CHAPTER 11

SEASONAL VARIATIONS IN THE CORPUSCLES OF STAINIUS IN **NOTOPTERUS NOTOPTERUS** (PALLAS)
The work on seasonal variations in endocrines is available mostly on the anadromous fish of salmon-ids (Wingfield and Grimm, 1977) but this approach for corpuscles of stannius in teleosts is almost nil (Subhedar and Prasada Rao, 1979; Swarup et al, 1986). However there is a report on the seasonal changes in the weight of gonads by Barr (1963a,b,c).

Previous workers (Subhedar and Prasada Rao, 1979) have also given a clue which is yet to be confirmed that corpuscles of stannius might have a special function during gonadal maturation. There are also few studies (Lopes, 1969; Subhedar and Prasada Rao, 1979) which showed that corpuscles of stannius become activated during gonadal maturation, which is very prominent in female, while others (Hiroi, 1970) considered that corpuscles of stannius can stimulate gonadal development.
This study is planned to note the seasonal variations in corpuscles of stannius during various stages of reproductive cycle of the fish, Notopterus notopterus (Pallas). An attempt has also been made to see if a correlation exists between changes in cell structure of corpuscles of stannius and the reproductive cycle of this fish.

MATERIALS AND METHODS

The fish Notopterus notopterus (Pallas) were collected locally from Sagar lake, Sagar, M.P., India, during the second week of every month for twelve consecutive months.

The mature specimens of this fish with both sexes ranging from 11 to 12 cm in length were kept in aquaria containing tap water and maintained under natural photoperiod and room temperature. They were acclimatised for one week prior to sacrifice. The fish were not fed throughout the experiment. About 10 fish were sacrificed for each month.

At the time of sacrifice, the fish were killed by a single blow on the head and kidney were dissected under binocular microscope. The portion of kidney with corpuscles of stannius were dissected and fixed in Alcoholic Bouin (24 hr), Bouin-Holland (24 hr), Carnoy's fluid (1-3 hr) and Kelly's fluid (24 hr). The tissues were dehydrated and embedded in paraffin wax after clearing in xylene. Serial sections were cut at 6 μm and those of corpuscles of stannius were stained with Hematoxylin.
eosin, Masson's Trichome (Masson, 1929), Mallory's Triple (Mallory, 1936) and Heidenhain's Azan (Curr, 1962) stain.

For the estimation of diameter of cells and the nuclei, an ocular micrometer disc (Japan) was invariably used.

HORMOMETRIC PROCEDURE

Tissues of every month were treated similarly and stained. Sections were prepared at 6 µm to study the hypertrophy of the cells and their nuclei. Sections used for counting the cells were stained with hematoxylin-eosin. The cells and nuclei were then counted to get the exact number of cells in a particular corpuscles of stannius. Total area of cells and nuclei in particular corpuscles of stannius were measured with the help of an ocular micrometer disc. The mean area for one cell and nucleus was calculated. Observations were made randomly from different sections or slides of each month. All statistics presented in this paper are mean ± standard errors.

RESULTS

Corpuscles of stannius in Otocerus totoxerus (Pallas) are round to oval and white small bodies found embedded on the kidneys. Only one corpuscles of stannius is observed throughout
the year except in the months of August, October, December and February (post-spawning phase) when two corpuscles of stannius are noted.

The corpuscles of stannius observed by light microscopy appear as a structure encapsulated by fibrous connective tissue sheath. It is a richly vascularised and glandular structure. The connective tissue penetrates the corpuscles of stannius dividing the parenchyma into lobes and lobules. Degree of penetration of connective tissue shows structural patterns in the corpuscles of stannius. In Nototerrus notorterus (Pallas), blood capillaries and nerve fibres enter the corpuscles of stannius along with the connective tissue. The lobes of corpuscles of stannius are irregular in shape. A central cavity between the lobes of corpuscles of stannius can be seen.

**Cells of the Corpuscles of Stannius**

The cells of corpuscles of stannius are spherical in shape, their cytoplasm contain secretory granules with prominent nuclei. A multinucleate condition in the cells has also been observed during enhanced secretory activity.

Vincent (1938), Nadkarni and Gorbman (1966), Krishnamurthy and Bern (1969), Lopez (1969) and Songa (1975) have also observed two types of cells in corpuscles of stannius according to their specific staining (Aldehyde-fuchsin (AF) and periodic Acid Schiff (PAS)) positive granules while the other type is devoid of any stainable granules. Recently Lopez (1984) has
reported three type of cells in corpuscles of stannius in eel.

The seasonal variations in corpuscles of stannius for a complete year are divided into three important stages of the reproductive cycle which are mentioned as follows:

1. **Pre-spawning Period (April-May)** (Fig. 5-8)

   During this period corpuscles of stannius are found enlarged, oval or elongated in shape. The connective tissue penetrates well in the corpuscles of stannius. Initial stage of this period is marked by the loss of cellular mass. Cells are immensely hypertrophied with enlarged nuclei. Cytoplasm is granular and deeply stained. Central canal and vascular supply is also visible.

2. **Spawning Period (June-July)** (Fig. 9-12)

   The corpuscles of stannius at this phase of reproductive cycle are comparatively smaller in size than the pre-spawning stage. Lobulation is incomplete. Cells are smaller with distinct nuclei, present in the centre of the cell. Cytoplasm is clear and agranular. Rich blood supply is also marked. In some cases inter-lobular space is found comparatively increased.

3. **Post-spawning Period (August-Onward)**

   The period spreads between eight months which is the longest in reproductive phase and as such divided into two consecutive stages depending on the size of corpuscles of stannius and the structure of the cells.
(1) **Initial Stage (August to November) (Fig. 13-19)**

During this stage a gradual reduction in size of corpuscles of stannius is visible which remained small till the end of November. Lobes are irregular. Cytoplasm is clear with very small nuclei. Interlobular space starts coming close together and make corpuscles of stannius more compact. Nucleoli are also visible and a limited number of granules are also seen in the cytoplasm.

(II) **Advanced Stage (December to March) (Fig. 20-23)**

This stage gradually advances to pre-spawning period and as such corpuscles of stannius are observed as active during this phase. The size of corpuscles of stannius starts gradually increasing thereby pointing a correlation between the size of corpuscles of stannius and the gonads at post-spawning period. A gradual atrophy in the cell and nucleus is also observed from August onwards while the cytoplasm is clear with moderate granulation which also follows the same patterns as marked in cells and nuclei. Lobes are clear and compact. Cells of corpuscles of stannius are enlarged than spawning phase. They are also active than the initial stage. Cytoplasm is clear and higher granular than the initial stage. Nuclei are well marked. Nucleoli are also visible. High vascularization is noted at this stage.

At this phase, two corpuscles of stannius are present only in female fish. The one is normal in size while the other
is very small (Fig. 26, 27, 28). Central canal is also visible.

**OBSERVATIONS**

(1) **Effect on Diameter of Cells of Corpuscles of Stannius**:  

The mean area for 30 cells of corpuscles of stannius comes to:

- Pre-spawning period = 5.62 ± .13 \(\mu\)m  
- Spawning period = 4.90 ± .14 \(\mu\)m

- Post-spawning Initial stage = 4.10 ± .09 \(\mu\)m  
- Advance stage = 4.82 ± .12 \(\mu\)m

(2) **Effect on Nuclear Size in Cells of Corpuscles of Stannius**:  

The mean area of 30 nuclei of corpuscles of stannius comes to:

- Pre-spawning period = 2.99 ± .08 \(\mu\)m  
- Spawning period = 2.27 ± .07 \(\mu\)m

- Post-spawning Initial stage = 2.51 ± .09 \(\mu\)m  
- Advance stage = 2.64 ± .09 \(\mu\)m

**DISCUSSION**

Hiroi (1970) has observed that corpuscles of stannius can stimulate gonadal development. Activation of corpuscles
of stannius occurs in female fish only during sexual maturation
due to stimulation of the type one cell. As such, changes in
the size of corpuscles of stannius are closely related to the
ovarian cycle (Bonga, 1963) and hypertrophy in corpuscles of
stannius also coincides with the size of gonads which are also
found increased at this phase of reproductive cycle. This also
agrees to the contentions of Hiroi (1970) and Bonga et al (1975).

Our study shows the hypertrophy of corpuscles of stannius,
when the cells of corpuscles of stannius are highly active in
pre-spawning period. During this period animals are sexually
mature. Some workers (Pang, 1973; Bjornsson, 1969; Bonga, 1963)
have suggested that female fish develop hypercalcemia during
sexual maturation, due to increased estrogen secretion by ovary.
The high state of corpuscles of stannius activity at this phase
also points to the probable hypercalcemia in the fish of our
experiments.

During the pre-spawning period, there is a clear hyper-
trophy in the cells and nuclei which indicates the stimulation
of these cells and presumably results in an enhanced production
of the hormones. The large cells with hypertrophied nuclei
alongwith the high cytoplasmic granulation are considered as a
reflection of high state of activity.

Many workers (Lopez et al, 1968; Oguri, 1973; Peignoux-
Deville et al, 1975) have observed the increased activity of
ultimobranchial gland during the late stage of the female
gonadal maturation. These findings suggest a role of calcitonin
in sexual maturation of female teleosts. In sexually matured female the level of plasma calcium is found increased (Bromage, Whitman and Breton, 1982) which is in coincidence with an enhanced ultimobranchial gland activity and also the higher level of calcitonin and plasma calcium during this period. This also indicates a direct role of calcitonin in calcium regulation (Oguri, 1973; Peignous-Deville et al., 1975).

However, it has been shown that the increase in total plasma calcium is due to the appearance of calcium containing yolk protein, vitellogenin in plasma. This yolk protein is synthesized in liver, secreted to the blood and transported to ovaries whereas the free plasma calcium levels are not affected (Bailey, 1957; Bjornsson and Haux, 1985). Thus it is not obvious that a direct relationship between plasma calcium and calcitonin exists during this period. Hypercalcaemia due to vitellogenin can be induced in both mature or immature males and females by estradiol injections (Bjornsson and Haux, 1985) or by the injection of hypophysial extract (Peignous-Deville et al., 1975; Yamauchi et al., 1977b). Estrogen is involved in gonadal development. Vitellogenin synthesis can also be induced in juvenile fish by administration of estradiol but since vitellogenin cannot be secluded by the oocytes, large amount accumulates in the blood. Concomitantly total plasma calcium increases as vitellogenin specially binds calcium in order to solubilise this large protein (Follett and Redshaw, 1974). However the binding of calcium to vitellogenin will affect the
calcium balance of fish, as calcium is mobilized from the endogenous calcium store (Oishi et al, 1972; Lopes et al, 1976; Mugniya and Watabe, 1977) or from the environment (Fleming et al, 1964). Recent study (Scott and Sumpter, 1983) has suggested that there is a feedback regulation between calcitonin and other hormones (gonadotropin and estrogen). Deftos (1983) and Catherwood et al (1983) have also supported the view that estrogen stimulates calcitonin secretion in mammals.

During the spawning period the corpuscles of stannius show atrophy in the cells and nuclei. Deftos et al (1974), Watt et al (1975), Yamauchi et al (1978b) have reported that plasma calcitonin level during spawning period in female is higher than in male.

The plasma calcitonin changes followed closely the changes in gonadosomatic index. Calcitonin level is found increased and reaches to its peak prior to ovulation and then falls sharply towards normal after ovulation. The atrophy of corpuscles of stannius along with its cells and nuclei during spawning also points towards the maximum increase in plasma calcitonin level which checks calcium level at one hand and also makes a coordination with gonadotropin and estrogen by a feedback mechanism (Scott and Sumpter, 1983).

During the late post-spawning period corpuscles of stannius become active and showed high physiological activity. Between this period cell and nuclear hypertrophy are well marked along with the granular cytoplasm and rich vascularization which
also indicate the progressive activity of the corpuscles of stannius in *Notopterus notopterus* (Pallas).

The interesting point to note during this period is the presence of two corpuscles of stannius only in female fish. Such sexual difference in the number of corpuscles of stannius is also marked in Chilean cling fish *Sicyases sanguineus* where two corpuscles of stannius are reported in male and three to four in female (Galligallardo et al., 1977).

Gonadotropin and estradiol contents fall in post-spawning period, when the gonadosomatic index increases. It is partly due to continued vitellogenesis (Barr, 1963a) and then the final period of vitellogenesis is independent of both high pituitary gonadotropin content and an enhanced plasma estradiol level.

Decrease in female estradiol and male testosterone levels follow closely the drop in pituitary gonadotropin activity from January to March in plaice, *Pleuronectes platessa* L. (Barr and Hobson, 1964).

Previous workers (Donaldson and Fagerlund, 1969, 1970) have observed the relations between salmonid reproduction and interrenal function. The high cortisol concentration is found to be a characteristic of the plasma in migratory and spawning salmon which was the result of increased secretion of interrenal tissue due to impaired clearance of hormone from the blood. Donaldson and Fagerlund (1968) have shown the rates of cortisol secretion and metabolic clearance before, during and
after spawning in salmon. This measurement indicates that cortisol secretion was higher in spawned fish than in mature salmon. Studies on the sexual maturation in *Oncorhynchus nerka* which results in an enhancement in the rate of cortisol secretion can be correlated with interrenal hyperplasia and cytological evidences of increased activity (Donaldson and Fagerlund, 1970; McBridge and Van Overbeeke, 1969). High cortisol secretion during maturation is a result of the high plasma level of sex hormones (Barr and Hobson, 1964).

By the above description, it can also be concluded that not only the timing of gonadal development and spawning coincides in plaice from Iris sea and northerly fish from the firth of Clyde (Barr and Hobson, 1964) but the spawning also coincides with high activity and hypertrophy of the cells in corpuscles of stannius, which in turn, are affected by the high titre of the gonadal hormone and this might have also alter the plasma cortisol.

This study suggests that there are prominent and clear seasonal changes in the structure of corpuscles of stannius in *Nototenia nototertius* (Pallas) during various phases of reproductive cycle. These changes can also be correlated with the gonadal maturation in this fish and further studies are required to give more emphasis on this interesting clue.
REFERENCES


Lopes, E. 1970 : L'os cellulaire d'un poisson teleosteen (Anguilla anguilla L.) II. Action de l'ablation des corpuscles de stannius.

Lopes, E.; J. Peignoux-Deville; F. Lallier; F. Martelly and C. Hilet, 1976 : Effects of calcitonin and ultimobranchiallectomy (UBX) on calcium and bone metabolism in the eel, Anguilla anguilla L.


Mugiya, Y. and N. Watabe, 1977 : Studies on fish scale formation and resorption - II. Effect of estradiol on calcium homeostasis and skeletal tissue resorption in the goldfish, Carassius auratus and the killifish, Fundulus heteroclitus.


Wingfield, J.C. and A.S. Grimm, 1977 : Seasonal changes in plasma cortisol, testosterone and estradiol-17β in the plaice, Pleuronectes platessa L.

Yamauchi, H.; M. Orimo; K. Yamauchi; H. Takahashi, 1978b : Increased calcitonin levels during ovarian development in the eel, Anguilla japonica.

* Not seen in original.
Fig-1

EFFECT OF SEASONAL VARIATIONS ON THE DIAMETER OF THE CELLS OF CORPUSCLES OF STANNIUS.
Fig-2

Effect of seasonal variations on the diameter of the nuclei of corpuscles of Stannius.
Fig-3

EFFECT OF MONTHLY VARIATIONS ON THE DIAMETER OF THE CELLS OF CORPUSCLES OF STANNIUS.
Fig 4. EFFECT OF MONTHLY VARIATIONS ON THE DIAMETER OF THE NUCLEI OF CORPUSCLES OF STANNIUS.

Nuclear diameter in \( \mu \)
Fig. 5: Section of kidney showing corpuscles of stannius in April month. The lobes are clear.

Hematoxylin-eosin stain X 150

Abbreviations
a = Kidney
b = Corpuscles of stannius
c = Interlobular space

Fig. 6: Magnified view of Fig. 5 showing hypertrophied cells with enlarged nuclei and granular cytoplasm.

Hematoxylin-eosin stain X 900

Abbreviations

c = Hypertrophied nuclei
i = Granular cytoplasm.
Fig. 7: Section of kidney showing corpuscles of stannius in the May month. The structure is enlarged.

Hematoxylin-eosin stain \( \times 150 \)

**Abbreviations**

- \( a \) = Kidney
- \( b \) = Corpuscles of stannius

Fig. 8: Magnified view of Fig. 7 showing enlarged cells with granular cytoplasm (arrow).

Haidenhain's Azan Stain \( \times 900 \)
Fig. 9: Section of kidney showing corpuscles of stannius in June month with incomplete lobulation. Numerous interlobular spaces are also seen.

Hematoxylin-eosin stain X 150

Abbreviations
a = Kidney
b = Corpuscles of stannius
e = Interlobular space

Fig. 10: Magnified view of Fig. 9 showing small cells with clear nuclei. A gradual degranulation (arrow) in cytoplasm also occurs.

Heidenhain's Azan Stain X 900
Fig. 11  :  Section of kidney showing in the July month rich blood supply. The lobes are incomplete.

Hematoxylin-eosin stain          X 150

Abbreviations
a = Kidney
b = Corpuscles of stannius
c = Central canal
d = Blood supply

Fig. 12  :  Magnified view of Fig. 11 showing small cells with distinct nuclei. The cytoplasm is clear and agranular.

Hematoxylin-eosin stain          X 900

Abbreviations
h = Distinct nuclei
j = Agranular cytoplasm.
Fig. 13: Section of kidney showing corpuscles of stannius in August month. Interlobular spaces are reduced and lobes are irregular. Rich blood supply is also visible.

Hematoxylin-eosin stain  X 150

Abbreviations

a = Kidney
b = Corpuscles of stannius
d = Blood supply

Fig. 14: Magnified view of Fig. 13 showing variable cell with vesicular nuclei (arrow). Cytoplasm possesses limited number of granules.

Hematoxylin-eosin stain  X 900
Fig. 15 : Section of kidney showing corpuscles of stannius in September month. The lobes are irregular. Rich blood supply is also seen.

Hematoxylin-eosin stain \hspace{1cm} X 150

Abbreviations
\(a\) = Kidney
\(b\) = Corpuscles of stannius
\(d\) = Blood supply.

Fig. 16 : Magnified view of Fig. 15 showing variable cells (arrow). Cytoplasm possesses limited number of granules.

Masson's trichrome stain \hspace{1cm} X 900
Fig. 17: Section of kidney showing small corpuscles of stannius in October month. The lobes are irregular.

Hematoxylin-eosin stain x 150

Abbreviations
a = Kidney
b = Corpuscles of stannius.

Fig. 18: Magnified view of Fig. 17 showing cells with clear nuclei. The cytoplasm is very poor in granulation (arrow).

Hematoxylin-eosin stain x 900
Fig. 19: Magnified view of the corpuscles of stannius in November month showing comparatively bigger cells with distinct nuclei. The cytoplasmic granulation is also increased.

Masson's trichrome stain X 900

Abbreviations
h = Distinct nuclei
i = Granular cytoplasm

Fig. 20: Section of kidney showing corpuscles of stannius in December month. The size is still small.

Hematoxylin-eosin stain X 150

Abbreviations
a = Kidney
b = Corpuscles of stannius.
Fig. 21: Magnified view of Fig. 20 showing clear nuclei (arrow). While the cytoplasm still possesses limited number of granules.
Hematoxylin-eosin stain X 900

Fig. 22: Section of kidney showing corpuscles of stannius in January month. The size of corpuscles of stannius is gradually increased with rich blood supply.
Hematoxylin-eosin stain X 150

Abbreviations
a = Kidney
b = Corpuscles of stannius
c = Central canal
d = Blood supply
Fig. 23  
Section of kidney showing corpuscles of stannius in February months. The size of corpuscles of stannius has also increased. The lobes are compact.
Heidenhain's Azan Stain  X 150

Abbreviations
a = Kidney
b = Corpuscles of stannius

Fig. 24  
Magnified view of Fig. 23 showing enlarged cells with distinct nuclei. Nucleoli (arrow) are also visible in some cells. The cytoplasmic granules are increased.
Heidenhain's Azan Stain  X 900

Abbreviations
h = Distinct nuclei
i = Highly granular cytoplasm.
Fig. 25 : Section of kidney showing corpuscles of stannius in March month. The size is remarkably increased. Lobes are compact.

Hematoxylin-eosin stain X 150

Abbreviations

a = Kidney
b = Corpuscles of stannius.
Fig. 26: Section of the kidney showing corpuscles of stannius in February month. Two corpuscles of stannius are clearly visible.
Heidenhain's Azan Stain x 150

Fig. 27: Section of kidney showing corpuscles of stannius in March month. Two corpuscles of stannius are present.
Heidenhain's Azan Stain x 150

Fig. 28: Section of kidney showing corpuscles of stannius in November month. Two corpuscles of stannius are also present here.
Heidenhain's Azan Stain x 150

Abbreviations
a = Kidney
b = I Corpuscles of stannius
c = IIInd Corpuscles of stannius
d = Blood supply.