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Determination of Some Enzymatic Indices of Yellowfin Sea Bream (*Acanthopagrus latus*) in Mahshahr Creeks (North West of Persian Gulf)

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Abstract: Enzymes catalyse physiological reactions by decreasing the activation energy level that the substrates must reach for the reaction to occur. Five creeks from Mahshahr were selected. ALP, ACP, AST and ALT were determined by enzymatic methods with a Metrolab 2300 Plus auto-analyze. Lipase activities were determined with Photometric method. Total protein levels were determined with bovine serum albumin serving as standard. Levels of ALT, AST and ACP in Majidieh had higher levels than other creeks, however, other enzymes were partly high at Majidieh. Ghazaleh had lower amount of ALT and AST, however the ALP level was at maximum amount. In Jafari the levels of ACP and Lipase was in lowest level. Finally our results confirm that enzymatic indices are suitable ecophysiological parameters for evaluated ecological features of Yellowfin Sea bream in natural condition and we can used these indices as biomarker of pollution.

Key words: Enzyme % Creek % Yellowfin Sea Bream

INTRODUCTION

Coastal ecosystems are subjected to discharge from anthropogenic activities. However, estuaries and creeks are the main contributors of fishing activities, but exposed to severe damage due to increased industrialization and urbanization near the coastal areas are very dangerous [1].

Mahshahr creeks, located in the north west of Persian Gulf, faces this problem because it is being surrounded by polluting industries, which deposit pollutants directly into the creeks.

Yellowfin sea bream, *Acanthopagrus latus* Houttuyn, (1782), is a protandrous hermaphroditic sparid (Porgies) from order Perciformes (perch-likes) and class Actinopterygii (ray-finned fishes) [2] and being distributed off southern Japan, southeastern China, Taiwan, southeastern Asia, Persian Gulf and Australia and in the Indian Ocean to southeastern Africa [3]. This Fish can live in freshwater, brackish and marine environment and have high potential for aquaculture in the Indo-Pacific region because of its high market value, easy adaptation to captivity and availability of production technology in experimental condition [4, 5]. *A. latus*, are found in the shallow water of the estuarine zone that experiences dual fluctuations in both temperature and salinity. Yellowfin sea bream is considered to be one of the most commercially important marine fish in Iran, due to its consumer preference and is newly successful cultured in the coastal area of Iran, especially in Khuzestan province.

These economically important fish are under threat because of their continuous exposure to toxic chemical-rich industrial effluents that are discharged into the Persian Golf and their enclosed creeks. Because wild stocks of sea bream have declined substantially in recent years, owing to overexploitation, pollution and illegal fishing practices, there is an urgent need to revive them through processes of conservation, management and mariculture, so knowledge of the mainly common pollution effect on vital organ of this Fish, is a basic prerequisite for successful management and also for correct interpretation of ecological investigations.

To determine the effect of undesirable materials on the fishes, in other research several strategies have been used. Among these, the change of the key enzymatic activities rates has been frequency used in fish and shellfih [6, 7]. So in this study we detect different levels of serum enzymes in five pollutant creeks of Khoor-Mousa in Mahshar region.

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MATERIAL AND METHOD

***In vivo* test:** According to our past data, from 26 creeks in Mahshahr region (northwest of Persian Gulf) we choose five more pollutant creeks (Jafari, Ghazaleh, Zangi, Majidieh and Petroshimi). For every creek we choose three station and for every station two yellowfin sea bream with the same size (170 g) and same sex (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.

Blood Analyses: Extracted blood (1 mL), was immediately placed in placed in non-heparinized tubes and left to clot at 4°C for 15 min, Afterwards, tubes were centrifuged at 3000 rpm (20000_g) using an Eppendorf centrifuge for 10 min to obtain serum. The serums were separated into aliquots and were frozen and stored at -80°C until enzymatic analyses. All samples were immediately immersed in liquid nitrogen and then transferred to a _80° C freezer until analysis. Biochemical analyses was done with the automate apparatus Autoanalyser, Metrolab 2300 plus, Argentina (Random Access Clinical Analyzer). Enzyme level units (IU), defined as micromoles of substrate converted to product at assay temperature per minute, were expressed per liter and mg/dl of serum protein (specific level). All enzyme assays were performed in duplicate.

Aspartate Aminotransferase (AST) or Glutamate Oxaloacetate Transaminase (GOT): AST (EC 2.6.1.1), was determined with Pars-Azmoon Diagnostics Infinity AST reagent kit (Procedure No. 1 400 018) and Sigma Diagnostics Infinity ALT reagent kit (Procedure No. 1 400 019), respectively by enzymatic methods with a Metrolab 2300 Plus auto-analyze. Results were expressed as units per gram of protein.

The AST level in the serum was estimated by Reitman and Frankel (1957) method using Pars Azmoon Kit (1 400 018). The oxaloacetate formed in the reaction is coupled with 2,4-dinitrophenyl hydrazine (DNPH) to give the corresponding hydrazone, which give brown colour in alkaline medium and this is measured colorimetrically at 340 nm and 37 C. A standard curve was obtained using different amounts of pyruvate and enzyme level was expressed as U/L. The limit of detection (LOD) of the procedure was 2 U/L. Intra-assay and Inter-assay coefficients of variation were of 3.25% and 4.40%, respectively. Intra-assay and Inter-assay Mean±SD were 25.1±0.82 and 25.7±1.13 U/L, respectively [8].

Alanine Aminotransferase (ALT) or Glutamate Pyruvate Transaminase (GPT): The ALT (EC 2.6.1.2) level was also estimated by the method of Reitman and Frankel (1957) using Pars Azmoon Kit (1 400 019) with DNPH as colour reagent. Pyruvate formed in the reaction is coupled with 2,4-DNPH to give the corresponding hydrazone, which give brown colour in alkaline medium and this is measured colorimetrically at 340 nm and 37°C. A standard curve was obtained using different amounts of pyruvate and enzyme level was expressed as U/L. The limit of detection (LOD) of the procedure was 2 U/L. Intra-assay and Inter-assay coefficients of variation were of 3.25% and 4.40% respectively. Intra-assay and Inter-assay Mean±SD were 25.1±0.82 and 25.7±1.13 U/L, respectively [8].

Alkaline phosphatase (ALP) and Acid phosphatase (ACP) Alkaline phosphatase (ALP) and Acid phosphatase (ACP), were determined by enzymatic methods with the automate apparatus Auto-analyzer, Metrolab 2300 plus, Argentina (Random Access Clinical Analyzer) with Darman Kave and Pars Azmoon kit at 37C and 410 nm and 405 nm respectively. The limit of detection (LOD) of the procedures was 3 IU/L. Acid and alkaline phosphatase in serum (two point method) were determined by the procedure described by Bergmeyer, 1983.

Intra-assay and Inter-assay coefficients of variation for ACP were of 2.60% and 2.80% respectively. Intra-assay and Inter-assay Mean±SD were 8.4±0.29 and 8.7±0.25 U/L respectively. Intra-assay and Inter-assay coefficients of variation for ALP were of 1.50% and 1.60% respectively. Intra-assay and Inter-assay Mean±SD were 114±1.71 and 120±1.93 U/L respectively.

Lipase: Lipase activities were determined with Photometric method in Pars-Azmoon Diagnostics Infinity reagent kit (Procedure No. 1 50 24) at 580 nm for detection. The limit of detection (LOD) of the procedure was 3 IU/L [8].

Total Protein: 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 *et al*. (1951) at 546 nm and 37C. The limit of detection (LOD) of the procedure was 5 mg/dl. Intra-assay and Inter-assay coefficients of variation were of 0.91% and

1.06% respectively. Intra-assay and Inter-assay Mean±SD were 5.27±0.05 and 5.24±0.06 g/dl respectively [8].

Statistical Analyses: 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). Data are reported as means±standard deviation ($\bar{X} \pm SD$). The software SPSS, version 11.5 (SPSS, Richmond, Virginia, USA) was used.

RESULTS

Results of pure enzyme level are presented in table 1. Significant changes occurred in the activities of all indices. Levels of ALT, AST and ACP in Majidieh had higher levels than other creeks, however other enzymes were partly high at Majidieh. Ghazaleh had lower amount of ALT and AST, however the ALP level was at maximum amount. In Jafari the levels of ACP and Lipase was in lowest level.

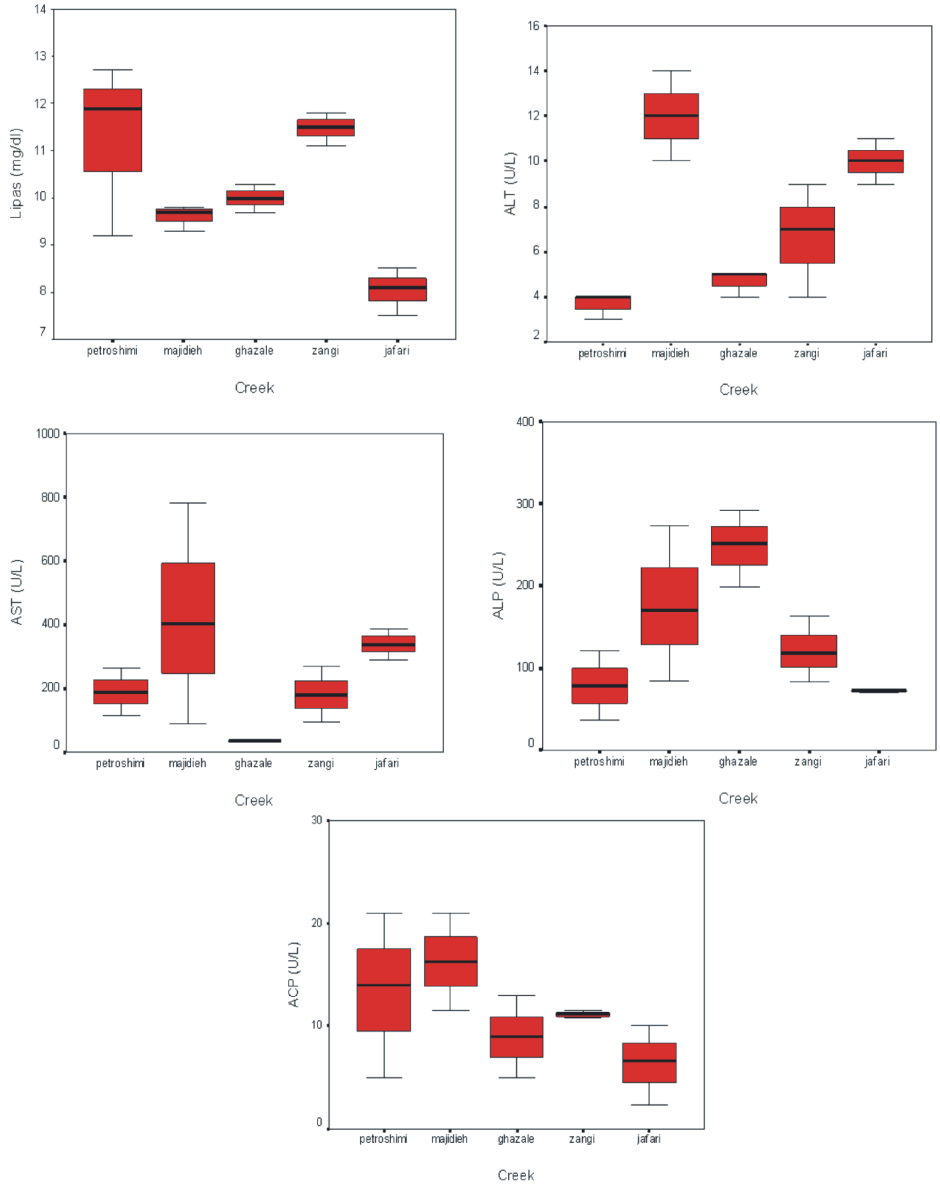

Fig. 1: Enzymatic response (ALT, ALT, ALP, ACP and Lipase) of the yellowfin sea bream in different creeks of Mahshahr (box plots contain mean and standard deviation). Values of specific enzyme levels are expressed in (U/mg Protein) except Lipase (mg/mg Protein)

Table 1: Pure enzyme activities of Yellowfin Sea bream in different creeks of Mahshahr

	ALT (U/ L)	AST (U/ L)	ALP (U/ L)	ACP(U/ L)	LIPASE (mg/dl)
Petroshimi	3.66±0.57^{c}	190.33±74.5^{ab}	78.66±42^{b}	13.33±8.02^{ab}	11.26±1.8^{ab}
Majidieh	12±2^{a}	425.33±345.9^{a}	176.33±94.1^{ab}	16.26±4.75^{a}	9.6±0.26^{bc}
Ghazaleh	4.66±0.57^{bc}	36.66±2.5^{b}	247±46.1^{a}	8.96±4^{ab}	10±0.3^{ab}
Zangi	6.66±2.51^{b}	182.33±88.5^{ab}	121.66±39.6^{b}	11.13±0.35^{ab}	11.46±0.35^{a}
Jafari	10±1^{a}	339±48^{ab}	72.33±2.08^{b}	6.36±3.8^{b}	8.03±0.5^{c}

Table 2: Enzyme/Protein portion levels of Yellowfin Sea bream in different creeks of Mahshahr

	ALT (U/mg protein)	AST (U/mg protein)	ALP (U/mg protein)	ACP (U/mg protein)	LIPASE (mg/mg protein)
Petroshimi	0.7±0.14^{c}	37.3±18.2^{ab}	14.4±6.4^{c}	2.43±1.2^{a}	2.12±0.14^{a}
Majidieh	2.48±32^{a}	76.5±48.3^{a}	33.7±9.2^{b}	3.68±2.0^{a}	2.04±0.55^{a}
Ghazaleh	0.88±0.09^{bc}	69.9±0.32^{a}	46.5±7.6^{a}	1.68±0.7^{a}	1.89±0.01^{a}
Zangi	1.35±0.53^{b}	36.5±16.9^{ab}	24.4±7.4^{bc}	2.24±0.02^{a}	2.31±0.12^{a}
Jafari	2.36±0.06^{a}	79.9±5.3^{a}	17.1±0.8^{c}	1.55±1.0^{a}	1.91±0.26^{a}

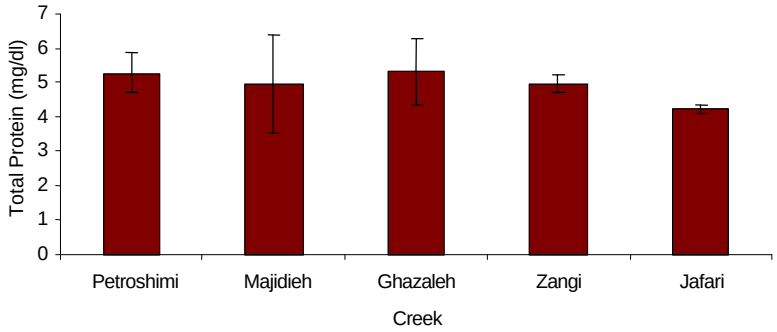

Fig. 2: Total portion levels of Yellowfin Sea bream in different creeks of Mahshahr

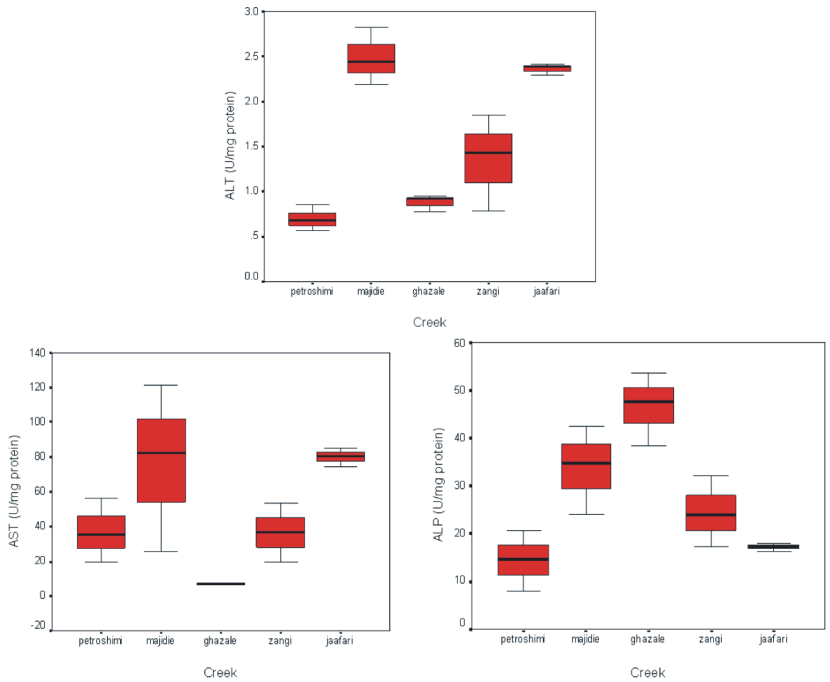

Fig. 3: Enzyme/Protein portion levels (ALT, ALT, ALP) of Yellowfin Sea bream in different creeks of Mahshahr (box plots contain mean and standard deviation). Values of specific enzyme levels are expressed in (U/mg Protein) except Lipase (mg/mg Protein)

All pure enzymatic indices had significance difference in all creeks. For Lipase and ACP, Petroshimi had the most standard deviation, however Majidieh had higher standard deviation of AST and ALP (Fig. 1).

Table 2 show the enzyme/protein portion level of all enzymatic indices. Significant changes occurred in the activities of ALT, AST and ALP. Levels of ALT and almost AST in Majidieh had higher levels than other creeks, however Ghazaleh had higher amount of ALP. ACP and Lipase had not any significance difference within the creek.

Amount of Protein is important for index of enzymes as portion (Fig. 2).Between all enzyme/protein portion levels, just ALT, AST and ALP had significance difference and were suitable indices, so box plot of these enzymes are in Fig 3. For ALP and AST, Majidieh had the most standard deviation, however Zangi had higher standard deviation of ALT.

DISCUSSION

Enzymes catalyse physiological reactions by depletion the activation energy level that the substrates must reach for the reaction to occur. The use of enzymatic indices has been advocated to provide an early warning of potentially damaging changes in stressed fish [8].

Any change in enzyme level is a very accurate index for diagnostic of quantity and quality of undesirable materials. Similar research on fish enzymes have demonstrated that antioxidant systems could provide relevant indices in explaining the sensitivity of some fish species to contaminants [9]. Antioxidants have a very sensitive role in maintaining cell homeostasis and, when these defenses are impaired or surmounted, oxidative stress products, namely reactive oxygen species (ROS), may induce DNA damage, enzymatic inactivation and peroxidation of cell constituents. Fish often increased the levels of protective antioidxants enzymes, as well as non-enzymatic free radical scavengers for prevent and cope again abnormality that cause by ROS [10].

As we know, Proteins are a major constituent in the metabolism of organisms, it is important to study the changes in protein metabolism after metal exposure in further detail. Changes that may occur are the increased synthesis or breakdown of proteins and the inhibition or activation of certain enzymes. These can be observed as alterations in the total protein content and portion of phosphatase and aminotransferase enzymes to protein [11], so Portion of ALT, AST, ACP and ALP to protein were discussed.

Serum ALT level, formerly known as serum glutamic pyruvic transaminase (SGPT), is found in many tissues, but its greatest concentration in most species is within hepatocytes. For practical purposes and in the absence of severe muscle necrosis, significant elevations of serum level result only from hepatocyte ALT.

The magnitude of serum level elevation is proportional to the number of affected hepatocytes and is not indicative of the reversibility of the lesion. For example, it is possible to have higher serum ALT level following reversibility of the lesion. It is also possible to have higher serum ALT level following reversible cellular hypoxia secondary to hypoolemic shock than might occur with focal necrosis caused by localized hepatic abscess. Of course, the greatest elevations result from severe lesions that affect a large portion of the liver tissue [8].

Elevations in serum AST level due to hepatotoxicity are not as pronounced as the elevations in serum ALT level; this might occur because a portion of AST is mitochondrial. Corticosteroids and anticonvulsants affect AST in a similar manner as ALT [8]. In current study ALT and AST levels in Majidieh were higher than other creek, than we can state Majidieh is one the pollutant creek.

Acid and alkaline phosphatase catalyses the hydrolysis of various phosphate-containing compounds and acts as transphosphorylases at acid and alkaline pHs, respectively. Acid phosphatases act as marker enzymes for the detection of lysosomes in cell fractions and can be changed by the presence of xenobiotics [12], whilst alkaline phosphatases are intrinsic plasma membrane enzymes found on the membranes of almost all animal cells. In current study ACP levels in Majidieh were higher than other creek and AST in Majidieh (after Ghazaleh) were higher than other creek, than we can state Majidieh is one the pollutant creek.

Finally our results confirm that enzymatic indices are suitable ecophysiological parameters for evaluated ecological features of Yellowfin Sea bream in natural condition and we can used these indices as biomarker of pollution.

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