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Probe on the Synchronized actions of Pituitary (GTH and TSH) and Thyroid Hormones in Controlling The Seasonal Reproductive Cycle of Female Asian Striped Catfish *Mystusvittatus* (Bloch, 1794)

Nilanjana Chatterjee
Assistant Professor,
Deptt. of Zoology,
Ramananda College,
Bishnupur, Bankura,
India

Baibaswata Bhattacharjee
Associate Professor,
Deptt. of Physics,
Ramananda College,
Bishnupur, Bankura,
India

Abstract

A quantitative approach has been employed to investigate the synchronized actions of Pituitary (GTH and TSH) and Thyroid hormones in controlling the seasonal reproductive cycle of female Asian striped catfish *Mystusvittatus* (Bloch, 1794).The analysis has been done using the histometric data obtained from the histological micrographs of pituitary and thyroid tissues.Histological and histometric evidences have clearly revealed that the cells of the pituitary and thyroid glands undergo changes in their dimensions throughout the female reproductive phases. Seasonal changes of cell dimensions can be related to the rate of secretion of the GTH, TSH and thyroid hormones. Quantitative histometric study thus throws light on the synchronized actions of GTH and TSH and Thyroid hormones in controlling the seasonal reproductive cycle of female *Mystusvittatus*. Knowledge acquired about the exact time of action of the hormones would helppeople to apply them from external source for increasing the fish productivity.

Keywords: Pituitary, Thyroid, Seasonal Reproductive Cycle, *Mystusvittatus.*

Introduction

Fish can be recognized as one of the major edible protein sources used in India. Therefore, significant heighteningin the fish production is expected to provide quality protein in cheap rate to the common people, helpingthem to fight more effectively against malnutrition, which is a huge problem of India today.Keeping this in mind, the science of fish reproduction has acquired prime importance to the researchers working in this niche.

Asian striped catfish, *Mystusvittatus*, is a species of freshwater catfish, found mainly in the rivers of Indian sub-continent. This species is easy to cultivate as this fish is strong enough to survive against the hostile climate. It is an important target species for the small-scale fishermen, who use a variety of traditional fishing gear [1, 2]. This small indigenous fish species has a high nutritional value in terms of protein, micronutrients, vitamins and minerals not commonly available in other foods [3] making it a very attractive candidate for aquaculture in the South East Asia. Therefore, increased production of this fish may help to tackle the malnutrition problem in India.

The main objective of this present research is to study the correlation between the secretions of the pituitary (GTH and TSH) and the thyroid glands of female *Mystusvittatus* during different reproductive phases. Hence, the present work attempts to fill the lacunae of the absence of a strong correlation between the exact time of secretion from the pituitary-thyroid axis during the seasonal reproductive cycle in teleost fish, employing histological and histometrical techniques. So, the present work targets at increasing the production of the fish by knowing the exact time of action of the pituitary and thyroid hormones.

Review of Literature

Effect of pituitary and thyroid glands in isolation on reproductive cycles of teleosts has been studied by several groups [4-15]. Reports are available on the seasonal effect of thyroid hormone on testis of *Daniorerio* [4], ovary of *Puntiussarana* [7], ovary [11] and testis [12] of *Mystusvittatus*, testis of *Tenualosailisa* [15].Histological changes in pituitary gonadotrophs in relation to the seasonal changes in fish reproductive cycles has been studied in detail in *Notopterusnotopterus* [8], *Liza parsia* [9], *Mystusvittatus* [10], *Oreochromismossambicus* [13], *Channastriatus* [14]. On the other hand, significantly less amount of work [16] has been found that deals with the combined effect of pituitary and thyroid glands on seasonal reproductive cycle of fish.

Most of the works in the field of fish endocrinology are based on the measurement of hormonal concentration in blood plasma, which is neither the site of synthesis nor the site of action of the respective hormones.Therefore, thedata thus acquired without periodic continuous assay may not portray the actual information which depends on the varied metabolic states of the target specimen. The uniqueness of the present work is that,here the actions of the hormones are assessedthrough the dimensions of the site of their synthesis (secretory cells), obtained from direct measurements.

Materials and methods

Fish Husbandry

Asian Striped catfish specimens were collected in each month during the period of September, 2013 to August, 2017from local fishermen. After collection, matured female fishes were separated and kept in watertight containers containing tap water that has been allowed to stand for a few days. A necessary supply of oxygen was provided by using a bubbler in regular intervals. Fishes were maintained at 25°- 30°C of temperature to ensure the natural environment. The fishes were fed on natural fish foods. Small, regular supplies of food were provided. Specimens were sacrificed in each month to study the seasonal variations of the pituitary and thyroid tissue layout.

Histology and Histometry

To study the seasonal variation of pituitary and thyroid histology, pituitary and thyroid tissues were dissected out and cut into small pieces for preservation in Bouin's fixative for 18 hours. The detailed histological techniques were reported elsewhere [10, 12].The histological sections were observed with a compound light microscope at different desired magnifications (400 X, 600 X) and photographed with a digital camera for identification of seasonal changes in pituitary and thyroid tissue layout.

Seasonal variation of diameters of Thyroid Stimulating Hormone secretory cells (δ_{TSH}) and Gonadotropic Hormone secretory cells (δ_{GTH}) in pituitary glandand the seasonal variation of diameters of Thyroid Stimulating Hormone secretory cells (δ_{TSH}) and heights of Thyroid Epithelial Cells (h_{TEC}) in thyroid gland were measured with reticulomicrometer and ocularmicrometer attached to the compound light microscope with a resolution of 0.04 μm.

Statistical Analysis

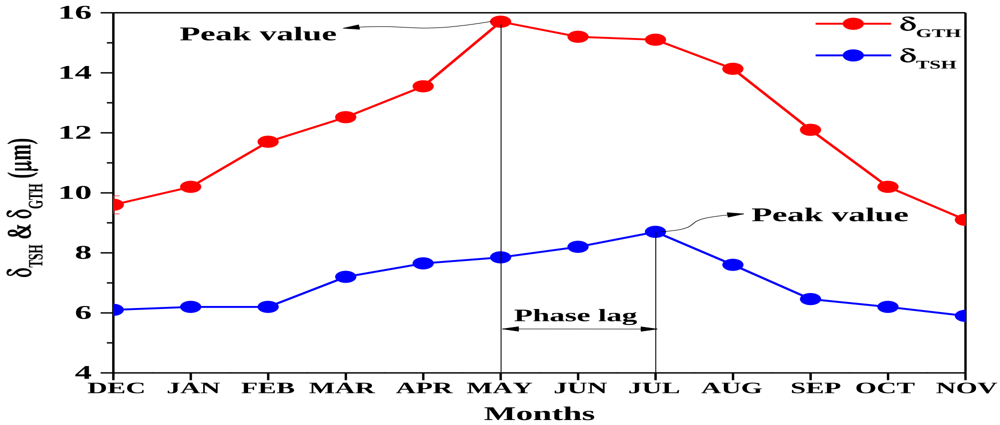
All data were expressed as means ± SE. Pearson's correlation coefficients (r) were calculated to determine the correlation, if any, between different experimental parameters at a significance level of 5%. Negative r values prefixed by negative (-) sign and positive values without any prefix are used in the manuscript. Calculation of the differential coefficients and Curve fitting to the experimentally obtained data were done using the software Origin 9.

Results and Discussions

Intra Pituitary Correlation

Histological micrographs showed prominent seasonal alterations of pituitary [10] and thyroid [11, 12] tissuesin *Mystusvittatus*and has been discussed in detail therein [10-12]. Figure 1 shows the seasonal variation of diameters of Thyroid Stimulating Hormone secretory cells (δ_{TSH}) and Gonadotropic Hormone secretory cells (δ_{GTH}) in pituitary gland of *Mystusvittatus* measured from histological micrographs. The figure divulges similar qualitative types of seasonal variation of the sizes of these two types of cells though the numerical values of δ_{GTH} are found to be always higher than that of δ_{TSH}. Calculation of Pearson's correlation coefficient at significance level of 0.05 reveals that there is a strong positive correlation (r = + 0.937) between the seasonal variation of $\delta_{TSH}$and δ_{GTH}. A comparison between the seasonal variations of the sizes of these two types of cells has been done using paired student t test at the significance level of 0.05. It can be inferred from the test the seasonal variation of the $\delta_{GTH}$are significantly different and sturdier than that of the δ_{TSH}.

Figure 1: Seasonal variation of δ_{TSH} and δ_{GTH} in pituitary gland of *Mystusvittatus*

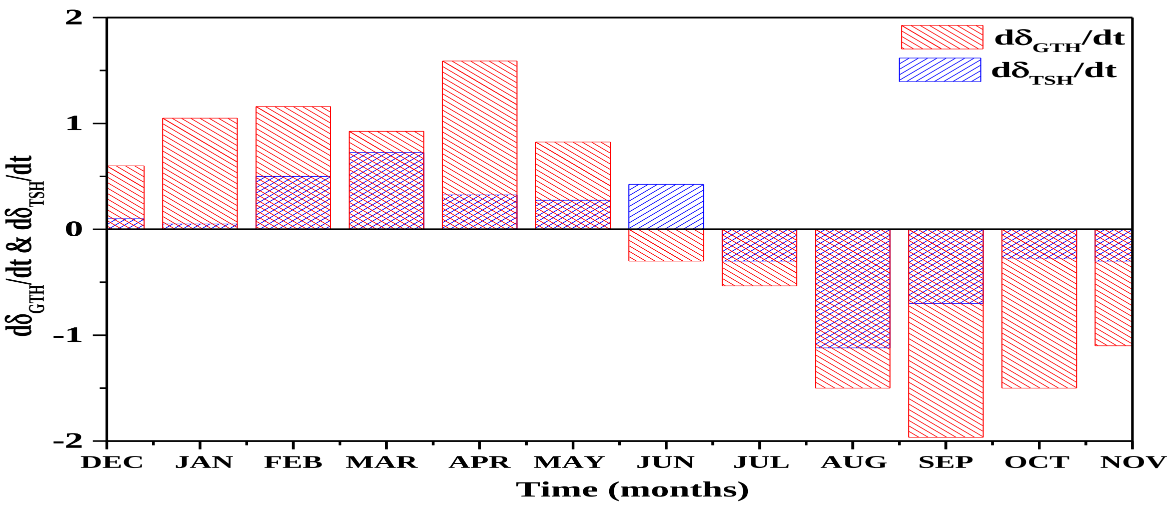


A careful inspection of figure 1 discloses the fact that there is a clear phase difference between the seasonal variation of $\delta_{TSH}$and δ_{GTH}. The peak value of $\delta_{GTH}$is found to reach in the month of May, while the peak value of $\delta_{TSH}$is found to reach during the month of July. The nature of seasonal variation of δ_{TSH}andδ_{GTH} can be interpreted more conspicuously by calculating the first order differential coefficients of these parameters with respect to time, which is shown in figure 2.

Figure 2 depicts that during the months of December to May, the values of both the coefficients are positive indicating increase in $\delta_{TSH}$and δ_{GTH} with time in this tenure. During July to November, the values of both the coefficients are found to be negative indicating decrease in $\delta_{TSH}$and δ_{GTH} with time in this tenure. It can be also noticed that the absolute value of$\frac{d\delta_{GTH}}{dt}$is always higher than the value of $\frac{d\delta_{TSH}}{dt}$ in these stretches of time demonstrating greater activity of δ_{GTH} with respect to the seasonal changes. An interesting case arises during the month of June when the value of $\frac{d\delta_{TSH}}{dt}$ is found to be positive while $\frac{d\delta_{GTH}}{dt}$ shows its negative value. This observation indicates the fact that during the month of June δ_{GTH}already starts decreasing after reaching its peak in May, while $\delta_{TSH}$is still increasing that makes it to reach its peak in July as shown in figure 1.

In teleosts, the GTH and TSH cell sizes are increased with accumulation of their secretory products in the cytosol and gradually decrease with discharge of its secretory products [14, 16]. Therefore, the secretory activity of GTH and TSH cells can be treated as somewhat proportional to the secretory cell sizes. Consequently, the secretory cell sizes can be used conveniently as an indirect measure of the secretory activity of GTH and TSH cells. Theobservations portrayedin figures 1 and 2 can thus be interpreted by the fact that the accumulation of the secretory products in the GTH cells reach its peak during May while that is in the TSH cells happens during July.The rate of cellular accumulation of secretory products is found to be highest for GTH cells during April and that is during March for the TSH cells. On the other hand, the cellular discharge rate is found to be maximizing during September for GTH cells and during August for TSH cells.

Figure 2: Seasonal Variation of the First Order Differential Coefficient of δ_{TSH}($\frac{d\delta_{TSH}}{dt}$) and δ_{GTH}($\frac{d\delta_{GTH}}{dt}$) with Respect To Time



The histometric data of the experiment (Figure 1) shows that during the growth phase (December-February), the δ_{TSH}values remain practically constant indicating less activity of these cells during this phase, whileδ_{GTH} values start increasing indicating their gradually increasing impact on gonads even in the growth phase. In the onset of the maturation phase (March –May), both the cell sizes are found to increase but δ_{GTH} shows much steeper slope (figure 2) when compared to theδ_{TSH}. This observation reveals superior control of GTH cells on gonad maturation overTSH cells during the maturation phase. After the spawning phase is reached, the GTH cell activity is found to decrease while the TSH cells remain in a higher stage of activity until July, when mid of the spawning phase is reached. As the secretions fromboth GTH and TSH cells acts simultaneously during the maturation and spawning phases of the fish [13], it can be inferred from the above study that the onset of the gonad maturation is activated by the secretion from the GTH cells during the commencement of the maturation phase while the secretion from TSH cells acts upon the gonads via thyroid and starts acting on gonads after a phase lag from that of GTH secretion. The TSH secretion helps to maintain the spawning phase, though the gonad maturation is triggered by the GTH secretion.The fact that the secretions from the TSH cells also influence the secretions from the GTH cells cannot be underestimated, as with the onset of the spawning phase when the GTH activity experiences a decline the TSH activity still continues till a major portion of the viable gametes are released

Hypothetically, in case of absence of the action of the GTH cells, the growth of the ovarian and testicular cells might not be significantly affected as the TSH cells are held to be almost equally responsible for this phase. However, the maturation phase of the ovary or the vitellogenesis and the repeated meiotic divisions of the testicular cells might not be possible, since the GTH secretions reach their maxima at this time and perhaps work in concert with the TSH secretions. However, strong research at the molecular level is required for the firm establishment of such conclusions.

Pituitary-Thyroid interrelation

Figure 3 shows the seasonal variation of diameters of Thyroid Stimulating Hormone secretory cells (δ_{TSH}) and heights of Thyroid Epithelial Cells (h_{TEC}) in *M. vittatus*measured from histological micrographs. The figure shows similar qualitative types of seasonal variation of the sizes of these two types of cells though the numerical values of h_{TFC} are found to be always greater than that of δ_{TSH}. Calculation of Pearson's correlation coefficient at significance level of 0.05 reveals that there is a positive correlation (r = + 0.575), though not much significant, between the seasonal variation of $h_{TFC}$and δ_{TSH}. Figure 3 also discloses the fact that there is a clear phase difference between the seasonal variation ofh_{TFC}andδ_{TSH}. The peak value of δ_{TSH} is found to reach in the month of July, while the peak value of h_{TEC} is found to reach during the month of August.

The nature of seasonal variation of $h_{TEC}$and δ_{TSH}can be interpreted more clearly by calculating the first order differential coefficients of these parameters with respect to time, which is shown in figure 4.Figure 4 depicts that during the months of December to June, the values of both the coefficients are positive indicating increase in $h_{TEC}$and $\delta_{TSH}$with time in this tenure. During September to November, the values of both the coefficients are found to be negative indicating decrease in $h_{TEC}$and$\delta_{TSH}$with time in this tenure. It can also benoticed that the absolute value of$\frac{dh_{TEC}}{dt}$is always higher than the value of $\frac{d\delta_{TSH}}{dt}$ in these stretches of time demonstrating greater sensitivity of $h_{TEC}$with respect to the seasonal changes.An interesting case arises during the months of July andAugust when the values of$\frac{dh_{TEC}}{dt}$ are found to be positive while $\frac{d\delta_{TSH}}{dt}$showed its negative values. This observation indicates the fact that during the month of Augustδ_{TSH}already starts decreasing after reaching its peak in July, whileh_{TEC} is still increasing that makes it to reach its peak in September as shown in figure 3.The TSH cells acts directly on the thyroid follicles and are believed to affect the rate of colloid synthesis from the thyroid epithelial cells. The maximum activity of the thyroid epithelials cells have been observed during the peak spawning period (August).So, there is a clear phase lag between synthetic activity of the TSH of the pituitary and the colloid synthesis from the epithelial cells (assuming that the height of the thyroid epithelial cells corresponds to the secretory activity of the cells). This is probably because the former synthesis products acts on the latter after a phase lag or "action time".

The secretion from the thyroid epithelial cells are first incorporated and stored temporarily in the thyroidal lumen in the form of acidophilic colloid. Hence, we notice a rise in the size of the diameter of the follicular cells with respect to that of the ECH in *M. vittatus*. This trendcontinues until a maximum follicular size is attained. It has already been established by many workers [4, 11-12] that the thyroid gland influences the reproductive behaviour of the teleosts. However, the direct and indirect control of the pituitary GTH and TSH respectively have been established through this research and the possible route through which the thyroid is influenced and finally acts upon the gonads have also been studied histometrically in *Mystusvittatus*, unlike any other siluroid.

Figure 3: Seasonal variation of δ_{TSH} and h_{TEC} in pituitary gland of *Mystusvittatus*

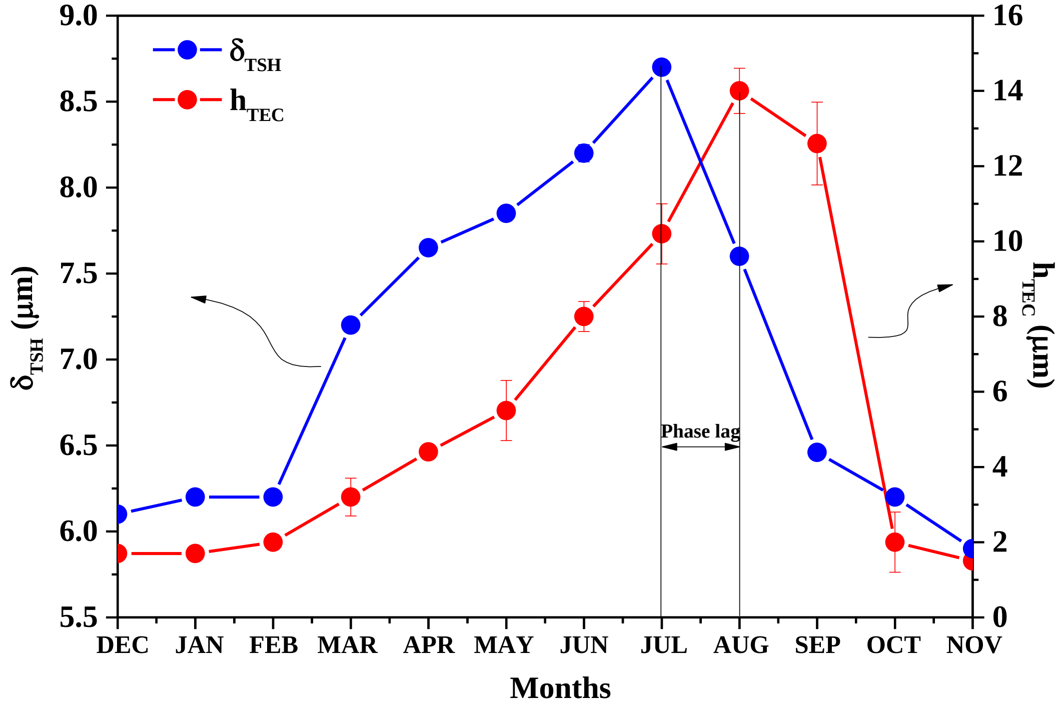
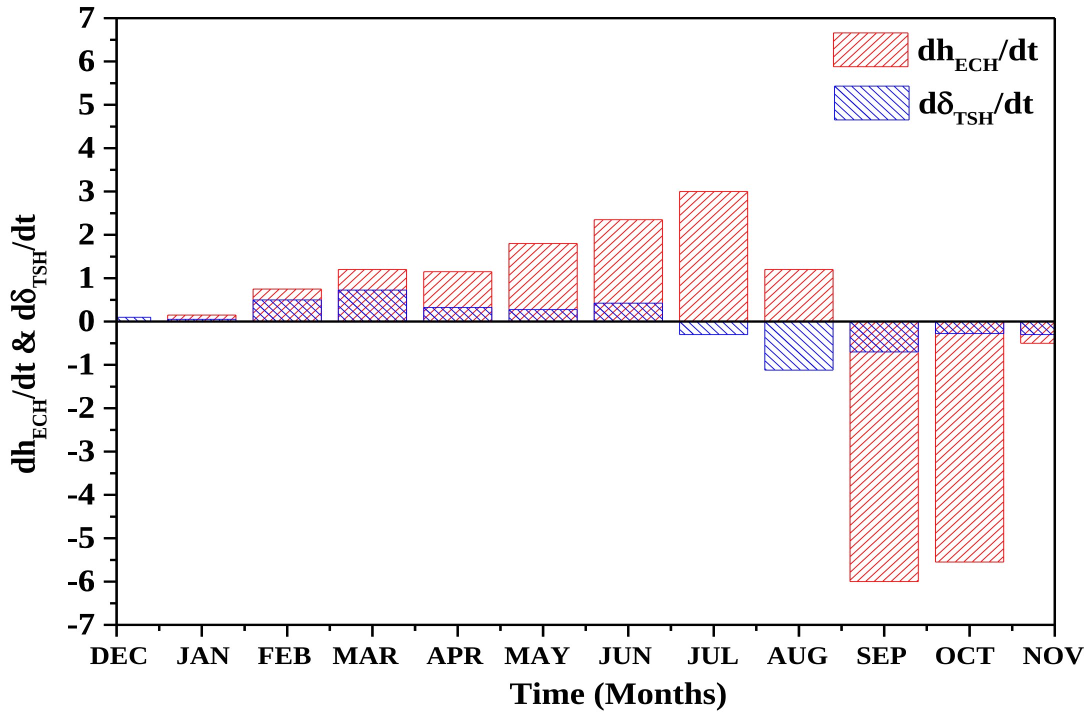


Figure 4: Seasonal Variation of the First Order Differential Coefficient of h_{TEC}($\frac{dh_{TEC}}{dt}$) and δ_{TSH} ($\frac{d\delta_{TSH}}{dt}$) with respect to Time



Intra Thyroid Correlation

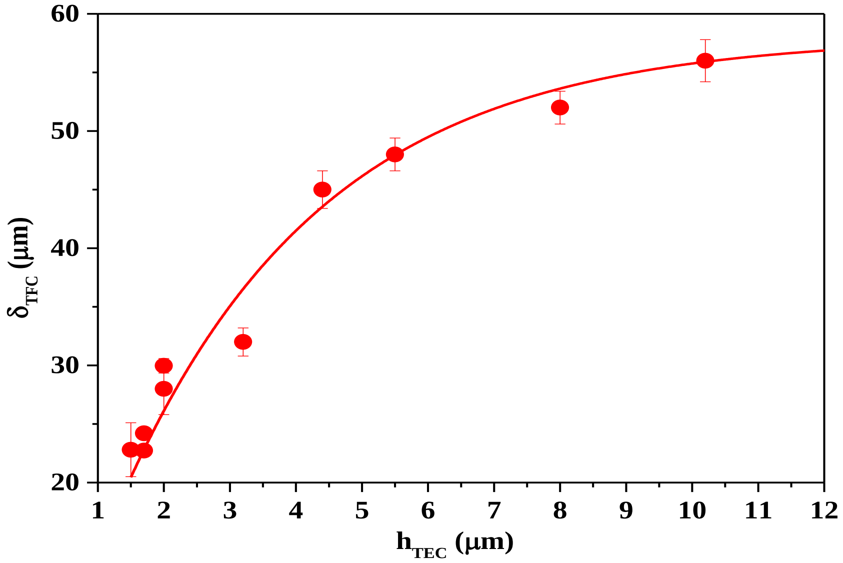
Figure 5 shows variation of the sizes of Thyroid Follicular Cells (δ_{TFC}) as a function of the heights of Thyroid Epithelial Cells (h_{TEC}) in female*Mystusvittatus*. Calculation of Pearson's correlation coefficient at significance level of 0.05 reveals that there is a strong positive correlation (r = + 0.928) between $h_{TFC}$and δ_{TSH}.

The figure shows initial steep rise of δ_{TFC} with increasing h_{TEC} up to h_{TEC} value reaches nearly 6 µm. Beyond this value of h_{TEC} a saturation effect can be observed in δ_{TFC} values. Therefore a specific value of h_{TEC} can be identified for which saturation effect in δ_{TFC} starts in a particular trial. The data in figure 5 are fitted well with the Boltzmann function depicted by the equation

$$\delta_{TFC} = a_2 + \frac{(a_1 - a_2)}{1+e^{\frac{(h_{TEC} - h_{TEC0})}{dh_{TEC}}}} \quad (1)$$

Where a_1, a_2 are constants for a particular experimental condition and h_{TEC0} corresponds to the specific value of h_{TEC} for which saturation effect in δ_{TFC} starts in a particular trial. For the present study the fitting parameters are obtained as a_1= -36.57 ± 3.65 µm, a_2=58.04 ± 3.504µm, h_{TEC0}=5.6 ± 0.87 µmand dh_{TEC}= 1.26 ± 0.54 µm.

Figure 5: Variation of δ_{TFC}as a function of h_{TEC} with corresponding theoretically fitted curve



Thyroid Epitelial Cells fill the lumen of the thyroid follicles by their colloidal secretions increasing the sizes of Thyroid Follicular Cells. An increase in h_{TEC} values indicates their enhanced activity as mentioned earlier. Therefore an increase in δ_{TFC} values can be associated with increasing h_{TEC} values. Initially the rate of increase of δ_{TFC} is higher and gradually the rate decreases leading to a near saturation effect, though the δ_{TFC} values keep increasing throughout the experimental tenure. In the present case, the saturation value of δ_{TFC} (~ 48 µm) can be corresponded to h_{TEC0} (5.6 ± 0.87 µm) from the theoretical fitting of the histometric data. As the δ_{TFC} increases, their activity increases and their secretion flows to the target organ through blood.

Conclusions

Histological and histometric evidences show that the cells of the pituitary and thyroid glands undergo changes in their dimensions throughout the female reproductive phases of *Mystusvittatus*.The TSH cells and the thyroid follicular cells show their activity throughout the growth and maturation phase and reach their climax or peak in the late spawning phase on the other hand the GTH cells of the pituitary reach its peak in the late maturation phase.A clear phase lag is observed between the peak of GTH and TSH cells of the pituitary indicating their specific role in the maturation of gonadal cells and the release of mature ovums respectively.In case of the thyroid gland, the height of the EC and the FC shows that there is a phase lag between the synthesis of the colloidal material and their release into the blood stream.Knowledge acquired about the exact time of action of the hormones from this study would facilitate their administration from external source to increase the fish productivity.

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