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Effect of Environmental Mercury on Some Hormonal Parameters of the Main Mariculture Fish of Persian Gulf

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Abstract: This study was conducted under two separate environmental and experimental conditions. The environmental component was carried out at several creeks in the Persian Gulf whilst the experimental phase was at conducted in recirculating seawater tanks. Mercury concentrations were determined. Results of the present investigation indicated that the sub- acute and chronic mercury concentrations tested may cause several changes in the biochemical and hormone parameters of the studied fish and we can use these changes as biomarkers of mercury contamination. The range of mercury concentrations found in the creeks and particularly in sediments along the Mahshahr coast was higher than other marine environments. It is suggested that the hormonal indices testosterone, T3 and T3/T4 and metabolite indices total protein can be considered as suitable and effective biomarkers of mercury pollution in yellowfin seabream.

Key words: Biomarker · Fish · Marine pollution · Serum biochemical

INTRODUCTION

Chronic pollution induced heavy metals in the marine ecosystems is a major problem particularly in shallow water like creeks. The hazardous influence of mercury on fish can be measured accurately by detection of biochemical and hormonal indicators. Although most similar study has been conducted on terrestrial vertebrates, there are a few studies related to the effects of mercury on biochemical activity in fish [1, 2].

Enzymes have an important role in physiological functions determinant for the survival and performance of the marine organisms. Enzymes catalyze physiological reactions by decreasing the activation energy level that the reactants must reach for the reaction to occur. The effects of heavy metals on enzymatic activity of fish are one of the most important biochemical parameters which are affected under exposure of toxicants [3]. In exposure to mercury, enzyme activity appears to be increased or it may be inhibited due to the active site being either denatured or distorted [2]. The increase or decrease in enzyme level in a very accurate index for diagnostic of quantity and quality of toxicant. For example, such effects have been observed after chronic exposure to low doses or acute exposure to high doses of mercury [4].

In fish, thyroid hormones have many important roles in maintaining proper physiological function and also in fish growth and early development [5]. When fish are exposed to environmental pollutants the levels of thyroid hormones have been demonstrated to be changed [6] and it well confirmed that chemicals have more affect thyroidal hormone status in a number of freshwater and marine fish species [7].

Biomarkers that can identify effects related to endocrine disruptors are changes in steroid hormone concentrations in fish serum content. The examination of fish reproduction and the potential adverse reproductive effects posed by chemical exposure can serve as a reasonable measure of potential ecologic risks. Indicators of long-term exposure to environmental chemicals in male fish are examining male gonads and spermatogenesis. Short-term measures can include steroidogenesis and pituitary activity among others [8], so because of all male yellowfin sea bream we examined testosterone as short-term indicator of mercury pollution.

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Glucose is a carbohydrate that has a major role in the bioenergetics of animals, being transformed to chemical energy (ATP), which in turn can be expressed as mechanical energy [9]. Levels of glucose can be measured as conventional stress markers in biomarker studies.

Most biochemical defenses respond to cellular injury by elevation amounts of defenses through self-regulating signal transduction mechanisms. These defenses are usually proteins that serve numerous cellular functions [10]. Thus, measuring these systems may provide early warning of danger to the cell as well as help elucidate potential mechanisms of cellular injury.

In contrast to mammalian, little is known about the biochemical parameters of stressed fish. In current study serum biochemical and hormones were measured in order to investigate patterns of response and to quantify the extent of alterations caused by the mercury pollution, so in this study, a multi factorial approach, involving determining serum parameters during the environmental and experimental exposure of mercury pollution was used. The information gained from this study may be useful for future strategies in monitoring and predicting the effects of mercury exposure and also in developing indices to measure stress during seabream culture.

MATERIALS AND METHODS

Environmental Test: This study was in two separate environmental and experimental conditions. For environmental test in natural condition, at first with mercury analysis of water and sediment (method details in bellow) of 26 creeks in Mahshahr region (northwest of Persian Gulf) we choose four more pollutant (Jafari, Ghazaleh, Majidieh and Petroshimi) and one less pollutant creeks (Zangi).

For every creek we choose three station and in every station one water and sediment sample collected, also two yellowfin sea bream with the same size (170 g) and same sexually (all immature male) were caught.

Experimental Test: Yellow fine sea bream all immature male in same size (150 g final body weight average) were maintained in seawater re-circulatory system (300-L tanks) equipped with physical/chemical filters and with aeration to the Mariculture Research Station of the South Iranian Aquaculture Research Center, Mahshahr, Iran.

Seventy five fish were randomly divided into five equal groups (15 per group) and each tank was randomly assigned to one of five experimental treatments and allocated to a 15 static cylindrical polyethylene tank filled with the appropriate concentration of an aqueous solution of Hg (standard solution for atomic absorbance spectrophotometer) in dechlorinated tap water [10].

Fish were exposed to mercury concentrations of 0 µg l, 10 µg l, 20 µg l, 40 µg 1, 80 µg l respectively and maintained for three weeks with aeration. These sub-lethal doses were chosen on the basis of preliminary toxicity tests and determinations of LC_{50} 96h for this species, suggestive of inducing toxic effects but not lethally so [11, 12].

Conditions within each experimental tank were monitored daily with the temperature 25°C ± 1, pH 7.8 ± 0.1 and salinity 46 ± 1 ppt under a natural photoperiod (12hL:12hD) in controlled room. Water was oxygen saturated through constant aeration in a static system. Voluntary feed intake was near to maintenance ration at the time of the maintenance; Fish were fed two times a day (08:30 and 17:30 h) but were starved for 48 h prior to the start of the experiment and throughout its duration. Fecal remains and food residues were removed by suction every other day. The food supply was provided to each predator fish with fresh prawn, collected from creeks without pollutants sources.

Mercury Analysis: In laboratory water samples were filtered with Millipore strainer mesh size 0.45 µm, the filtrate was then acidified with 2mg/l of 20% $K_2Cr_2O_7$(w/v) prepared at nitric acid [13] and soluble store at -4°C until mercury analyses.

For stabilize of weight, the sediments were freeze-dried [14], then were sieved through 63 µ mesh and were allowed to settle, the supernatant water decanted and homogenized, finely powdered sediment subsamples were dissolved in 60 ml container 4 ml of concentrated nitric acid and 2 ml of concentrated sulfuric acid. The mixture was digested at 90°C for l-2 h in hot plate. Upon cooling, 1 ml $K_2Cr_2O_7$ or 0.5 ml BrCl was added. The solution was filtered using Whatman No.1 filter paper and diluted to 50 ml with deionized water [15] and preserved prior to Hg analysis.

Mercury concentrations were determined by the Department of Marine Chemistry Laboratory, Khorramshahr University of Marine Science and Technology using a standard cold vapor atomic absorption (CV-AAS) method (Unicam 919) equipped with Hg cold vapor generator (VGA 77) [16].

Blood Sampling: To obtain blood samples, fish were quickly taken out from the water and held firmly on a bench with a cloth covering the head and blood was

withdrawn from caudal vessels [17] were for hematology and leukocyte analysis and the second were centrifuged to obtain serum. Serums were separated into aliquots and were frozen and stored at -80°C until metabolite analyses.

Metabolite Analyses: Serum glucose was measured photometrically according to a method modified from Banauch *et al*. [18] based on the quantification of NADH after a glucose oxidation catalyzed by glucosedehydrogenase. The quantity of NADH formed is proportional to the glucose concentration.

Serum total protein levels were determined using Pars Azmoon, Iran (1 500 028) kit, with bovine serum albumin serving as standard by the method of Lowry [12].

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 [6]. Absorbance was measured using a Monobind T3 and T4 Eliza instruments at 450 nm for detection of both hormones. Serum testosterone were assay using pre-coated ELISA kits purchased from IBL Testosterone Enzyme Immunoassay Kit, Hamburg, Germany according to supplier's instructions [19].

Statistical Procedures: For each biomarker, the data were tested for normality and homogeneity. One-way analysis of variance ANOVA with Duncan Post Hoc was used to determine significant differences to evaluate the effect of mercury on parameters. To investigate associations between bioaccumulation and its effects, Pearson correlation coefficients (r) were calculated between mercury concentrations and blood parameters. The differences between means were analyzed at the 5% probability level. Data are reported as means ± standard deviation ($\bar{x}$ ± SD). The software SPSS, version 11.5 (SPSS, Richmond, Virginia, USA) was used as described by Dytham [20].

RESULTS

Environmental Results

Mercury: In different creeks significant differences were found between the sampling stations. The range of mercury concentrations found in the creeks water and specially sediments along the Mahshahr coast was high. From the stations it was possible to observe a gradient of metal contamination. Station Zangi had lower levels of mercury contamination in all measurements and chosen as clean station. Other Stations had higher levels of mercury contamination in all measurements and were chosen 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.

The analytical data were normalized to the distance from the creeks with water and sediment mercury (Fig. 1). Concentrations of both water and sediment mercury were strongly higher in infected creeks than the clean one, however this increase in water mercury was realizable. In general, the highest concentrations of mercury for water and sediment had same progress. These observations strongly suggest that anthropogenic activities can significantly increased mercury levels in the water and sediment even in closed creek. These differences denote

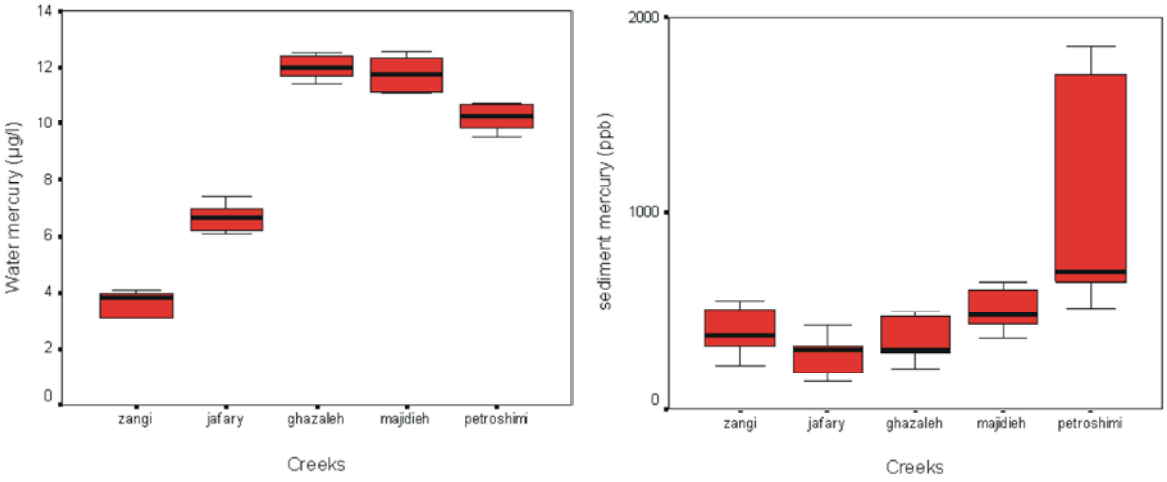

Fig. 1: Environmental concentrations of mercuric chloride (µg/l) in the water and sediment of different creeks in Mahshahr coast with different sources of pollutants (box plots contain mean and standard deviation)

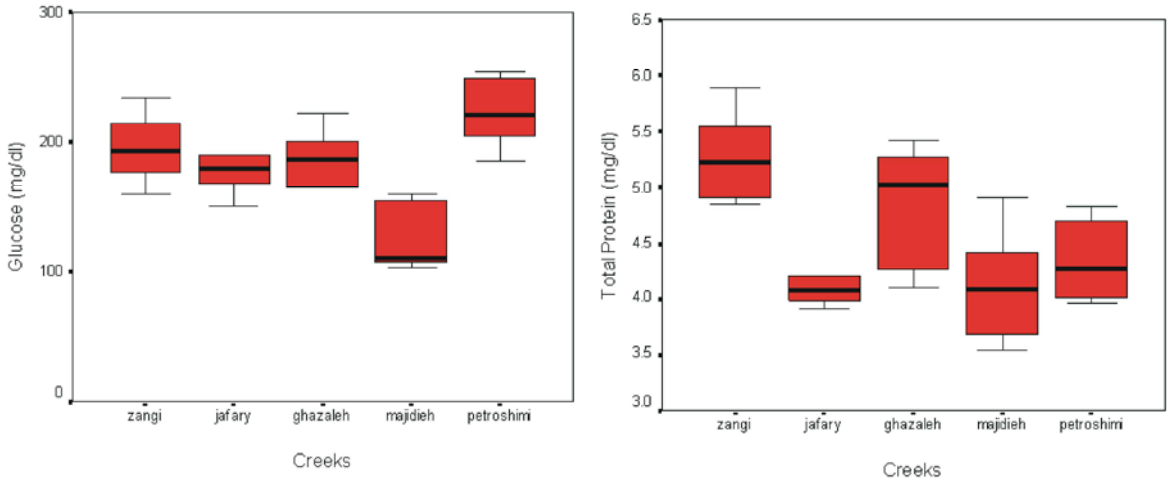

Fig. 2: Metabolites response (Glucose and Total protein) of the yellowfin seabream during environmental exposure to different concentrations of mercuric chloride (box plots contain mean and standard deviation)

Table 1: Environmental correlation of metabolites activities of yellowfin sea bream with water mercury

	Glucose	Total protein
Pearson correlation (r)	-0.29	-0.38*
Sig (p)	0.11	0.03

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

Table 2: Environmental correlation of metabolites activities of yellowfin seabream with sediment mercury

	Glucose	Total protein
Pearson correlation (r)	0.39*	-0.22
Sig (p)	0.03	0.24

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

a contamination gradient according to the distance to the point source of mercury into the system, Petroshimi and Majidieh being the closest and Zangi and Jafari the furthest creeks to the anthropogenic activities near the Mahshahr coast.

Metabolite Analysis: Values recorded for activity of total protein show significant depletion in infected creeks with respect to clean creek. Glucose was significantly increased in infected creeks with respect to clean creek (Fig. 2).

Correlation coefficients between water mercury concentrations and biochemical indices wasn't significant (P<0.05), however both correlation were negative in correlate with water mercury (Table 1). Result of sediment correlation show only glucose had significant positive correlate whereas glucose parameter had negative correlation with sediment mercury (Table 2).

Hormone Analysis: All hormonal indices exhibited high significant analysis of variance (P<0.01). Hormone result declared significance increase of T4 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 (Table 3).

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 (Table 4). Results of sediment correlation show that all parameters had significant negative correlate with sediment mercury (Table 5).

Experimental Results: No differences were observed in body weight gain at 21 days of mercury exposure, but the food intake was decreased with increased concentrations of mercury. During maintenance and acclimation period, no mortality was observed in any experimental group.

Metabolite Analysis: $HgCl_2$ intoxication causes significant variation ($P < 0.05$) in total protein and glucose with respect to control treatment without mercury induction. Results revealed significant $P<0.05$ variations in glucose levels among the experimental samples with same elevation process in treatments, however depletion process in total protein show significant $P < 0.05$ variations with same depletion from treatment II-V (Fig. 3).

During experimental results, the correlation between mercury with biochemical parameters were statistically tested by analyzing the data obtained during the mercury

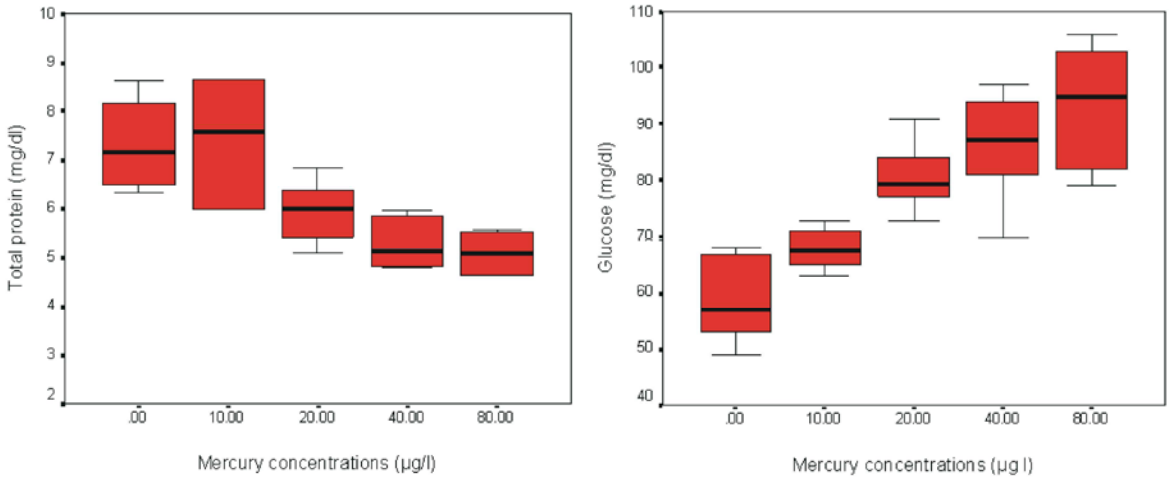

Fig. 3: Metabolites response (Glucose and Total protein) of the yellowfin seabream during experimental exposure to different concentrations of mercuric chloride (box plots contain mean and standard deviation)

Table 3: Environmental hormone activities of yellowfin sea bream 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}
Testosterone (ng/dl)	0.43±0.03	0.45±0.04	0.31±0.04	0.34±0.01	0.34±0.02

Table 4: Environmental correlation of hormonal activities of yellowfin seabream with water mercury

	T3	T4	T3/T4	Testosterone
Pearson correlation (r)	-0.83**	-0.24	-0.66**	-0.84**
Sig (p)	0.00	0.19	0.00	0.00

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

Table 5: Environmental correlation of hormonal activities of yellowfin seabream with sediment mercury.

	T3	T4	T3/T4	Testosterone
Pearson correlation (r)	-0.55**	-0.44*	-0.40*	-0.40*
Sig (p)	0.00	0.01	0.02	0.02

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

Table 6: Experimental correlation of metabolites activities of yellowfin seabream with mercuric chloride

	Glucose	Total protein
Pearson correlation (r)	0.77**	-0.71**
Sig (p)	0.00	0.00

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

exposed. Both glucose and protein parameters show significant correlation (P<0.05) with mercury exposed, that also both correlations were negative (Table 6).

Hormone Analysis: With respect to experimental raw data, the Kolmogorov-Smirnov normality test was significant at a P<0.05, for all our measured parameters. between hormonal indices T3, T4, T3/T4 and testosterone activities exhibited significant analysis of variance (P<0.5) with lower considerable values than those of the control group for T3, T3/T4 and testosterone and higher considerable values than those of the control group for T4 (P<0.05) (Table 7).

Compare of laboratory and field compare mean of hormonal results show that among 4 hormonal indices, T3, T3/T4 and Testosterone had same process in both test area with decline progress for Testosterone, T3 and T3/T4 and increase progress for T4 (P<0.05), all other indices in both conditions had significant differnce, so in this section we can introduce 3 candidate hormonal biomarkers as T3, T3/T4 and Testosterone.

Table 7: Experimental hormone activities of yellowfin seabream exposed to mercuric chloride

	Control	10 µg l	20 µg l	40 µg l	80 µg l
T3 (ng/dl)	5.74±0.67^{a}	4.79±0.41^{b}	4.77±0.73^{b}	2.04±0.21^{d}	3.56±0.36^{c}
T4 (ng/dl)	0.41±0.14^{c}	0.52±0.07^{bc}	0.55±0.08^{bc}	1.10±0.72^{ab}	1.42±0.81^{a}
T3/T4 (ng/dl)	14.92±4.45^{a}	9.21±1.4^{b}	8.74±1.45^{b}	2.46±1.34^{c}	4.10±4.16^{c}
Testosterone (ng/dl)	0.39±0.05^{a}	0.38±0.10^{ab}	0.34±0.05^{bc}	0.33±0.04^{c}	0.32±0.10^{c}

Table 8: Experimental correlation of hormonal activities of yellowfin seabream with mercuric chloride

	T3	T4	T3/T4	Testosterone
Pearson correlation (r)	-0.61**	0.86**	-0.67**	0.50**
Sig (p)	0.00	0.00	0.00	0.00

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

During experimental results, the correlation between mercury with all hormonal parameters was statistically tested by analyzing the data obtained during the mercury exposed. All parameter show significant correlation ($P<0.05$) with mercury exposed, that among all correlation was positive except T3 and T3/T4 (Table 8).

DISCUSSION

The range of mercury concentrations founded in the creeks water and specially sediments along the Mahshahr coast was higher than other marine environment, so it reveals that is an area requiring a special concern in order to avoid future environmental problems. Correlation results and regression models show that in different test condition (laboratory, field water and sediment) high correlation between surrounded mercury, total protein, testosterone, T3 and T3/T4 was well confirmed.

Our result show same progress of biochemical indices in both test condition with elevation in glucose and depletion in total protein (Hyperglycemia and Hypoproteinemia respectively).

The most frequently encountered reasons of hyperglycemia are failure to fast an animal and catecholamine release secondary to excitement or fear [21]. In polluted conditions the chromaffin cells release catecholamine hormones, adrenaline and noradrenaline toward blood circulation [22]. Those stress hormones in conjunction with cortisol mobilize and elevate glucose production in fish through glucogenesis and glycogenolysis pathways [23] to cope with the energy demand produced by the stressor, Glucose is then released toward blood circulation and enters into cells through the insulin action [24].

Amounts of glucose often elevate during the first phase of the stress response due to an elevated breakdown of glycogen [25]. Significant increases in glucose were observed as a result of toxic effect of cooper on African catfish [26]. These results may be attributed to the hepatocellular damage. The serum glucose concentration of gilthead seabream exposed to acute confinement was increased [27]. From aspect of elevation, our findings show no disagreement with the literature values.

Under pollutant conditions may constitute a physiological mechanism with an important role in providing energy to cope with the stress situation. So depletion of total protein (hypoproteinemia) content might also be attributed to the destruction or necrosis of cellular function and consequent impairment in protein synthetic machinery [28]. During exposure to xenobiotics, diversification of energy occurs to accomplish the impending energy demands and hence the protein level is depleted [1]. The depletion of total protein content may be due to breakdown of protein into free amino acid under the effect of mercury chloride at the lower exposure period [29].

Some studies indicate a decrease in total protein content during heavy metal exposure. Such depletion were found in the edible crab *Scylla serrata* exposed to cadmium or in the freshwater fish *Sarotherodon mossambicus* and the common carp exposed to mercury [30]. Decrease in total protein content of *Catla catla* exposed to sub-lethal concentrations of mercuric chloride was estimated [31].

Our result indicate depletion of T3 in contrast to elevation of T4 during mercury exposure in both environment, also portion indices, T3/T4 and T4/T3, show decrease and increase respectively that all results strongly confirmed disrupt in the production of the active form of thyroid hormone (T3) from the prohormone (T4) and function of enzyme monodeiodinase [29].

A variety of environmental xenobiotics have been shown to change the thyroid system in fishes by causing decreases in the circulating levels of thyroid hormones and hepatic 5'-monodeiodinase activity, declines in monodeiodinase activity have been fined in fishes after exposure to metals [32].

Since it was previously observed that heavy metals inhibit the conversion of T4 into T3 by 5'monodeiodinase [33], the T3 serum depleted level can indicate a lower capability of converting T4 into T3. Thyrotropin stimulates the synthesis and release of T4 from the thyroid gland. The production of the active form of thyroid hormone (T3) from the prohormone (T4) is mainly under peripheral control by the enzyme monodeiodinase. Environmental pollutants may disrupt the TH axis by binding to TH transporter proteins [34].

Heavy metals (like mercury) can modify hormone production and activity through the Blocking the synthesis of hormones, mimicking the natural hormones and providing receptors that inhibit cell synthesis of hormones [35]. Some studies reported that exposure of fish to heavy metals having endocrine disruptor effects lead to decreased levels of sex steroid hormones [36].

Our result show depletion of testosterone in both test area that is same to almost all toxicological studies, for example serum testosterone levels in male croakers were also reduced [37, 38]. Webb *et al*. [39] find significant negative correlations between serum testosterone and mercury content. The obtained results suggest the ability of the mercury to disrupt endogenous hormone levels in yellowfin seabream.

Environment pollutants mimic the sex hormone by binding to androgen receptors (AR) and influencing cell signaling pathways. Alternatively, they can block, prevent and alter androgen binding to AR and interfere with cell signaling pathways. Contaminants that block or antagonize androgens are labeled antiandrogens [38].

Results of the present investigation indicated that mercury is a toxic substance in yellowfin seabream and the sub- acute and chronic mercury concentrations tested may cause several changes in the enzymatic, biochemical and hormonal parameters of the studied fish and we can use these changes as biomarkers of mercury detection. In conclusion, total protein, testosterone, T3 and T3/T4 could provide useful indicators of mercury pollution and are suitable biomarkers in yellowfin seabream.

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