.E., Greven, J.A.A. and Veenhuis, M. (1977). Vascularization, innervation, and ultrastructure of the endocrine cell types of Stannius corpuscles in the teleost. Gasterosteus aculeatus. Journal of Morphology 153 225-244. | Wendelaar Bonga, S.E., Greven, J.A.A. and Veenhuis, M. (1976). The relationship between the ionic campaign ion of the environment and the secretary activity of the endocrine cell types of Stannius corpuscles in the teleost Gasterosteus aculeatus. Cell Tissue Res., 175: 297-312. | Wendelaar Bonga, S.E. and Greven, J.A. (1975). A second cell type in Stannius bodies of two euryhaline teleost species. Cell. TissueRes. 159, 287-2 |