Effect of Seasonal Temperature Changes on Thyroid Structure and Hormones Secretion of White Grouper (*Epinephelus Aeneus*) in Suez Gulf, Egypt

Hossam H. Abbas[1], Mohammad M. Authman[1], Mona S. Zaki[1] and Gamal F. Mohamed[2]

[1] Hydrobiology Department, National Research Centre, Cairo, Egypt
[2] Food Processing Department, National Research Centre, Cairo, Egypt

Abstract: The thyroid is the largest and one of the phylogenetically oldest endocrine glands in vertebrate species. It is the first endocrine structure to become recognizable during an animal's development. Although the thyroid gland is structurally conserved in all vertebrate species, exhibiting a similar follicular structure and function. Seasonal temperature changes on the thyroid gland structure and hormones secretion was examined in white grouper; *Epinephelus aeneus* in Seuz Gulf, Egypt. 60 male of white grouper; *E. aeneus* (138.5±6.05 g) were netted from Suez Gulf during a year from July 2008 to June 2009. Water temperature and salinity were ranging from 12 to 34°C and 39 to 40 ppt during cold and warm seasons, respectively. Blood samples were collected from the caudal vein for thyroid hormones analysis. Samples of *E. aeneus* were dissected to expose the internal organs, histological examination and measuring the cell height of the thyroid epithelium. Thyroid gland composed of follicles scattered around the ventral aorta, near the gills. Follicular cells varied according to secretion of the gland during warm and cold seasons. Thyroid hormones [Triidothyronine (T_3) and Thyroxin (T_4)] were detected in the fish serum in levels ranged from 1.28-4.08 ng/ml for T_3 and 0.22-1.11 ng/ml for (T_4) in the warm and cold seasons, respectively. The results showed that the height of thyroid epithelium and plasma concentration of thyroid hormones (thyroid activity) in *Epinephelus aeneus* increased significantly during spring and summer. The peak of these factors occurred in midsummer (August). Then, the thyroid activity decreased significantly during autumn and early winter from October to December according to the decreasing of temperature. T_3 and T_4 increased significantly from January to April.
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Key Words: White grouper, *Epinephelus aeneus*, Thyroid Gland, Triidothyronine,Thyroxin, Histology, Serum.

1. Introduction

The thyroid is the largest and one of the phylogenetically oldest endocrine glands in vertebrate species **(Dickhoff and Darling, 1983)**. It is the first endocrine structure to become recognizable during an animal's development. Although the thyroid gland is structurally conserved in all vertebrate species, exhibiting a similar follicular structure and function, there are some gross morphological differences among species, and the responses of this structure to environmental influences are also differ across the phylum **(Rupik, 2011)**.

Thyroid hormones (THs) include triiodothyronine (T_3) and thyroxin (T_4); are essential for regulating normal growth, development, differentiation, metabolism, and maintenance of normal physiological functions (e.g., homeostasis) in vertebrates **(Szisch *et al.*, 2005; Zoeller *et al.*, 2007; Schnitzler *et al.*, 2012)**. In all vertebrates embryogenesis, organogenesis and growth acutely depend on thyroid hormones **(Power *et al.*, 2001)**.

In fish, thyroid hormones are involved in the control of osmoregulation, metabolism, somatic growth and post-hatching metamorphosis **(Power *et al.*, 2001; Yamano, 2005; Schnitzler *et al.*, 2012)**. Although there is an extensive diversity in teleosts, developmental stages in most of them include larva, juvenile, and adult, which appear to regulate by THs **(Wright and Alves, 2001)**. As it seems, thyroid hormones (THs) involve in many physiological processes in teleosts. It has been suggested that photoperiod, temperature, and food intake may play species specific role in regulation of seasonal thyroid cycles **(Comeau *et al.*, 2000)**, and these seasonal changes may act to promote growth, migratory activity, and reproductive development **(Leatherland, 1994)**. It has been found that the changes of thyroid gland depend on species or population and are sensitive to food intake and diet composition models **(MacKenzie, 1998)**.

Groupers of the genus *Epinephelus* are widely distributed throughout the tropical and subtropical waters of the world. They are commercially important and highly regarded as a favorite marine food fish. The groupers possess excellent biological characteristics: they are fast-growing and disease resistant **(Yeh *et al.*, 2003)**. As no detailed study has been carried out on the thyroid patterns of *E. aeneus*, in Suez Gulf, Egypt, the present study was conducted on annual changes of the morphometric structure and hormones secretion of thyroid gland, triidothyronine (T_3) and thyroxin (T_4) in *E. aeneus*, in two seasons (cold and warm).

2. Material and Methods

Sampling:

60 male of white grouper; *E. aeneus* (138.5±6.05 g) were netted from Suez Gulf during a year from July 2008 to June 2009. Water temperature and salinity were ranging from 12 to 34°C and 39 to 40 ppt during cold and warm seasons, respectively.

Blood sampling:

Blood samples were collected from the caudal vein by a syringe with a little saturated solution of sodium citrate to prevent blood coagulation. The blood samples were kept on ice for up to 30 min and then, serum was separated using centrifuge (3000 rpm for 15 minutes) and frozen at -20°C for thyroid hormones analysis.

Thyroid gland histology:

E. aeneus was dissected to expose the internal organs and the jaws were cut at the corners to expose pharyngeal region. All tissues between the gills were fixed in Bouin's fixative for 72 hrs and then stored in 70% ethanol. Tissues were dehydrated using an ethanol series and embedded in paraffin **(Biswas *et al.*, 2006)**. Samples were then sectioned at 5-6µm and were stained with hematoxylin and eosin (H&E) for basic histological analyses. The cell height of the thyroid epithelium was measured under an Olympus microscope with a camera Lucida attachment according to **Halasz and Martin, 1985** in a total of 15 follicles per fish. Measurements were made at four points within each follicle at 90° from one another and reported as the mean ± SEM.

Thyroid hormones analysis

Triiodothyronine (T_3):

The gamma coat [^{125} I] T_3 Radioimmunoassay kit purchased from DiaSorin, Stillwater, Minnesota, USA was used for the quantitative determination of triiodothyronine (T_3) level in serum as previously described by **Van der Geyten *et al.* (2001)**.

Thyroxin (T_4):

The gamma coat [^{125} I] total T_4 Radioimmunoassay kit purchased from DiaSorin, Stillwater, Minnesota, and USA was applied for the determination of total thyroxin (T_4) levels in serum as previously described by **Van der Geyten *et al.* (2001)**.

Statistical analysis:

All values of thyroid hormone levels were represented as means±SE. The significant difference between warm and cold season values was analyzed using the t-test (Software Program of Statistical Analysis, **SPSS, 2008**).

3. Results

White grouper (*Epinephelus aeneus*) fish from Suez Gulf, Egypt is shown in figure (1) and inactive thyroid structure is shown in Photomicrograph (1) showing lobules, follicles and interlobular connective tissues of the thyroid gland.

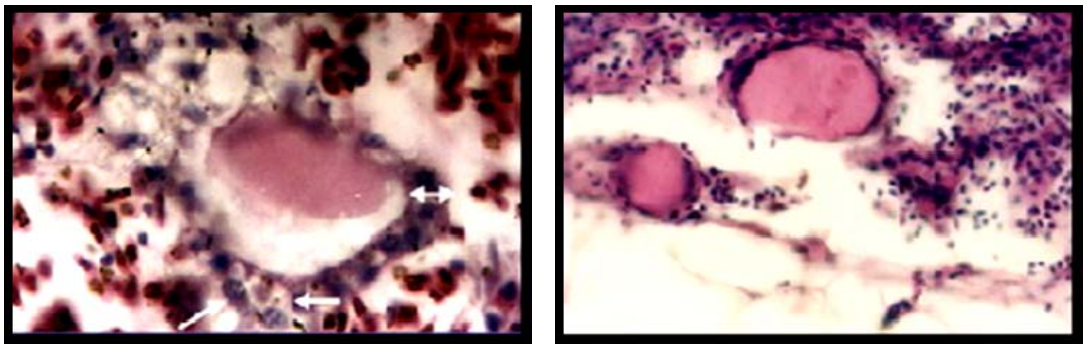
Structure of thyroid tissue:

The obtained results showed that the thyroid gland of *E. aeneus*, such as other teleosts is not capsulated. It was composed of follicles, which scattered throughout the pharyngeal region along with the dorsal surface of ventral aorta and bronchial arteries near the gills. The follicles were round and their walls were consisted of epithelial cells, include follicular cells and a few parafollicular cells, surrounding the central lumen full of colloid fluid. The epithelial cells were cuboidal to squamous during warm and cold seasons, respectively. The mean water temperature of Suez Gulf and the mean epithelial cell height for fishes during a year is shown in Table 1.

The results showed that there is 20% correlation between epithelial cell height and water temperature. Follicular epithelial cells had maximum height in August, then their height significantly decreased to January, after which it slowly increased throughout the winter (P<0.05). Fish thyroid gland was characterized by predominance of macrofollicles rich in colloid material during warm months (especially July to August) (Photomicrograph 1), whereas in cold months (especially October to December) thyroid gland showed some microfollicles with less colloid content and more interstitial connective tissue (Photomicrograph 1). There was a significant increase in ratio of parenchyma to stroma in summer in comparison with winter (P<0.05).

Seasonal changes of serum triidothyronine (T_3) and thyroxin (T_4): The results obtained with the RIA method are shown in Table 2. This method confirms that the serum level of T_3 and T_4 increased significantly from January to April, and again from April to June. This level was maintained up in summer and the peak of them in serum occurs during August (4.08±0.33 and 1.11±0.02 ng mL^{-1}, respectively), then declining significantly during autumn and early winter from October to December (P<0.05) to reach their lowest level in November (1.28±0.28 and 0.22±0.04 ng mL^{-1}, respectively). Both hormones varied similarly across seasons and there was 99% correlation (at the level of 0.01) between two hormones. The increasing of T_3 and T_4 were correlated with increase of temperature (98 and 82%, respectively) and with the height of thyroid epithelial cell.

Figure 1: Showing the white grouper (*Epinephelus aeneus*) from Suez Gulf, Egypt



Photomicrograph 1: Inactive thyroid follicle in December (left); Active thyroid follicle in August (right), Parafollicular cells (white arrows) and follicular epithelial height (two head arrows) (H&E 400X)

Table 1: Changes in heights of thyroid epithelial cells (Mean ± SE) according to the changes of water temperature during a year.

	Months											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Water Temperature (°C)	12	14	19	25	28	31	33	34	32	24	21	15
Epithelial Height (µm)	1.75 ±0.7	1.95 ±0.6	2.25 ±0.4	2.50 ±0.5	2.80 ±0.8	3.30 ±0.7	3.62 ±0.6	3.71 ±0.4	3.41 ±0.4	2.51 ±0.5	1.58 ±0.8	1.37 ±0.4

-Data are represented as mean ± SE Y= 0.0491x + 2.8568 R^2 =0.0472

Table 2: Seasonal variations of the thyroid hormone concentrations (ng/ml) during a year in the White Grouper (Mean ± SE).

	Months											
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Water Temperature (°C)	12	14	19	25	28	31	33	34	32	24	21	15
T_3	2.57 ±0.23	2.77 ±0.30	2.94 ±0.31	3.38 ±0.21	3.51 ±0.33	3.62 ±0.25	3.96 ±0.21	4.08 ±0.33	3.78 ±0.18	1.44 ±0.19	1.28 ±0.28	1.64 ±0.26
T_4	0.62 ±0.05	0.73 ±0.03	0.79 ±0.06	0.81 ±0.02	0.86 ±0.08	0.87 ±0.04	1.04 ±0.02	1.11 ±0.02	0.96 ±0.03	0.35 ±0.04	0.22 ±0.04	0.37 ±0.03

-Data are represented as mean±SE Y= -0.0003x + 0.0018 R^2 =0.0018

4. Discussion

The synthesis of thyroid hormones (THs) occurs in the thyroid follicle, a single layer of epithelial cells enclosing a colloid-filled space and thyroxin (L-T_4) is the predominant hormone secreted. T_4 has few direct actions and is considered to act principally as a precursor for triiodothyronine (T_3), the biologically active form of the hormone **(Power *et al.*, 2001)**. The conversion of T_4 to T_3 occurs in the peripheral tissue by the enzymatic removal (5-monodeiodination of one of the iodide units of the outer ring of T_4. THs circulate in serum bound to thyroid hormone-binding proteins that include, albumin, transthyretin (TTR) and thyroxin-binding globulin in vertebrates **(Power *et al.*, 2000)**.

The present study showed that, thyroid gland of *E. aeneus* is not compact organ and is found in the subpharyngeal region, such as other teleosts. However, in some species thyroid follicles are found in heart, head kidney and kidney**.**

According to micrometric data, thyroid follicular cells of *E. aeneus* vary in size in cold and warm seasons. Also in Atlantic stingray, *Dasyatis Sabina*, follicular cells vary in size and shape, according to the activity of the gland **(Volkoff *et al.*, 1999)**. The surrounding epithelial cells are flattened, cuboidal, or columnar, depending on their activity. Tall, columnar epithelial cells with basophilic colloid containing vacuole-like spaces, characteristics of an active thyroid gland, were seen in warm season. In *Solea senegalensis*, thyroid represented colloid-filled follicles surrounded by a cuboidal epithelium during summer, suggesting a high activity state of this organ **(Ortiz-Delgado *et al.*, 2006)**.

Although seasonal cycle of thyroid hormones have been observed in numerous fish species, but the seasonal changes in thyroid hormones in *E. aeneus* have not been studied. Circulating thyroid hormone concentrations represent just one component of the multilevel control of target tissue metabolism by the hypothalamic-pituitary-thyroid axis. In the present study, significant monthly changes were observed in circulating levels of thyroid hormones in *E. aeneus* during a year. Thyroid hormones are a component of a large complex network of responses to a number of environmental and physiological factors, many of which also influence growth, development, and metabolism **(Hadley, 2000)**. They are involved in the regulation of energy management, functioning primarily to help control basal metabolic rate by regulating lipid metabolism **(Hadley, 2000)**.

Levels of thyroid hormones can be influenced by many factors including age, gender, diet, nutritional status, season and physiological condition **(Rolland, 2000; Schnitzler *et al.*, 2012)**. Stimuli such as the lunar cycle, rainfall, turbid water, temperature shock, chemicals, water quality, and swimming activity induce an increase in serum thyroid hormones concentration **(Iwata *et al.*, 2003)**. **Swift (1960)** suggested that the seasonal changes in thyroidal activity in many teleosts are regulated primarily by water temperature. This relationship of glandular activity and water temperature is interpreted as further evidence that the basic function of the thyroid is concerned in the control of the animal's metabolism, to compensate for changes in the environmental temperature. Thus the release of thyrotropic hormone from the pituitary would seem to be influenced by the environmental temperature. Serum levels of thyroid hormones were sensitive to temperature in starved eels *Anguilla anguilla* L. **(Leloup and De Luze, 1985)** and also in trout fed specific diets **(Latherland *et al.*, 1980)**. In the present study, mean serum T_3 and T_4 showed similar seasonal changes patterns. Both hormones decreased significantly during autumn and early winter from October to December according to decrease of temperatures, feed consumption and somatic growth.

In general, fasting and food restriction decrease both T_3 and T_4 levels in most animals **(Janan *et al.*, 1995)**. **Loter *et al.* (2007)** also reported minimum thyroid hormones in cold months. T_3 and T_4 increased significantly from January to April, and again from April to July. Thyroid activity increase in the winter corresponds with intermediate temperatures and feed consumption during rapid reproductive development and spawning period of *E. aeneus*.

E. aeneus spawns during late winter and early spring **(Abou-Seedo *et al.*, 2003)**. In normal diploid catfish, *Heteropneustes fossilis*, a general inverse relationship between thyroid hormone levels and advanced reproductive state has been observed **(Cyr *et al.*, 1988)**, which suggested involvement of thyroid hormones in reproductive maturity. **Weber *et al.* (1992)** found that accumulation of thyroid hormones into oocytes of tilapia, *Oreochromis mossambicus*, was against its concentration gradient, which could be a reason for depletion of thyroid in serum of normal diploid female specimens during the spawning period.

Increase of T_3 and T_4 serum concentrations in spring coincides with increasing ambient temperature but the results of the present study showed that the peak activity occurs during midsummer when temperature increase precipitously from July to September with elevating of feed consumption and somatic growth. These requirements vary seasonally in a poikilothermic animal such as a fish, increasing with the rising temperature of the water in summer and decreasing in winter **(Swift, 1955)**. **Loter *et al.* (2007)** reported that increased both T_4 substrate availability (higher serum T_4 levels) and increased temperature would lead to much greater enzyme activity and T_3 production in summer. In summary, the activities of the hepatic thyroid hormones deiodination pathways appear to be regulated to provide a much greater availability of T_3 in summer, when fish are eating and

growing most actively, than in winter **(Loter *et al.*, 2007)**. Decreased food consumption during cold season may depress thyroid hormone cycles in many fishes. The seasonal trend is consistent with the hypothesis that thyroid hormone production is activated during periods of increased nutrient assimilation **(MacKenzie *et al.*, 1998)**.

Conclusion

All together, high magnitude seasonal changes of thyroid hormones in *E. aeneus* suggest that this species provides an excellent opportunity to examine the relative contributions of the generation mechanisms of dynamic cycles in circulating thyroid hormone levels. This study was designed to determine basal concentrations of thyroid hormone in *E. aeneus*, utilizing assays which have been validated for this species. The relationships between these hormones and food deprivation, reproductive state, other circulating hormones, immunoglobulins and contaminants can now be identified by further investigations.

Correspondence author name
Hossam H. Abbas
Hydrobiology Department, National Research Centre, Cairo, Egypt
h3abbas@yahoo.com

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