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Detection of Some Hormonal Responses of Yellowfin Sea Bream (*Acanthopagrus latus*) in Mahshahr Creeks (North West of Persian Gulf)

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Abstract: Hormones are specific chemical messengers which are secreted by endocrine glands directly into the blood. In this study we detect different levels of hormones parameters in five creeks with different levels of pollutant of Khoor-Mousa in Mahshar region, that located in the north west of Persian Gulf and surrounded by polluting industries. Serum thyroxine T4 and triiodothyronine T3 were assayed using diagnostic ELISA direct immunoenzymatic kits. Serum testosterone were assay using pre-coated ELISA kits. From five sampling stations (Zangi, Jafari, Ghazaleh, Majidieh and Petroshimi) from Mahshahr coastal waters, Station Zangi had lower levels of mercury contamination in all measurements and chooses as clean station. Other Stations had higher levels of mercury contamination in all measurements and were choose as contaminated station. Stations Majidieh and Petroshimi were noticeably close to an area of industrial activities (oil and petrochemistry, respectively) and higher amount were predictable. All hormonal indices exhibited high significant analysis of variance. *In vivo* result declared significance increase of T4 and T4/T3 within higher considerable values than those of the clean station, beside significance decrease of T3, T3/T4 and testosterone with lower considerable values than those of the control group. It was concluded that hormones are affected by environmental condition and are different in close ecosystem.

Key words: Hormone · Yellowfin Sea Bream · Creek · Persian Gulf

INTRODUCTION

Hormones are specific chemical messengers which are secreted by endocrine glands directly into the blood. Although these hormones are distributed in the bloodstream throughout the body, they only act at specific target tissues. This specificity of hormone action is due to the presence of specific receptor molecules that preferentially bind the hormone in the target cells. Hormone binding activates the receptor, causing stimulation of intracellular second messenger pathways or direct binding of the hormone–receptor complex to regulatory elements on genes, ultimately resulting in alterations in cell function. Most of the information reported to date on endocrine toxicity indicates that xenobiotic chemicals primarily interfere with gonadal, thyroid and adrenocortical (interrenal) functions in fish and other vertebrate species [1].

Thyroid hormones have essential functions in the regulation of embryological development, metabolism and reproduction in vertebrates and also have important roles in metamorphosis and osmoregulation in fishes. The secretory activity of the thyroid in fishes, like that in mammals, is regulated by a pituitary glycoprotein hormone: thyrotropin (TSH). Unlike mammals, however, only minor amounts of the active form of thyroid hormone, triiodothyronine (T3), are secreted in fishes, the majority being in the form of the prohormone thyroxine (T4); consequently, the production of the active form of thyroid hormone (T3) from the prohormone (T4) in several fish species is largely under peripheral control by the enzyme monodeiodinase [2].

Testosterone secreted by the testes is the main androgen in males, along with its similarly active metabolite dihydrotestosterone. In principle, altered levels of sex steroids in serum might be due to interferences with the control of steroid synthesis via the pituitary-gonadal axis, or to effects on steroid metabolism and excretion. Testosterone is essential for reproduction, but it is also important in sexual behaviour.

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Disturbances of this hormone may affect reproduction both directly and indirectly [1]. Glucocorticosteriods are involved in a range of physiological processes including reproduction, immune function, behaviour and metabolic adaptation to stress. Steroid hormones affect sex differentiation, maturation, spawning, sexual behavior and secondary sexual characteristics. Some studies reported that exposure of fish to substances having endocrine disruptor effects lead to decreased levels of sex steroid hormones [3].

To date, little is known about the hormonal parameters of yellowfin sea bream, so we have revealed an interesting pattern of response on the hormonal variables in stressed fish. serum hormonal parameters are very sensitive to environmental pollutant, so in this study we detect different levels of hormones parameters in five creeks with different levels of pollutant of Khoor-Mousa in Mahshar region, that located in the north west of Persian Gulf and surrounded by polluting industries which deposit pollutants directly into the creeks, to Characterized hormonal response of Yellowfin Sea Bream to environmental pollutant and undesirable materials.

MATERIALS AND METHODS

***In vivo* Design:** According to our past data, from 26 creeks in Mahshahr region (northwest of Persian Gulf) we choose four more pollutant creeks (Jafari, Ghazaleh, Majidieh and Petroshimi) and one less pollutant as control treatment (Zangi). For every creek we choose three station and for every station two yellowfin sea bream with the same size (170 g) and same sexually (all immature male) were caught with hooks and transferred immediately in a fiberglass tank equipped with specific creek water and chargeable aeration (SOBO, China) to the Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran.

Serum Collecting: Blood was placed in non-heparinized tubes and left to clot at 4°C for 15 min, Afterwards, tubes were centrifuged at 3000 rpm using an Eppendorff centrifuge for 10 min to obtain serum. The serums were separated into aliquots and were frozen and stored at -80°C until metabolite analyses. All samples were immediately immersed in liquid nitrogen and then transferred to a 80°C freezer until analysis.

Hormone Analysis: Serum thyroxine T4 and triiodothyronine T3 were assayed using diagnostic ELISA direct immunoenzymatic kits purchased from Monobind, USA according to supplier's instructions [4]. Absorbance was measured using a Monobind T3 & T4 Eliza instruments at 450 nm (Reference 620) for detection of both hormones.

T3 and T4 methodology requires immobilized T3 or T4 antibodies, as well as HRP-T3 or HRP-T4 conjugates. Regarding TSH, an antibody specific to the β-chain of TSH molecule is immobilized on microwell plates and other antibodies to the TSH molecule are conjugated with HRP. TSH from the sample is bound to the plates. The enzymatic reaction is proportional to the amount of TSH in the sample.

Serum testosterone were assay using pre-coated ELISA kits purchased from IBL Testosterone Enzyme Immunoassay Kit (RE52151), Hamburg, Germany according to supplier's instructions. Absorbance was measured using a Testosterone Eliza (RE52151) instruments at 420 nm for detection. The limit of detection (LOD) of the procedure was 100 pg/ml mL. Intra-assay and Inter-assay coefficients of variation were of 9.5% and 11.6% (T) respectively.

Statistical Procedure: One-way analysis of variance ANOVA with Duncan Post Hoc was used to determine significant differences. The differences between means were analyzed at the 5% probability level (p value of less than 0.05 was considered as statistically significant).

RESULTS

Five sampling stations (Zangi, Jafari, Ghazaleh, Majidieh and Petroshimi respectively) from Mahshahr coastal waters which w ere considered important for their commercial and recreational potential were selected to enumerate the presence of mercury and its synergetic action on the liver of A. latus. Station Zangi had lower levels of mercury contamination in all measurements and choose as clean station. Other Stations had higher levels of mercury contamination in all measurements and were choose as infected station. Stations Majidieh and Petroshimi were noticeably close to an area of industrial activities (oil and petrochemistry respectively) and higher amount were predictable.

With respect to *In vitro* raw data, the Kolmogorov-Smirnov normality test was significant at a $P<0.05$, for all our measured parameters. Results of hormone activity analysis are presented in Table 1. All hormonal indices exhibited high significant analysis of variance ($P<0.01$).

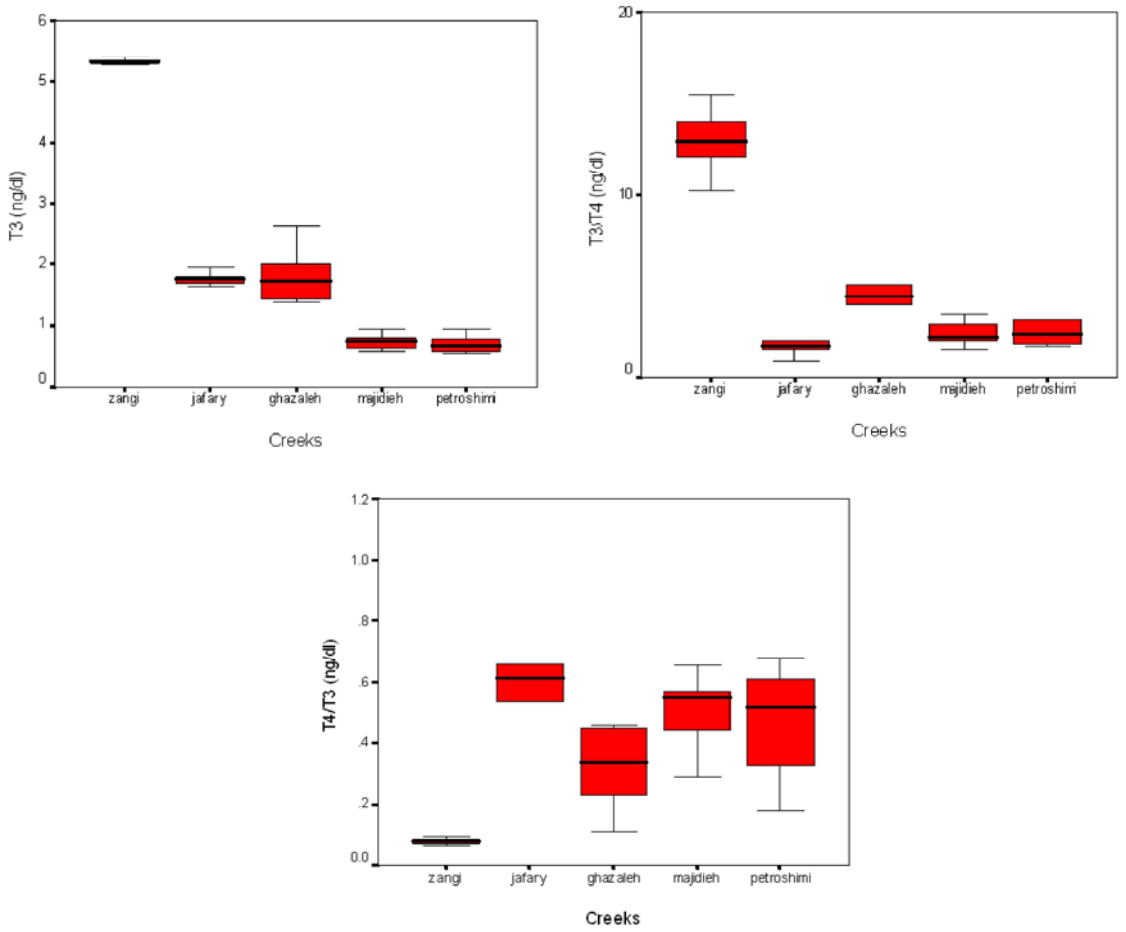

Fig. 1: T3, T3/T4 and T4/T3 of the yellowfin seabream during in vivo exposed to different creeks of Mahshahr coast (box plots contain mean and standard deviation)

Table 1: *In vivo* hormone activities of yellowfin seabream exposed to mercury

	Zangi	Jafari	Ghazaleh	Majidieh	Petroshimi
T3 (ng/dl)	5.3±0.04^{a}	1.7±0.12^{b}	1.8±0.46^{b}	0.73±0.12^{c}	0.70±0.14^{c}
T4 (ng/dl)	0.41±0.06^{b}	1.08±0.45^{a}	0.53±0.13^{b}	0.36±0.05^{b}	0.31±0.09^{b}
T3/T4 (ng/dl)	12.92±1.8^{a}	1.93±0.98^{c}	4.56±1.59^{b}	2.38±0.68^{c}	2.84±1.46^{c}
T4/T3 (ng/dl)	0.07±0.01^{c}	0.62±0.29^{a}	0.31±0.13^{b}	0.50±0.12^{ab}	0.47±0.18^{ab}
Testosterone (ng/dl)	0.43±0.03	0.45±0.04	0.31±0.04	0.34±0.01	0.34±0.02

In vivo result declared significance increase of T4 and T4/T3 within higher considerable values than those of the clean station, beside significance decrease of T3, T3/T4 and testosterone (P>0.01) with lower considerable values than those of the control group (Fig. 1).

Comparison of laboratory and field mean of hormonal results showed that among 5 hormonal indices, T3, T3/T4, T4/T3 and Testosterone had same process (but T4 hadn't predictable process in field condition and increase wasn't at linear model) in both test area with decline progress for Testosterone, T3 and T3/T4 and increase progress for T4/T3 (P<0.05).

The correlation between mercury with hormonal parameters was statistically tested by analyzing the data obtained during the five sampling creeks for *In vivo* indices. Correlation coefficients between water mercury concentrations and hormonal indices were significant in T3, T3/T4 and testosterone (P<0.05), that all significant parameters had negative correlate with water mercury and among insignificant indices T4/T3 had positive and T4 had negative correlation (Table 2).

Results of sediment correlation show that any parameters hadn't significant correlate whereas within

Table 2: *In vivo* correlation of hormonal activities of yellowfin seabream with water mercury

	T3	T4	T3/T4	T4/T3	Testosterone
Pearson correlation (r)	-0.81**	-0.24	-0.66**	0.32 -0.76**	
Sig. (p)	0.00	0.19	0.00	0.08	0.00

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 3: *In vivo* correlation of hormonal activities of yellowfin seabream with sediment mercury

	T3	T4	T3/T4	T4/T3	Testosterone
Pearson correlation (r)	-0.31	-0.31	-0.17	0.20	-0.26
Sig (p)	0.09	0.08	0.35	0.28	0.15

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 4: *In vivo* curve fit linear regression of hormonal activities of yellowfin seabream with water mercury

	T3	T4	T3/T4	T4/T3	Testosterone
R square (r^2)	0.66	0.06	0.44	0.10	0.58
F	56.04	1.80	22.21	3.29	38.91
Sig (p)	0.00**	0.18	0.00**	0.08	0.00**

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

Table 5: In vivo curve fit linear regression of hormonal activities of yellowfin seabream with sediment mercury

	T3	T4	T3/T4	T4/T3	Testosterone
R square (r^2)	0.09	0.13	0.02	0.04	0.07
F	2.98	3.52	0.53	1.16	2.17
Sig (p)	0.09	0.07	0.47	0.28	0.15

* Correlation is significant at the 0.05 level, ** Correlation is significant at the 0.01 level

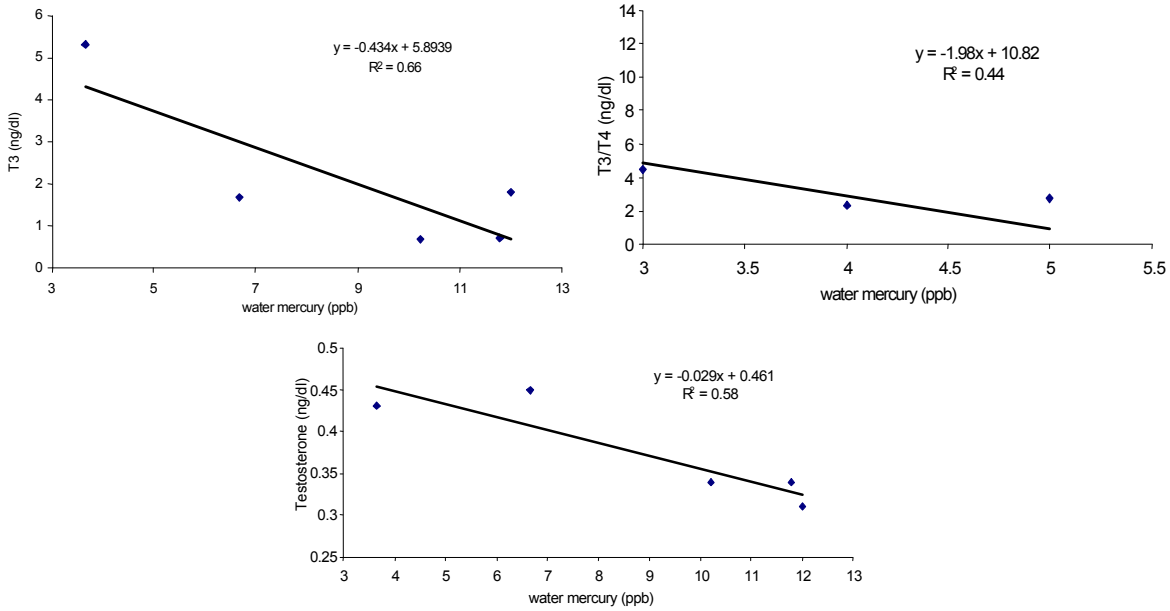

Fig. 2: Regressions model (Y = a ± bX) of T3, T3/T4 and Testosterone in the yellowfin seabream during in vivo exposed to different concentration of water mercury

insignificant parameter T4/T3 had positive correlation and other indices had negative correlation with sediment mercury (Table 3).

In order to evaluate the response of A. latus to mercury concentrations, a linear model and a sigmoid model were tested and compared. The variation of mercury concentration in the *In vivo* and *In vitro* condition were best fitted by a linear model equation, so linear regression model was found to fit well the relations between the concentrations of mercury with hormonal parameters in the both condition of our test. Curve estimation regressions data were used to determine

the relationship between water mercury and hormonal content in different creeks. Except T4 and T4/T3, other parameters show significant linear regression ($P<0.05$) with water mercury (Table 4). Regressions model $Y = a \pm bX$ of significant indices are in Fig. 2.

Curve estimation regressions data were used to determine the relationship between sediment mercury and hormonal content in different creeks. Any parameters did not show significant linear regression ($P<0.05$) with sediment (Table 5).

DISCUSSION

The physiological functions of the gonads and interrenal and thyroid glands in fishes are under complex neuroendocrine control by hormones synthesized by the hypothalamus and pituitary. The hormones secreted by the gonads and interrenal and thyroid glands in turn exert negative feedback effects on neuroendocrine function; however, the thyroid hormone function in fishes is largely controlled peripherally by monodeiodinases, the enzymes that convert T4 to the active form of the hormone (T3) or to inactive metabolites. Sex steroid hormones synthesized by the gonads control critical functions in this tissue during gametogenesis (paracrine control) in addition to exerting their actions at other target tissues. Chemicals could potentially disrupt these complex endocrine systems at multiple sites along their axes and via a variety of mechanisms. Chemicals can disrupt endocrine function by altering the circulating levels of hormones, by decreasing hormone secretion, by increasing hormone metabolism, or by interfering with hormone action [1].

A variety of environmental chemicals have been shown to alter the thyroid system in fishes by causing decreases in the circulating levels of thyroid hormones and hepatic 5'-monodeiodinase activity, although the sites and mechanisms of chemical interference with thyroid function remain poorly understood. Declines in monodeiodinase activity have been reported in fishes after *In vivo* exposure to metals and insecticides [5], but it is unclear whether these chemicals are acting directly on the enzyme or indirectly by altering other endocrine systems that influence monodeiodinase activity [6]. Deiodinases contain selenium bound to cysteines and therefore are potentially susceptible to interference by heavy metals that displace selenium from these sites in proteins such as cadmium, mercury and copper. Deiodination has been proposed as a valuable marker of interference of the thyroidal system in fish [6]. Other potential sites of xenobiotic interference with thyroid function include interference with neurotransmitter control of neuroendocrine function [7] and binding to the thyroid hormone receptor or plasma transport protein, although direct evidence is lacking [8].

Both the steroid hormones and thyroid hormones are under control of the hypothalamus and pituitary. Thyroid-stimulating hormone acts on the thyroid gland and signals the synthesis and release of thyroid hormone. To date, thyroid hormone in fish has been characterized as thyroxine (T4), which is metabolized to triiodothyronine (T3) by means of enzymatic deiodination by iodothyronine 5'-monodeiodinase type 1 (5'-ID1) [9]. T3 appears to be more biologically active, having a greater affinity for the receptor than T4. The mechanistic action of T3 in fish is largely unexplored, but mammalian studies suggest that T3 binds with nuclear receptors, creating a T3-receptor complex which, in turn, binds to a thyroid response element to initiate DNA transcription [10].

A major role of thyroid hormone in fish is regulation of growth and development. However, in a large majority of teleost species examined, there is an association between the thyroid hormones and reproduction. In most fish studied to date, thyroid activity increased during early gonadal development, remained elevated during the reproductive cycle and decreased after spawning [10]. This relationship does not directly indicate that thyroid hormones influence reproductive function, but does suggest that there is a potential for such interactions to occur. A role for T3 in male fish has also been reported whereby the inhibition of thyroid hormone resulted in an inhibition of testicular growth in developing male fish [9].

Only few researches have been invested in examining the effect of thyroid hormones as a result of environmental chemical exposure. Ruby *et al.* [11] examined plasma estradiol, T3 and T4 in rainbow trout following exposure to cyanide. These investigators demonstrated that plasma estradiol and T3 levels were lower in cyanide-treated fish, whereas no difference was observed in plasma T4 levels. Thus, the investigators suggested that, although cyanide inhibited the conversion from T4 to T3, our result likewise show that amount of T3/T4 was decreased but T4/T3 was increased during polluted creek, so the lowered T3 levels may have been the result of chemical interaction along the hypothalamic-pituitary-ovarian axis. Thyroid hyperplasia in salmon and herring gull populations has also been reported in select regions of the Great Lakes [12]. It is suggested that this goiter condition may be a result of the inability of the organism to produce T3.

The sharp depletion on serum testosterone levels in exposed individuals was one of the most sensitive responses of juvenile turbot to fuel exposure [13]. Flammarion *et al.* [14]. found a decrease in testosterone as a result of the exposure of chub to effluents containing organic pollutants and heavy metals. The present study has confirmed these findings.

Webb *et al.* [15] find significant negative correlations between serum androgens (T and KT) and mercury content. Exposure to the fuel oil sharply reduced circulating levels of testosterone in serum [13]. Nonetheless, no effect on testosterone levels detected in juvenile cod exposed to the same oil concentration in the same tanks as turbot [16]. In Atlantic croaker (*Micropogoinas undulates*) exposed to Aroclor during gonadal recrudescence, there was depressed serum testosterone levels in males [17]. Our results were similar to those of previous studies, thus the hypothesis of a decrease of circulating testosterone in exposed individuals due to an inhibition of steroid biosynthesis might be considered. Indeed, one might hypothesize increased energy requirements for detoxifying functions and xenobiotic clearance in exposed fish, increased physiological stress and hence, decreased energy for growth and reproduction [18].

Decreased serum levels of testosterone indicated that factors in the aquatic environment have affected important physiological functions of yellowfin sea bream.

In conclusion, results of the present investigation showed that pollutants have direct correlation with fish hormones abnormalities and also indicated that the different in environmental condition of marine ecosystems may cause several changes in the hormonal parameters of the studied fish.

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