

## Seasonal change of thyroid histomorphological structure and hormone production in yellowfin seabream (*Acanthopagrus latus*) in the Persian Gulf

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Received: May 2012 Accepted: September 2012

### Abstract

Seasonal changes of the thyroid gland structure and hormones secretion was examined in yellowfin seabream (*Acanthopagrus latus*) in the northwest of Persian Gulf (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 (Triiodothyronine [T3] and Thyroxine [T4]) were detected in the fish serum in levels ranged from 4.09-1.30 ng/mL for T3 and from 1.10-0.21 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 *A. 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 decrease of temperature. T3 and T4 increased significantly from January to April.

**Keywords:** Yellowfin seabream, *Acanthopagrus latus*, Thyroid gland, Triiodothyronine, Thyroxine, Histology, Plasma

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## Introduction

Thyroid hormones (THs) include of triiodothyronine (T3) and thyroxine (T4), regulate growth, development, differentiation, metabolism, and maintenance of homeostasis in vertebrates (Szischa et al., 2005). In all vertebrates embryogenesis, organogenesis and growth acutely depend on thyroid hormones (Power et al., 2001). Although there is an extensive diversity in teleosts, developmental stages in most of them include of 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 teleost fishes. 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). 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 et al., 1998). *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 (By et al., 2003). As no detailed study has been carried out on the thyroid patterns of *A. latus*, in the Persian Gulf (Zangi branch, Musa creek), the present study was conducted on changes of the morphometric structure of thyroid gland and the secretion of thyroid hormones, T3 and T4 in *A. latus*, in two cold and warm seasons.

## Materials and methods

### *Sampling conditions*

The number of 60 male (all yellowfin seabreams are protandrous namely are male in the first age of their life and then will become females) with weight of  $138.5 \pm 6.05$  g were collected from the northwest Persian Gulf (Musa creek) during a year from July 2007 to June 2008. The water temperature and salinity were ranging from 11 to 34°C and 40 to 43 ppt during cold and warm seasons, respectively.

### *Blood sampling*

Fish were euthanized with MS222 (200 ppm/100 L). Blood samples (2 cc) 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 in ice for up to 30 min and then, plasma was separated using centrifuge (1000 rpm in 10 minutes) and frozen at -20°C for further thyroid hormones analysis.

### *Thyroid gland histology*

The *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 (Biswas et al., 2006). The samples were then sectioned at 5-6µm and were stained with hematoxylin and eosin (H&E) and PAS for further 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.

#### Thyroid hormones analysis

The 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. (2001). Their concentrations were computed in ng mL<sup>-1</sup> plasma. 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 ± SE. The significant

difference between warm and cold season values was analyzed using t test. Also correlation was taken between thyroid epithelial cell height and water temperature (Confidence level of 0.05).

## 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, scattered throughout the pharyngeal region along with the dorsal surface of ventral aorta and bronchial arteries. 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 and squamous during warm and cold seasons, respectively. The mean water temperature of Musa creek, Zangi branch and the mean epithelial cell height for fishes (*A. latus*) during a year is shown in Figure 1.

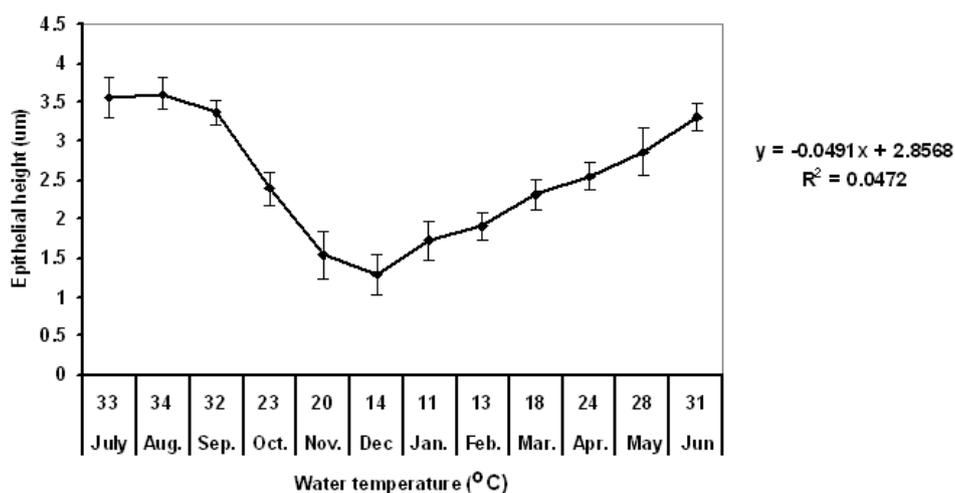
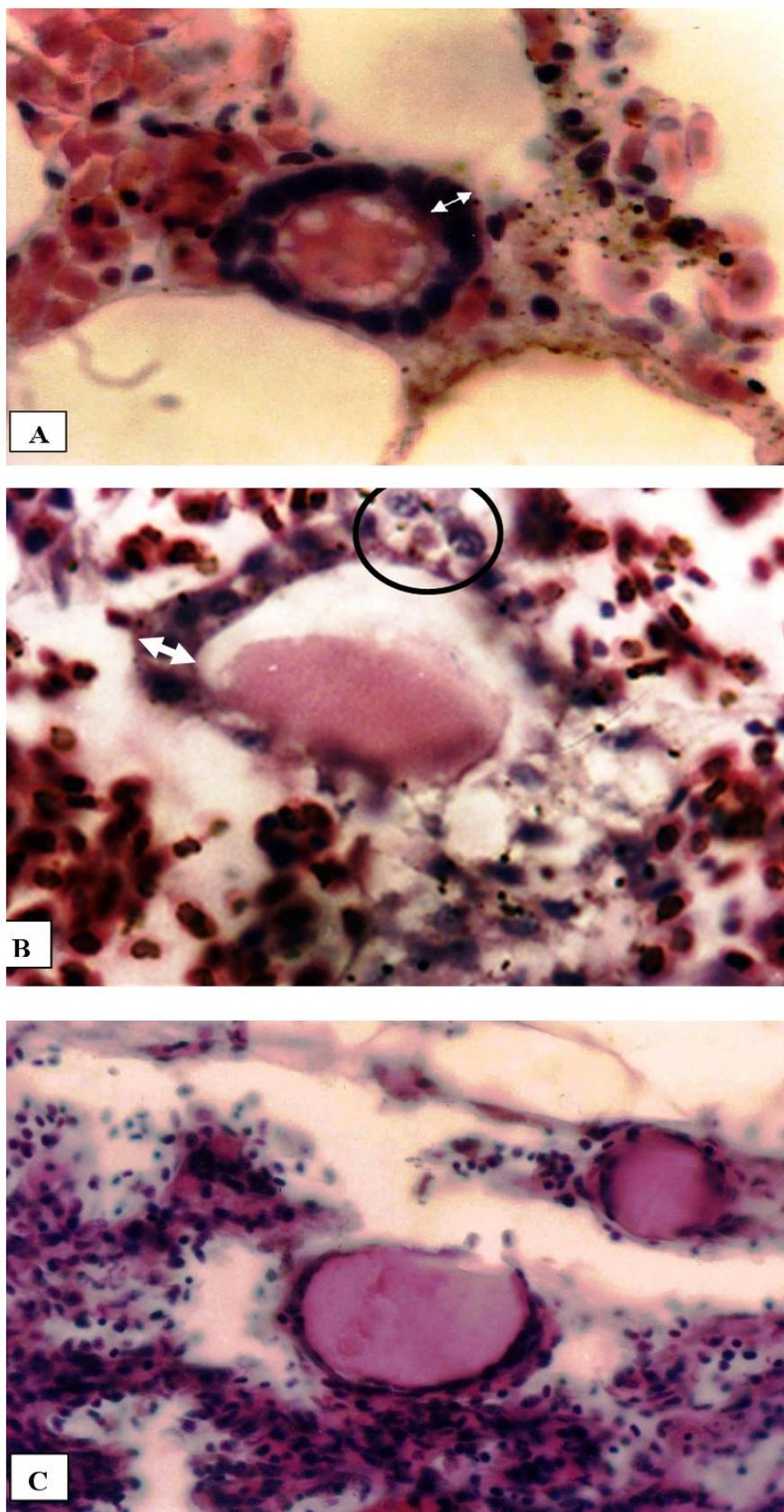


Figure 1: Changes of thyroid epithelial cell heights (mean ± SE) according to water temperature changes during a year in *Acanthopagrus latus*



**Figure 2: Active thyroid follicle in August (A and B), Parafollicular cells (black circle), follicular epithelial height (two head white arrows); Inactive thyroid follicle in December (C). (H&E; ×40)**

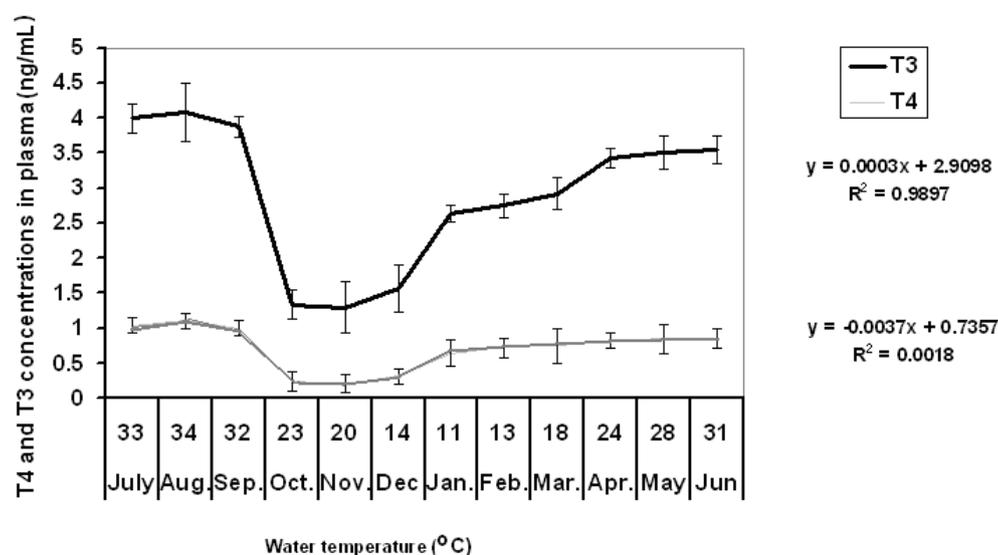
The results showed that there is 20% correlation between epithelial cell height and water temperature. Follicular epithelial cells had maximum height in August, then their height significantly decreased to January, after which it slowly increased throughout the winter ( $P < 0.05$ ). Fish thyroid gland was characterized by predominance of macrofollicles rich in colloid material during warm months (especially July to August) (Fig. 2A and B), whereas in cold months (especially October to December) thyroid gland showed some microfollicles with less colloid content and more interstitial connective tissue (Fig. 2C). There was a significant increase in ratio of parenchyma to stroma in summer in comparison with winter ( $P < 0.05$ ).

#### *Seasonal change of plasma T3 and T4*

The results recorded with the RIA method are shown in Fig. 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 ( $4.09 \pm 0.41$  and  $1.10 \pm 0.11$  ng mL<sup>-1</sup>, respectively), then declining significantly during autumn and early winter from October to December ( $P < 0.05$ ) to reach their lowest level in November ( $1.30 \pm 0.36$  and  $0.21 \pm 0.12$  ng mL<sup>-1</sup>, 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).

**Table 1: Seasonal variations of the T3 and T4 plasma concentrations during the year in the yellowfin seabream (mean  $\pm$  SE).**

	T3	T4
July	4.00 $\pm$ 0.20	1.04 $\pm$ 0.11
Aug.	4.09 $\pm$ 0.41	1.10 $\pm$ 0.11
Sep.	3.87 $\pm$ 0.15	1.00 $\pm$ 0.10
Oct.	1.33 $\pm$ 0.21	0.24 $\pm$ 0.13
Nov.	1.30 $\pm$ 0.36	0.21 $\pm$ 0.12
Dec.	1.56 $\pm$ 0.34	0.31 $\pm$ 0.11
Jan.	2.64 $\pm$ 0.11	0.64 $\pm$ 0.19
Feb.	2.75 $\pm$ 0.16	0.72 $\pm$ 0.14
Mar.	2.91 $\pm$ 0.23	0.75 $\pm$ 0.24
Apr.	3.43 $\pm$ 0.14	0.82 $\pm$ 0.11
May.	3.51 $\pm$ 0.24	0.85 $\pm$ 0.21
Jun.	3.55 $\pm$ 0.2	0.86 $\pm$ 0.15



**Figure 3: Seasonal variations of the thyroid hormones plasma concentrations during the year in the yellowfin seabream. Bars represent mean  $\pm$  SE.**

## Discussion

This study established information for two forms of thyroid hormone measured in yellowfin seabream in warm and cold seasons. No literature indicated any of these hormones, had been previously investigated for fishes in the Persian Gulf. Thyroid hormones are one 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 (Myers et al., 2006).

It was shown that in Atlantic sting ray, *Dasyatis sabina*, follicular cells vary in size and shape, according to the activity of the gland (Volkoff et al., 1999). This was in agreement with the finding of the present study. 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 et al., 2006). 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. In the present study, mean plasma T3 and T4 showed similar seasonal changes patterns, which was in agreement with the finding of Pavlidis et al. (1991). They also reported that similar seasonal variations occurred in circulating thyroid hormones in two strains of rainbow trout (*Oncorhynchus mykiss* Shasta and Kamloops). The results showed that both

hormones decreased significantly during autumn and early winter from October to December according to decrease of temperature. Loter et al. (2007) also reported minimum thyroid hormones in cold months.

In the present study, T3 and T4 increased significantly from January to April, and again from April to July. Duncan et al. (1989) reported a significant increase in the concentrations of both thyroxine and triiodothyronin in mature female channel catfish (*Ictalurus punctatus*) in February, which was coincident with the steroid hormone peak. They suggested that endocrine interactions between thyroid activity and reproduction may be occurring at this time. Thyroid activity increase in the winter corresponds with intermediate temperatures and feed consumption during rapid reproductive development and spawning period of *A. latus* which spawn 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). It seems that thyroid hormones involve in reproductive maturity. 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. Loter et al. (2007) reported that both increased T4 substrate availability (higher plasma T4 levels) and increased temperature would lead to much greater T3 production in summer. In

summary, the activities of the 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 (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).

All together, high magnitude seasonal changes of thyroid hormones in male *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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