ISSN: 2347-5129
IJFAS 2014; 1(4): 79-84
www.fisheriesjournal.com
Received: 09-01-2014
Accepted: 13-02-2014

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Comparative histology of corpuscles of Stannius in freshwater and sea water fishes

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ABSTRACT
Morphology, number, location and histology of corpuscles of Stannius in six freshwater fishes and six sea water fishes have been investigated as these fishes have different aquatic habitat. The six fishes from freshwater are *Notopterus notopterus, Labeo rohita, Tilapia mossambica, Channa punctatus, Channa marulius* and *Clarias* gariepinus. The other six sea water fishes are Rastrelliger *kanagurta, Hemiramphus far, Parastromateus niger, Sillago sihama, Pampus argenteus* and *Scoliodon laticaudus*.
The histology of corpuscles of Stannius shows that the cells are active in both freshwater and seawater fishes. In marine fishes witch are associated with coral reefs the concentration of calcium increases in the reefs, the CS cells are more active than the fresh water fishes of fresh water, thus indicating calcium is a factor for the activity of corpuscles of Stannius in fishes. The histological observation shows that three types of cells found and one type (type-I) of cell is predominant and found to be active.

Keywords: Corpuscles of Stannius, Morphology, Freshwater, *Notopterus notopterus*.

1. Introduction
The corpuscles of Stannius from various fishes have been extensively examined morphologically [18, 9, 22, 21, 2].
[1] Studied comparative anatomy of the corpuscles of Stannius in different species of fishes including both marine and freshwater forms and found that the primitive fishes have corpuscles is on anterior one about midway of the length of the kidney, and the most evolved, a posterior one. With the experimental results [19] has shown by the cytological evidence for a role of the corpuscles of Stannius in the osmoregulation of teleosts.

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 [4]. 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 [12]. Amongst the endocrine glands studied, the corpuscles of Stannius have been most consistently implicated in the control of plasma calcium metabolism [23]. Histological examination of the CS has revealed that the glands are consistently more active in sea water adopted fish than fish in freshwater fish [7, 24, 13].
In view of calcium is the environment as a regulatory factor for functioning and structural differences of corpuscles of Stannius. Hence, in the present investigation the histological studies on the corpuscles of Stannius of the fishes collected from freshwater and marine environment is made.

3. Materials and Methods:

This study was carried out on 12 species of fishes, The six fishes were collected from Karwar coast (marine habitat) and the other six fishes were collected from Bheema river, Khaja Kotanoor reservoir (freshwater habitat) for comparative studies of corpuscles of Stannius. All the fishes were photographed by Sony digital camera. The fishes were weighed and the weights ranged from 150-220 gm and measured 30-45 cm in length, in each species, six fishes were used to demonstrate the gross morphological features. They were transported to nearby laboratory in an hour. Firstly, the fishes were sacrificed and dissected and removed the CS from each fish. The tissue (CS) was fixed in Bouin's fluid, about an hour later the tissue was kept in the fresh fluid. This procedure insured rapid fixation of the rather labile granules of the glandular tissue. After fixation, the samples were extensively washed in 70 % alcohol (3 × 24 hr) to get rid of the fixative before the subsequent step of tissue processing. The tissue samples were then dehydrated in graded series of ethanol (80%, 95% and absolute), cleared in xylene and impregnated and embedded in paraffin wax. Sections of 5-7 µm were cut using Rotary microtome and mounted on glass slides. Paraffin sections were kept in incubator at 40 ^{0}C until used for conventional staining (H and E).

4. Observations:

In the present investigation the morphology, number, location and histology of corpuscles of Stannius in six freshwater fishes and six sea water fishes has been studied. The calcium concentration of freshwater and sea water is presented in the Table-1. The higher concentration of calcium along with phosphate has been noticed in sea water. In the six freshwater fishes, ***Notopterus notopterus*** (Fig.1) the corpuscles of Stannius (CS) are two in number located in the anterior portion of the posterior kidney. The CS are 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. The cytoplasm looks homogenous with oval or round nucleus and contains nuclei. In ***Labeo bata*** (Fig2) the CS are two in number located in the middle portion of the dorsal side of the posterior kidney. One portion of the gland is closely embedded in the kidney. Each CS is invested by connective tissue. The cells are closely packed with conspicuous nucleus. The cytoplasm of these cells exhibit variation in the staining intensity, some are darkly stained and others are weekly eosinophilic. In some of the sections cell boundaries are not visible the gland is highly vascularized. In ***Tilapia mossambica*** (Fig. 3) fish the CS are two in numbers, the gland is embedded in the middle portion of the dorsal side of the posterior kidney. The gland is highly vascularised. The cells are closely packed. Two types of cells are noticed, are type having vesicular nucleus and highly vacuolated. The nucleus is elongated, the other type having round nucleus eosinophilic cytoplasm. The presence of big vacuoles is a common feature of most of the cell indicating the gland is very active In ***Channa punctatus*** (Fig.4) the CS the two CS are separately embedded in the middle portion of the posterior kidney (dorsal). The gland is completely embedded in the kidney. The cells have conspicuous big nucleus. The cytoplasm is eosinophilic. Amongst the cells, different types are seen, some are intensely eosinophilic and some others are weak to eosin stain. The cytoplasmic vacuoles are seen in the weakly stained cells. In **Channa *marulius*** (Fig.5) the two CS are separately embedded is the posterior middle portion of the kidney. The cells are eosinophilic, amongst them some are weakly stained and others are intensely stained. The cells have vacuoles in their cytoplasm, the two types of cells can easily differentiated based on staining response. The nuclei of cells are clearly visible with nucleolus. The African cat fish collected local pound near Gulbarga. In ***Clarias gariepinus*** (Fig.6) the CS of this fish are two in numbers they are embedded in the posterior region on the dorsal side of the posterior kidney. The CS are although embedded, some portion is clearly visible from outside. Each CS is invested by connective tissue. The gland is highly vascularised. Two types of cells are clearly visible with homogenous cytoplasma. Some cells are quite big with vesicular type of nucleus having large sized vacuolar and the cytoplasm is moderately eosinophilic, the other type of cells have small nucleus and vaculated cytoplasm. Some cells seem to dividing.

In the six Seawater fishes,

Rastrelliger kanagurta. **[India mackerel].** (Fig.7) the CS of this has two in number and are found embedded in the anterior region of the posterior dorsal kidney. The CS glands are quite larger in size and colored white. The connective tissue septa are enters the gland. The CS cells are arranged in cords. The gland is highly vascular. The cells are having homogenous cytoplasm with different shapes of nucleus. The nucleus in the cells round to elongated with abundant chromatin positive to hematoxylin. Morphologically all the cells look alike, some cells are different having small nucleus and sparse cytoplasm indicating two types of cells are present. However, one type of cell is predominant .In the ***Hemiramphus far*** **[Black-barred half beak]** (Fig.8) the CS are in two numbers, embedded in the anterior region of the posterior kidney on the dorsal side. The glands are small and highly vascularised. The CS cells are having has vacuolated cytoplasm. Although two different cells are found, majority of the cells belong to one type which have eosinophilic cytoplasm, vacuoles and vasicular type of nucleus. The nucleus has nuclei. In the ***Parastromateus niger*** **[Black pomfret]**. (Fig.9), the CS are two in number embedded in the middle portion of the posterior kidney on the dorsal side. The connective tissue septa invades inside the gland. The CS cells form islands, surrounded by connective tissue. The cells have homogenous cytoplasm with smaller nucleus. In some cells vacuolated cytoplasm is seen which cells are hypertrophied. Although two different cells are there, only one type of cell is predominantly noticed. In ***Sillago Sihama*** **[Silver sillago]** (Fig.10) the CS are two in number and are embedded in the middle region of the posterior kidney on the dorsal side. The colours of the CS are yellow and are larger. The CS cells are round to elongated to some cells are larger in size and some are smaller. The cytoplasm of these cells is eosinophilic. Although two types of cells are differentiated based on cytoplasm and nuclear size, majority of these cells are one type. The cells have vacuolated cytoplasm which is seen clearly in some cells. In the ***Pampus argenteus*** **[Silver pomfret] (Euphrasen).** (Fig.11) the CS of this fish are round and are small in size embedded in the anterior region of posterior kidney on the dorsal side. The glands are white in colour and can be easily located. The CS

cells are heterogenous in distribution. Small and larger sized cells are clearly differentiated. Some cells are having vesicular type of nucleus and eosinophilic cytoplasm with vacuoles. The other cells which are less in number has small nucleus. The peripheral cells of the gland are intensely eosinophilic, nucleus has nucleoli. Amongst the two types of cells, one type is prominently distributed throughout the gland. The CS of fish, ***Scoliodon laticaudus*** **[Spadenose shark]** (Fig.12) cells are two in number forming two bunches which are larger in size and cream coloured. The gland is embedded in the anterior region of posterior kidney on the dorsal side. The CS cells are having vesicular nucleus with nucleoli clearly visible. Most of the cells are similar in size and cytoplasma has vacuoles and are very active. Only one type of cell can be differentiated. The corpuscles of Stannius (CS) of freshwater and sea water fishes studied above indicates that in all the fishes pair of CS are presented and are clearly located either in the anterior, middle or posterior region of the posterior kidney on the dorsal side. The colour of the gland varies from white to yellow or cream.

Table 1: Showing phosphate and calcium concentration in freshwater and seawater.

Water	Phosphate	Calcium
Fresh water	0.018	3.9
Sea Water	0.030	85.5

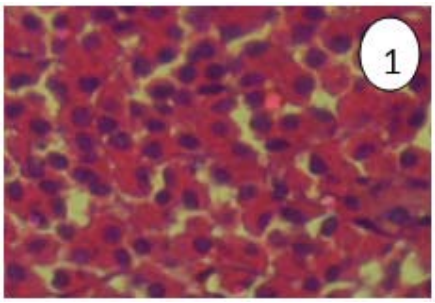

Fig 1: Section of corpuscles of Stannius in the freshwater fish, *Notopterus notopterus* H & E × 1200.

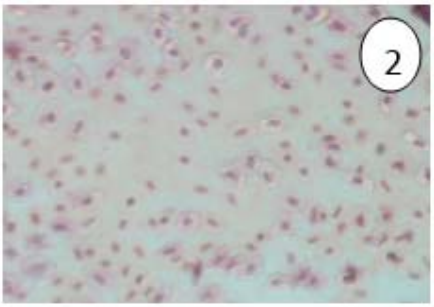

Fig 2: Section of corpuscles of Stannius in the fish, *Labeo bata* H & E × 1200.

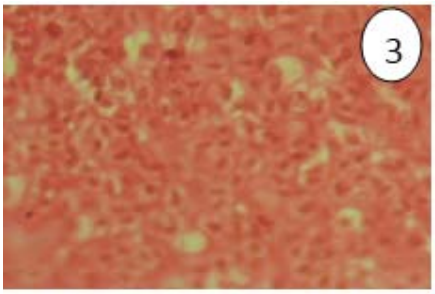

Fig 3: Section of corpuscles of Stannius in the fish, *Tilapia mossambica* H & E × 1200.

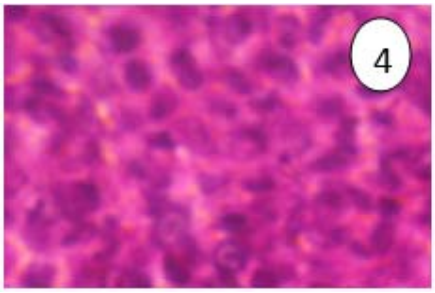

Fig 4: Section of corpuscles of Stannius in the fish, *Channa punctatus* H & E × 1200.

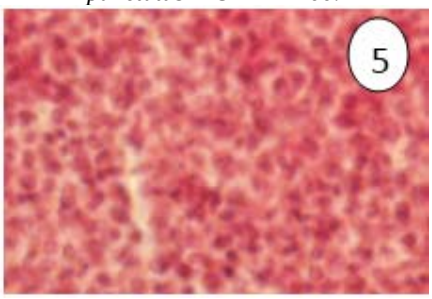

Fig 5: Section of corpuscles of Stannius in the fish, *Channa marulius*. H & E × 1200.

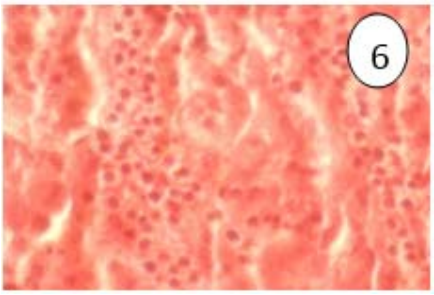

Fig 6: Section of corpuscles of Stannius in the fish, *clarias gariepinus* H & E × 1200.

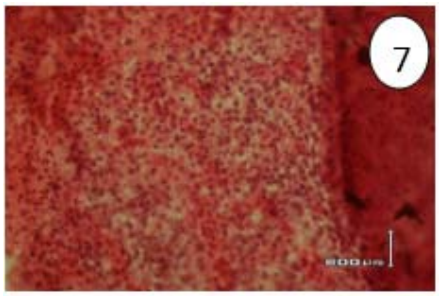

Fig 7: Section of corpuscles of Stannius in the sea water fish, *Rastrelliger kanagurta* H & E × 1200.

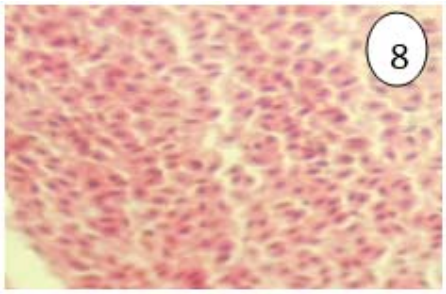

Fig 8: Section of corpuscles of Stannius in the sea water fish, *Hemiramphus far* H & E × 1200.

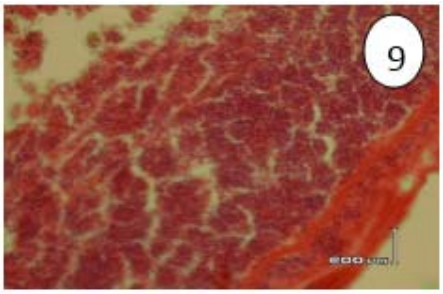

Fig 9: Section of corpuscles of Stannius in the sea water fish, *Parastromateus niger* H & E × 1200.

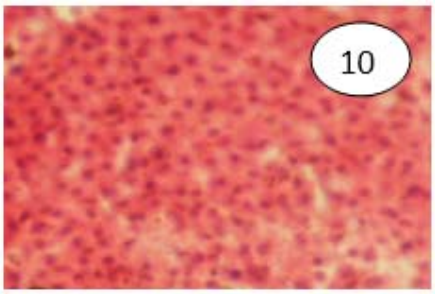

Fig 10: Section of corpuscles of Stannius in the sea water fish, *Sillago sihama* H & E × 1200.

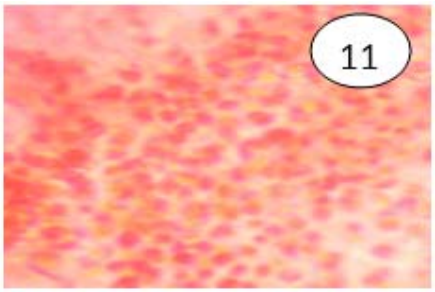

Fig 11: Section of corpuscles of Stannius in the sea water fish, *Pampus argenteus* H & E × 1200.

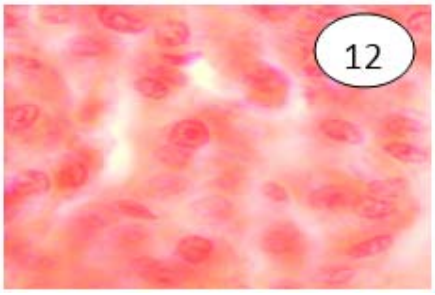

Fig 12: Section of corpuscles of Stannius in the sea water fish, *Scoliodon laticaudus* H & E × 1600.

The size of the gland compared between freshwater fish and sea water fish indicates that the fish from sea water has larger glands than the freshwater fish. This suggests that calcium concentration of sea water is higher than that of freshwater. A relation may exist between calcium concentration and CS. It is known that CS is involved in calcium regulation by secreting a hormone called stanniocalcin.

The histological structure of the CS shows that the CS cells are active in both freshwater and sea water fishes having cell cytoplasm laden with varying sizes of vacuoles and nucleolus hypertrophy. In freshwater fish the CS cells have more than one type while the CS of marine fish has large number of single type of cells recognised. The CS cells of four freshwater fishes such as *N. notopterus, C. punctatus, C. Marulius* and *C. gariepinus* are active compared to the CS cells of *L. bata* and *T. mossambicus* indicating that the fishes spending more time in the bottom are continuously exposed to more ions and the CS cells of the above four freshwater fishes have active CS cells since they are bottom feeders. Similarly in marine fishes such as *H. far, S. siama* and *P. niger* are associated with coral reefs and have migration to estuary, the concentration of Ca increases in the reefs and also changes when they migrate to estuary, the CS cells are more active than the CS cells of fish having pelagic habitat. Thus indicating calcium concentration is factor for the activity of CS in the fishes.

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 marine fish CS *R. kanagurta* is 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 increase in the calcium concentration of sea water.

5. 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 six fishes collected from freshwater and six fishes collected from sea water, the number and location of the CS has been observed and found that in all the fishes studied has a pair of CS embedded in the posterior portion of the kidney. The numbers of CS in several teleostean fishes have been studied. In *Onchorhynchus gorbuscha*-2 pairs *Oncorhynclus tshawylscha* and *Oncorhynchus kisutch* – 5-6 CS [15]. In *Salmo airdnerii* 4-6 CS *Oncohynchus kisutch* 4-5, *Atheronopsisi californiensis*, -2,3 or 4 and *Sepastodes auriasbiva* -3 CS [10]. The CS may vary sfrom 4-10 in *Salmo solar* (Heyl, 1970) and 6-8 in *Salmo trutta* [1]. In catfish *Heteroprenstes fossilis*, the CS are as many as 4 corpuscles [20]. 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 fishes studied in the present investigation a pair of CS is present in the fishes of freshwater and sea water and all these fishes are advanced fishes.

The location of CS in the teleostean kidney presents several variations and it has been suggested by [1] that it is related to the taxonomic position. In fishes such as *Carassius auratus* [16] 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* [15]. In salmonid fish *A. californiensis* the CS are situated near the middle of Mesonephros [10]. 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 [6]. In view of localization of the CS in the kidney of the fishes studied in the present investigation that other than *N. notopterus*, all other freshwater fish have the CS situated at the middle portion of the posterior kidney this is also true with the location of CS in marine fishes.

The present study is undertaken by selecting fish from freshwater and marine water under natural condition to assess the activity of CS through histological and electron microscopic study. There are quite few reports available on the histological examination of CS in the fish from freshwater and marine water showing that the CS are consistently more active in sea water adapted fish than in freshwater fish [17, 7, 24, 13]. In the fish kept in calcium deficient sea water, the CS found to be inactive [3, 21]. In our study in the fishes from the freshwater such as *N. notopterus*, *Labeo bata*, *Tilapia mossambica*, *Channa punctatus*, *Channa marulius* and *Clarias gariepinus*, the CS on histological examination revealed that the cells are moderately active having homogenous eosinophic cytoplasm and vesicular muscleus indicating the cellular activity. In the marine fishes such as *R. kanagurta*, *H. far*, *P. niger*, *S. siama*, *P. argenteur* and scoliodon the cells are though small are large in number with homogenous eosinophilic cytoplasm and very active vesicular nucleus also suggests that the CS cells are very active indicating that since these fishes live is a calcium rich environment. From this study it is important to notice that the CS cells are active in both freshwater as well as sea water, though the CS cells of sea water fish are in large number compared to freshwater fish CS cells. Therefore, CS is an important endocrine gland regulating calcium level in the blood in both marine and freshwater fish studied in the present investigation.

The observation made in the present study also indicates that the cells of corpuscles of Stannius in particular are, more active in fishes collected from sea water than freshwater.

6. Acknowledgements

Authors are very grateful to Dr. Vasantkumar .Karwar for providing facilities to conduct sea water research work.

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