World Journal of Fish and Marine Sciences 4 (1): 37-41, 2012
ISSN 2078-4589

DOI: 10.5829/idosi.wjfms.2012.04.01.6169

Seasonal Changes of Morphometric Structure and Plasma Hormone Levels of Thyroid Gland in Persian Gulf Yellowfin Seabream (*Acanthopagrus latus*)

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Abstract: Seasonal changes of the thyroid gland structure and hormones secretion was examined in yellowfin seabream *Acanthopagrus latus* in Persian Gulf (Zangi branch, Musa creek). 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 [T3] and Thyroxine [T4]) were detected in the fish serum in levels ranged from 4.09-1.3 ng/mL for T3 and 1.1-0.21 ng/mL for (T4) 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 *Acanthopagrus latus* 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 decreasing of temperature. T3 and T4 increased significantly from January to April.

Key words: Yellowfin Seabream % Acanthopagrus Latus % Thyroid Gland % Triidothyronine % Thyroxine % Histology % Plasma

INTRODUCTION

Thyroid hormones (THs) include of triiodothyronine (T3) and thyroxine (T4), regulate growth, development, differentiation, metabolism and maintenance of homeostasis in vertebrates [1]. In all vertebrates embryogenesis, organogenesis and growth acutely depend on thyroid hormones [2]. 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 [3]. 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 [4] and these seasonal changes may act to promote growth, migratory activity and reproductive development [5]. 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 [6]. *Acanthopagrus latus* is a valuable seabream, which has distributed off Indo-West Pacific included Persian Gulf to Australia. It is also recorded from the Gulf of Tadjourah, Djibouti [7]. As no detailed study has been carried out on the thyroid patterns of *A. latus*, in Persian Gulf (Zangi branch, Musa creek), the present study was conducted on annual changes of the morphometric structure of thyroid gland and the secretion of thyroid hormones, triidothyronine (T3) and thyroxine (T4) in *A. latus*, in two cold and warm seasons.

MATERIALS AND METHODS

Sampling Conditions: 60 male yellow seabream, *A. latus* (138.5±6.05 g) were netted from Persian Gulf (Musa creek, Zangi branch) during a year from July 2007 to June 2008. Water temperature and salinity were ranging from 11 to 34°C and 40 to 43 ppt during cold and warm seasons, respectively.

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Blood Sampling: Fish were euthanized with MS222 (200 ppm/100 liter basin). Blood samples were then collected from the caudal vasculature in heparinized syringes (to prevent blood coagulation and the separation of serum, although serum also can be used). The blood samples were kept on ice for up to 30 min and then, plasma was separated using centrifuge and frozen at -20°C for further thyroid hormones analysis.

Thyroid Gland Histology: *A. latus* were 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 h and then stored in 70% ethanol. Tissues were dehydrated using an ethanol series and embedded in paraffin [8]. Samples were then sectioned at 5-6µm and were stained with hematoxylin and eosin (H&E) and PAS for basic histological analyses. The cell height of the thyroid epithelium was measured 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: Radioimmunoassays (RIAs) were performed using T3 and T4 RIA kits (Immunotech, Beckman Culture Company, France) and gamacounter to determine T3 and T4 levels in plasma, as previously described by Van der Geyten *et al*. [9] and their concentrations were computed in ng/mL plasma and were expressed as means ± SEM [9]. The T3 RIA had an intra assay variability of 2.2% and an inter assay variability of 9.6%. The T4 RIA had an intra assay variability of 2.8% and an inter assay variability of 11.0%. For the T3 RIA cross-reactivity with T4 was 0.1-0.5%, whereas for the T4 RIA cross-reactivity with T3 was 3.5%. For both RIA systems, plasma dilution tests and loading tests showed good parallelism with the standard curve.

Statistical Analysis: All values of thyroid hormone levels and the height of follicles epithelium were represented as means ± SEM. The significant difference between warm and cold season values was analyzed using the t and u test.

RESULTS

Structure of Thyroid Tissue: The results showed that the thyroid gland of *A. latus*, 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 Musa creek, Zangi branch and the mean epithelial cell height for fishes during a year is shown in Figure 1.

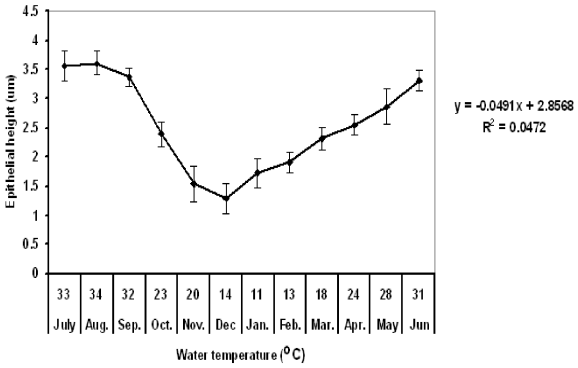

Fig. 1: Changes in heights of thyroid epithelial cells (Mean ± SEM) according to the changes of water temperature during a year.

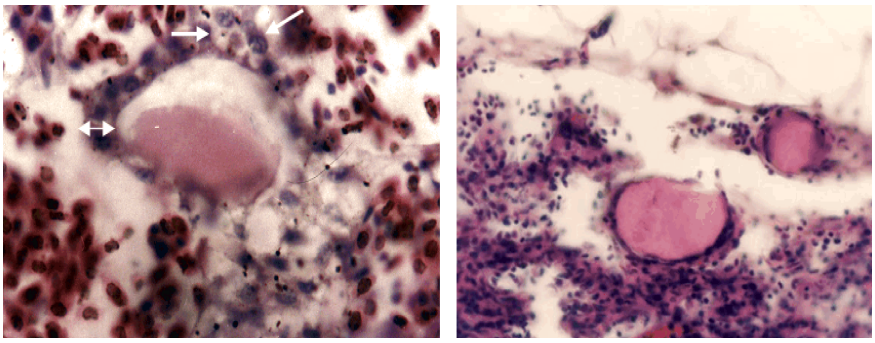

Fig. 2: Active thyroid follicle in August (left), Parafollicular cells (white arrows), follicular epithelial height (two head arrows); Inactive thyroid follicle in December (right)

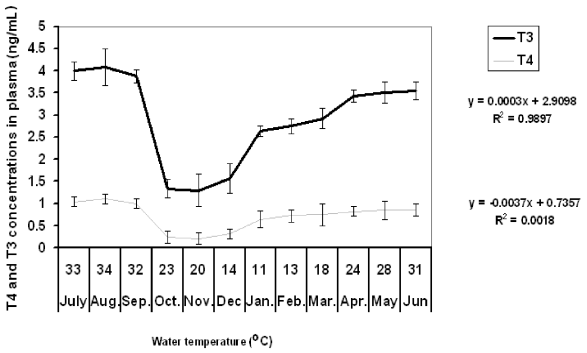

Fig. 3: Seasonal variations of the thyroid hormones plasma concentrations during the year in the yellowfin seabream. Each point represents Mean ± SEM.

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) (Figure 2), whereas in cold months (especially October to December) thyroid gland showed some microfollicles with less colloid content and more interstitial connective tissue (Figure 2). There was a significant increase in ratio of parenchyma to stroma in summer in comparison with winter ($P<0.05$).

Seasonal Changes of Plasma Triidothyronine (T3) and Thyroxine (T4): The results recorded with the RIA method are shown in Figure 3. This method confirms that the plasma level of T3 and T4 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 plasma occurs during August (4.09±0.41 and 1.1±0.11 ng/mL, respectively), then declining significantly during autumn and early winter from October to December ($P<0.05$) to reach their lowest level in November (1.3±0.36 and 0.21±0.12 ng/mL, respectively). Both hormones varied similarly across seasons and there was 99% correlation (at the level of 0.01) between two hormones. The increasing of T3 and T4 were correlated with increase of temperature (98 and 42%, respectively) and with the height of thyroid epithelial cell (84 and 82%, respectively).

DISCUSSION

The present study showed that, thyroid gland of *A. latus* 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 *A. latus* 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 [10]. 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 [11].

Although seasonal cycle of thyroid hormones have been observed in numerous fish species, but the seasonal changes in thyroid hormones in *A. latus* 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 *A. latus* 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 [12]. They are involved in the regulation of energy management, functioning primarily to help control basal metabolic rate by regulating lipid metabolism [12]. Stimuli such as the lunar cycle, rainfall, turbid water, temperature shock, chemicals, water quality and swimming activity induce an increase in plasma thyroid hormones concentration [13].

Swift [14] suggested that the seasonal changes in thyroidal activity in many teleosts are regulated primarily by water temperature [14]. 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. Plasma levels of thyroid hormones were sensitive to temperature in starved eels *Anguilla anguilla* L. [15] and also in trout fed specific diets [16]. In the present study, mean plasma T3 and T4 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 T3 and T4 levels in most animals [17]. Loter, *et al*. [18] also reported minimum thyroid hormones in cold months [18]. T3 and T4 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 *A. latus*. *A. latus* spawns during late winter and early spring [19]. In normal diploid catfish, *Heteropneustes fossilis*, a general inverse relationship between thyroid hormone levels and advanced reproductive state has been observed [20], which suggested involvement of thyroid hormones in reproductive maturity. In other study, accumulation of thyroid hormones into oocytes of tilapia, *Oreochromis mossambicus*, was against its concentration gradient, which could be a reason of depletion of thyroid in plasma of normal diploid female specimens during the spawning period [21].

Increase of T3 and T4 plasma 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 [22]. Loter *et al*. [18] reported that both increased T4 substrate availability (higher plasma T4 levels) and increased temperature would lead to much greater enzyme activity and T3 production in summer [18]. In summary, the activities of the hepatic thyroid hormones deiodination pathways appear to be regulated to provide a much greater availability of T3 in summer, when fish are eating and growing most actively, than in winter [18]. 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 [6].

CONCLUSION

All together, high magnitude seasonal changes of thyroid hormones in *A. latus* 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 *A. latus*, 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 analysis.

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