JBAAR
Journal of Bioscience and Applied Research
WWW.JBAAR.ORG

Physiological perturbations in gills and liver of *Siganus canaliculatus* as potential biomarkers of hydrocarbons pollution in Arabian Gulf of Saudi Arabia

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Abstract

The present study aimed to investigate the state of health of gills and liver tissues of Rabbitfish, *Siganus canaliculatus*, inhabiting three different coastal localities in the Saudi coast of the Arabian Gulf, namely Al-Dammam, Dareen and Maniefa, with varying degrees of pollution affected by anthropogenic inputs including fractionated hydrocarbons. Al-Dammam coast was the less impacted site, and thus considered as a reference location. High concentrations of aliphatic and aromatic hydrocarbons were detected in sediments and tissues at sites with anthropogenic activities (Dareen and Maniefa). Also, biochemical indicators were used to assess the impact of different levels of environmental pollution in gills and liver of the fish. The biomarkers: glycogen, total lipid, total protein, alkaline phosphatase, acid phosphatase, acetylcholinesterase, superoxide dismutase, glutathione peroxidase, glutathione reductase and reduced glutathione were found to be significantly lower in tissues of Rabbitfish caught from polluted locations compared to the reference values. However, aspartate aminotransferase, alanine aminotransferase, catalase, glutathione-s-transferase and lipid peroxidation displayed significantly higher levels in the Rabbitfish caught from polluted locations. Overall, our results highlight the importance of estimating a set of related biomarkers to gain a preferable comprehend of protective mechanisms activated under a given environmental situation. It can be concluded that marine contamination can affect the antioxidant defense status of the gills and liver of studied fish. This has led to the suggestion that the marine Rabbitfish *S. canaliculatus* could be considered as a good bioindicator of environmental contamination by aliphatic and aromatic hydrocarbons.

Key words: bioindicators, fractionated hydrocarbons, gills, liver, oxidative stress, *Siganus canaliculatus*.

1 Introduction

The marine environment is a sink for a great variety of potentially hazardous chemical contaminants released from domestic and industrial sources. Recently, emphasis has been focused on the evaluation of causal relationships between pollutant exposure and observable effects in aquatic biota (Costa et al., 2009; Lyons et al., 2010). The sediment quality is considered the primary factor controlling the status of the marine environmental health. Contamination of the organic and inorganic chemicals play a significant role in threatens to marine organisms, including fish (Zyadah, 2010). It is important to understand the impact and effects of these chemicals on aquatic life forms (Almroth et al., 2008). These pollutants, over time, have dangerous consequences for the organisms that might not become apparent until changes occur at the population or ecosystem level, a point at which it may be too late to take effective countermeasures (Gaber et al., 2013).

The Arabian Gulf is a shallow and semi-enclosed basin, therefore, the impact of contaminants on marine environment as a consequence of intense anthropogenic activities may be significant (Naser, 2013). The Gulf has been subject to inputs of oil pollution from a diversity of sources and it has been evaluated that oil pollution in the Gulf represents 4.7% of total petroleum pollution in the world. This figure has increased even more after the Gulf war. The Gulf region has about two-thirds of the world's

proven petroleum reserves (El-Sorogy and Youssef, 2015). Problems associated with petroleum contamination appear to be of greater importance in the Gulf compared with other regions. This region has undergone considerable development, industrialization, increased urbanization, and refineries have become major sources of contamination to the maritime environment. Accidental spills and increasing tanker traffic are also contributing factors. In addition, wastewater discharges from desalination plants are considered as anthropogenic sources that may contribute to damage the environment of the Arabian Gulf (Sheppard et al., 2010). It has been reported that oil can also be regarded as a substantial and chronic pollution source in the Gulf environment (Naser, 2013). Additionally, chimney emissions of many industrial complexes may contribute to the metal contamination in the Gulf, leading to disturbance of the coastal environment (Sadiq and Alam, 1989).

The introduction of persistent toxic substances (PTS) into the environment is a major issue that gives rise to concerns at local, national, regional and global scales (Scarpato et al., 2010). In aquatic animals, many xenobiotics can induce an imbalance between the production of reactive oxygen species (ROS) and their removal, and as a result oxidative stress occurs (Halliwell and Gutteridge, 2007). Many environmental contaminants are responsible for oxidative stress induction in animals by disturbing the antioxidant capacity and reinforcement the intracellular ROS, which often prelude in DNA damage, lipid peroxidation (LPO) and enzyme suppression (Van der Oost et al., 2003). The most important antioxidant defense systems include antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR), as well as non-enzymatic antioxidants (e.g. reduced glutathione- GSH) which have been extensively used as biomarkers of oxidative stress (Sahan et al., 2010).

Aliphatic (ALIP) and polycyclic aromatic hydrocarbons (PAHs) are a group of organic chemicals characterized by their toxicity, persistence, long-range transport and bioaccumulative potential (Ritter et al., 1995; Essumang et al., 2012). Due to their hydrophobic properties, these pollutants tend to strongly partition to particulate matter in the marine environment and settle through the water column to the sediments, which act as their final sink (Pimentel et al., 2014). Contaminated sediments may constitute a particular threat for the associated biota (e.g. macrophytes and benthic organisms) and even for demersal fish and marine birds through the marine food web. However, there are few published articles on the fate and effects of hydrocarbon pollutants on marine organisms in the Arabian Gulf, Saudi Arabia (Zyadah and Abu Taweel, 2013; Youssef et al., 2016). Levels of pollutants in the marine environment have increased as a result of anthropogenic activities. The diminishing of water and sediment quality can involve a reduction in natural resources. For this reason there is an increasing demand to acquire methods for the identification and assessment of the risks posed by chemical contaminant discharges to the environment and natural resources (Li et al., 2010). As recognized in the last years by international organizations, risk assessment can not be solely based on chemical analysis of environmental samples because this approach does not provide any indication of deleterious effects of pollutants on the organisms (Carlisle et al., 2008). Therefore, the estimation of the biological effects of contaminants has become of major importance for the assessment of the quality of the environment by using biomarkers which are sensitive tools for biological effect measurement in environmental quality management (Lama and Gray, 2003).

Fish species have attracted considerable interest in studies assessing biological and biochemical responses to environmental pollutants (Das and Chakrabarty, 2007). Fish play a major ecological role in the aquatic food-webs because of their function as a carrier of energy from lower to higher trophic levels (Van der Oost et al., 2003). Therefore, contaminant loading in fish is well reflective of the state of pollution in surrounding environments (Lanfranchi et al., 2006, Mora et al., 2008). Rabbitfish (Siganidae) are widely distributed and some species are considered to be an excellent food fish in the Indo-Pacific and eastern Mediterranean regions. The distribution of Rabbitfish is influenced by the quality of the invertebrate benthic community, which constitutes their food supply (Wassef and Abdul Hady, 1997). Because benthic fauna are intimately related to sediment, which accumulates sources of pollution, they constitute an indicator of environmental quality (Peterson et al., 2000; Salas et al., 2004). In this work, the Rabbitfish *Siganus canaliculatus* was selected as an indicator species due to its great economic and commercial interest. It is dominantly shallow water inhabitants and considered as one of the most abundant and representative species of Arabian Gulf coasts as well as exposed to sediment contaminants by foraging on benthic fauna, also by direct contact (e.g., through gill epithelia) with sediment particles or surrounding water (Al-Saleh and Shinwari, 2002). The gills are the first target to waterborne contaminants (Perry and Laurent, 1993) due to the complexity and constant contact with the surrounding water. In fact, contaminants enter the fish through the gills and exert their primary toxic effects on the branchial epithelium (Sakuragui et al., 2013). Therefore, changes in fish gills are among the most commonly recognized responses to environmental stressors and are indicative of chemical stress (Au, 2004). In addition, the liver is known to be the major detoxification organ and is the site of multiple oxidative reactions and maximal free radical generation (Avci et al., 2005).

The current study aims to assess the biochemical responses in the gills and liver, as biomarkers of exposure, of a population of *S. canaliculatus* inhabiting three different localities along the Saudi coast of the Arabian Gulf affected by anthropogenic inputs. Estimation of hydrocarbon concentrations in sediments as well as the gills and liver tissues of fish caught from the inspected locations was conducted in parallel to the biological investigations to discriminate the load of hydrocarbons pollution status of sampling locations.

2 Materials and Methods

2.1. Location and sampling

From March to August 2014, samples of sediments and *S. canaliculatus* fish were collected at three selected locations along the coastal Arabian Gulf of the Saudi Eastern Province as illustrated in Fig.1. Al-Dammam coast is relatively free of mining and industrial activities and was thus considered as a reference location. Dareen coast is often extensively contaminated by receiving various pollutants. The major anthropogenic sources which contribute significantly to the pollution in Dareen coastal region are mining, smelting, industrial sources, urban waste, wastewater discharges and shipping activities (Youssef et al., 2016). An approximate distance of 26 km between Al-Dammam and Dareen locations. Industrial production was mainly the source of pollution at Maniefa coast. It receives a huge amount of wastewater and other pollutants from drilling oil, global oil transportation, human and industrial activities (El-Sorogy and Youssef, 2015). Maniefa was located 160 km far from Al-Dammam and Dareen locations.

Sediment samples were collected using a stainless steel grab sampler and stored in clean polyethylene bags and frozen in a deep freezer at -20°C until analysis. *S. canaliculatus* fish samples were collected by trawl from the selected locations. Total lengths of specimens ranged between 17-36 cm and their weights fluctuated between 90-145 gm. The rabbitfish were transported from the field to the laboratory in an ice box. From each sampling location, about 65 fish were employed in this study. Fish were dissected and fresh samples of gills and liver tissues were kept frozen and stored at -80°C until used.

2.2. Analytical method for determination of fractionated hydrocarbons

The samples were analysed following well established technique (UNEP/IOC/IAEA, 1992). Sediment samples were freeze-dried, dry/wet ratio determined and then sieved through a stainless steel mesh (250 μm). Each sediment sample (30 g) was extracted and concentrated by rotary evaporation. About 10 g from each fish gills and liver tissue were homogenized, blended. The mixture was extracted by using soxhelt apparatus. Anhydrous sodium sulfate (30 g) was extracted in the same manner as the sample and used as the blank. The extracted volumes of sediment as well as fish tissue were passed through the silica column prepared by slurry packing 10 g of silica, followed by 10 g of alumina and finally 1 g of anhydrous sodium sulphate. Eluted samples with hexane were concentrated under a soft flow of purified nitrogen to about 0.2 ml, prior to injection into GC/FID for analysis. The samples were analyzed by a Hewlett Packard 5890 series II GC gas chromatograph fitted with an electron capture detector and flame ionization detector (FID).

2.3.Biochemical assessment

2.3.1. Tissue homogenate preparation

A composite tissue samples from either gills or liver were weighed and homogenized with 10 volumes (w/v) of ice-cold saline solution (0.9%) using a homogenizer (Tekmar tissumizer) for 30 s. The homogenates were centrifuged at 6500 rpm for 30 min at 4 °C using IEC-CRU5000 centrifuge.

2.3.2. Assay of glycogen, total lipids and total protein levels

Total lipids were determined following the method of Folch et al. (1957) after extraction with dichloromethane-methanol solution. After lipid extraction, the lipid-free residues were filtered and digested in NaOH solution (1N) for protein and glycogen measurement. Protein was measured following Lowry et al. (1951), using bovine serum albumin as a standard. Glycogen, after ethanol precipitation, was hydrolyzed by amyloglucamylase and measured by the glucose oxidase method (Hugget and Nixon, 1957).

2.3.3. Measurement of transaminase activities (AST and ALT)

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) enzyme activities were measured spectrophotometrically following the method of Reitman and Frankel (1957). Result was expressed as μ moles pyruvate/mg protein/h.

2.3.4. Measurement of acid phosphatase (ACP) and alkaline phosphatase (ALP)

ACP and ALP activities were determined according to the method of Andersch and Szcypinski (1947). Homogenates (50 mg/mL) were prepared in ice-cold 0.9% sodium chloride solution. *P*-nitro phenol was used as standard. Result was expressed as *P*-nitro phenol formed/30 min/mg protein.

2.3.5.Measurement of Acetylcholinesterase (AChE)

The rate of AChE activity was measured photometrically by monitoring the appearance of thiocholine at 412 nm (Ellman et al., 1961). The reaction mixture (3.0 ml) consisted of 0.05 M Tris–HCl buffer (pH 8.0), 0.34 mM DTNB, 1 mM acetylthiocholine and suitable amount of tissue homogenate. The reaction was followed by measuring the formation of thiocholine–DTNB complex at room temperature. The AChE activity was expressed as nmole thiocholine formed/min/mg protein.

2.3.6.Measurement of antioxidant enzymes

SOD activity was estimated by the method of Kakkar et al. (1984). The enzyme activity was calculated as nmol NADPH/min/mg protein. CAT activity was assayed by the method of Claiborne (1985) with some modifications as described by Ahmad et al. (2000). CAT activity was calculated in terms of nmol H_2O_2 consumed/min/mg protein. GPx activity was assayed

according to the procedure described by Mohandas et al. (1984). The enzyme activity was calculated as nmol NADPH oxidized/min/mg of protein. In addition, GST activity was determined by the method of Habig et al. (1974). The enzyme activity was calculated as nmol CDNB oxidized/min/mg protein. GR activity was determined by the procedure of Carlberg and Mannervik (1975). GR activity was expressed as nmol NADPH oxidized/min/mg protein.

2.3.7. Measurement of reduced glutathione (GSH*)*

GSH was determined as described by Ellman (1959) by measuring the rate of formation of chromophoric product in a reaction between 5,5́-dithiobis-2- (nitrobenzoic acid) (DTNB) and free sulfhydryl groups.

2.3.8. Assay of lipid peroxidation (LPO)

LPO was determined by the method of Utley et al. (1967) with some modifications as adopted by Fatima et al. (2000). The level of LPO was expressed as nanomoles of thiobarbituric acid reactive substance (TBARS) formed/h/mg of protein.

2.4.Statistical analysis

Data were analyzed with one-way analysis of variance (ANOVA). In case of significant differences the ANOVA was followed by the LSD post hoc test. All statistical analyses were conducted using the software package SPSS, version 16.0 (SPSS Inc., Chicago, I1., USA). To all tests, significance was assigned for $P<0.05$.

3 Results

3.1. Analysis of fractionated hydrocarbon residues in sediments and tissue samples

Most of the measured compounds were found at levels higher than their detection limits. As the distribution of hydrocarbons data were markedly skewed, logarithmic transformations of the data were applied. The concentrations of ALIP (pristane and phytane), and PAHs (Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene and Chyrsene) were detected in most of the analyzed samples. The minimum value of ΣALIP in sediment samples was recorded at Al-Dammam while the maximum value was recorded at Dareen coast. Eight PAHs members were identified in the sediments where important qualitative differences among the different sampling locations were observed. The minimum value of ΣPAHs was detected at Al-Dammam while the maximum value was detected at Maniefa. During the study period, the sum of five IARC (1987) probable and possible human carcinogenic PAHs (ΣPAH_{CARC}) {phenanthrene, anthracene, fluoranthene, pyrene and chrysene} was highest at Dareen coast as compared with the other locations (Table 1). The chemical analysis of the gills and liver revealed the presence of ALIP and PAHs in both organs of varying concentrations among the sites. The minimum value of ΣALIP was recorded at Al-Dammam while the maximum value was recorded at Maniefa. A comparison between the concentrations of PAHs in gills and liver samples during the study period is presented in table 1. Generally, concentrations of PAHs among the different locations was ranked as follows: Maniefa > Dareen > Al-Dammam. Moreover, ΣPAH_{CARC} was the highest at Maniefa in both tissue samples during the study period.

3.2. Biochemical assessment:

3.2.1. Glycogen, total lipids, and total protein levels:

Data for levels of glycogen, total lipids and total protein in gills and liver of *S. canaliculatus* caught from the selected locations were summarized in table 2. It is clear that most of these tested parameters were significant ($P<0.05$) lowest in polluted locations Dareen and Maniefa as compared to the reference location (Al-Dammam). Multiple comparisons between means (LSD) revealed that most of these tested parameters exhibited significant changes between all studied locations except for lipid levels in the gills which displayed an insignificant ($P>0.05$) decrease between Dareen and Maniefa. In addition, the levels of these parameters in gill tissues displayed the lowest mean value at Maniefa (-43.75%, -29.17%, and -22.05% of the reference site) respectively. However, in liver, the levels of these parameters decreased significantly ($P<0.01$) by -72.53%, -44.79%, and -34.23% respectively in fish caught from Maniefa when compared to those of Al-Dammam.

3.2.2. Enzyme activities:

Biochemical estimations of the activity of the enzymes (AST, ALT, ALP, ACP, and AChE) in gills and liver of *S. canaliculatus* were illustrated in table 3. All tested enzymes were lowest excluding AST and ALT in *S. canaliculatus* caught from polluted locations (Dareen and Maniefa) as compared to the reference site. Concerning AST and ALT, the highest mean values were recorded in fish caught from Maniefa (+5.11% and +52.13% of the reference site) in gills and +70.89% and +43.31% in liver tissues respectively. As regards ALP, ACP and AChE, they displayed the lowest mean value of its activities in gills at Maniefa (-16.61%, -20.38% and -21.10% of the reference site respectively). However, in the liver tissues, these enzymes decreased significantly ($P<0.05$) by -43.24%, -38.95% and -21.40% respectively when compared to those of Al-Dammam.

3.2.3. Oxidative stress biomarkers:

Evaluation of some antioxidant biomarkers (SOD, CAT, GPx, GST and GR) and TBARS in gills and liver were presented in table 4. A marked decrease in the activities of antioxidant enzymes (SOD, GPx and GR) was apparent in gills and liver of fish caught from the polluted locations Dareen and Maniefa. In gills, SOD, GPx and GR displayed the lowest mean value of its activities at Maniefa (-36.35%, -19.27% and -30.92% of the reference site respectively).

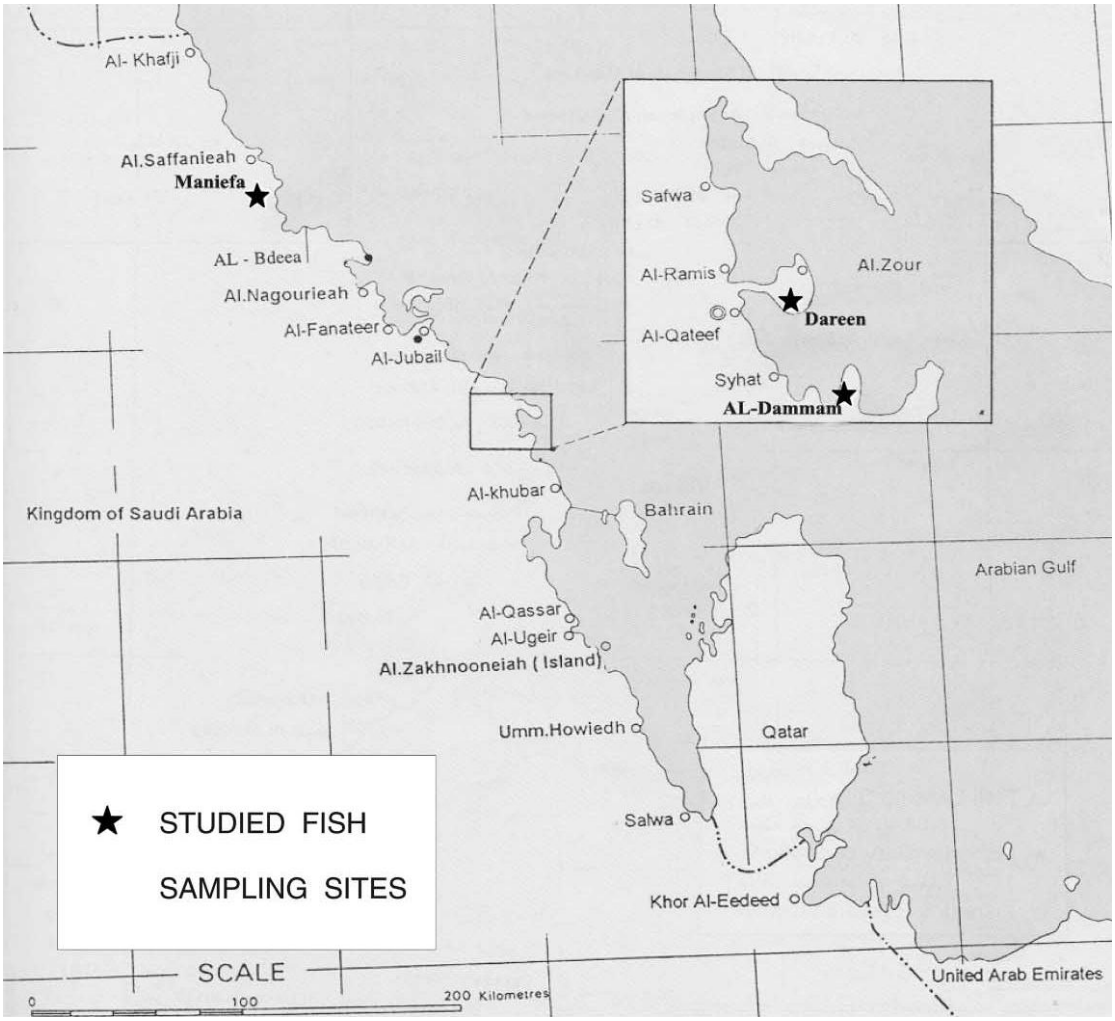

Fig.1. Map showing the locations of the sampling area on the Saudi coastline.

However, in liver tissue, these enzymes decreased significantly (P<0.01) by -31.03%, -43.95% and -33.97% respectively when compared with fish from reference location. Moreover, a tendency towards an increase in CAT and GST activities according to the pollution gradient was observed. As shown in table 4, the average activities of gills CAT and GST of *S. canaliculatus* caught from Maniefa were significantly (P<0.05) higher by +33.07% and +49.64% respectively than that found in the reference fish caught from the Al-Dammam. In addition, these enzyme activities in liver tissue showed significant (P<0.001) increase by +39.60% and +46.39% respectively at Maniefa coast. In addition, the highest GSH content in investigated tissues was observed at Al-Dammam. GSH level decreased significantly (P<0.05) by -20% and -22.22% of rabbitfish caught from Maniefa in gills and livers, respectively when compared to those caught from the reference location. However, the highest TBARS level was observed in the liver of fish caught from Maniefa. It increased significantly (P<0.001) by +41.79% and +67.25% of fish caught from Maniefa in gills and livers, respectively, when compared to those caught from the reference location (Al-Dammam).

4 Discussion

Pollution by ALIP, PAHs residues and persistent organic pollutants (POPs) has spread all over the world as evidenced by their detection both in humans and wildlife. Within the marine environment, the coastal areas deserve special consideration as primary receivers of urban and industrial chemical inputs. However, little attention has been paid to shallow closed water basins of the marine ecosystem (Storelli and Marcotrigiano, 2006; Paulino et al., 2014).

Table 1. Residual levels of measured aliphatic and aromatic hydrocarbons (µg/g) in sediments, gills and liver of *Siganus canaliculatus* collected from the studied locations along the Saudi coast of the Arabian gulf and the results of one-way ANOVA assessing the effects of location on these variables.

Compounds[•]		Al-Dammam (Reference location)	Dareen	Maniefa
Pristane	sediments	ND	0.06 ± 0.03^{a}	0.15 ± 0.12^{b}
	gills	0.04 ± 0.03^{a}	0.25 ± 0.05^{b}	0.44 ± 0.21^{c}
	liver	ND	0.13 ± 0.02^{a}	3.14 ± 1.22^{b}
Phytane	sediments	0.01 ± 0.01^{a}	0.02 ± 0.01^{a}	0.03 ± 0.01^{b}
	gills	0.29 ± 0.04^{a}	$0.27 \pm 0.08^{a,b}$	0.31 ± 0.05^{b}
	liver	ND	1.16 ± 0.14^{a}	3.11 ± 1.70^{b}
ΣALIP	sediments	1.27 ± 0.12^{a}	6.54 ± 2.120^{b}	4.81 ± 1.18^{b}
	gills	1.25 ± 0.52^{a}	11.20 ± 2.14^{b}	17.26 ± 4.61^{c}
	liver	0.62 ± 0.18^{a}	6.27 ± 2.62^{b}	10.35 ± 3.85^{c}
Naphthalene	sediments	ND	0.25 ± 0.14^{a}	0.62 ± 0.22^{b}
	gills	ND	ND	ND
	liver	ND	ND	0.56 ± 0.31
Acenaphthene	sediments	0.47 ± 0.16^{a}	0.33 ± 0.05^{a}	ND
	gills	ND	0.37 ± 0.11	ND
	liver	ND	0.81 ± 0.16^{a}	1.21 ± 0.25^{b}
Fluorene	sediments	0.15 ± 0.01^{a}	0.21 ± 0.05^{b}	0.24 ± 0.03^{b}
	gills	0.26 ± 0.01^{a}	0.28 ± 0.02^{a}	0.33 ± 0.12^{a}
	liver	0.12 ± 0.01^{a}	ND	0.25 ± 0.03^{b}
Phenanthrene	sediments	0.25 ± 0.01^{a}	1.37 ± 0.02^{b}	0.28 ± 0.01^{a}
	gills	0.33 ± 0.02^{a}	0.37 ± 0.18^{a}	0.41 ± 0.02^{a}
	liver	0.07 ± 0.02^{a}	0.25 ± 0.13^{b}	0.31 ± 0.10^{c}
Anthracene	sediments	0.06 ± 0.02^{a}	0.07 ± 0.01^{a}	0.12 ± 0.02^{b}
	gills	0.14 ± 0.03^{a}	0.17 ± 0.05^{a}	0.21 ± 0.01^{a}
	liver	0.41 ± 0.07^{a}	0.57 ± 0.06^{a}	0.72 ± 0.02^{b}
Fluoranthene	sediments	0.47 ± 0.07^{a}	0.75 ± 0.01^{b}	1.03 ± 0.03^{c}
	gills	0.05 ± 0.02^{a}	0.18 ± 0.04^{b}	0.22 ± 0.06^{b}
	liver	0.13 ± 0.24^{a}	0.22 ± 0.12^{b}	0.74 ± 0.13^{c}
Pyrene	sediments	0.49 ± 0.04^{a}	0.62 ± 0.02^{a}	1.03 ± 0.52^{b}
	gills	0.07 ± 0.04^{a}	1.25 ± 1.37^{b}	0.08 ± 0.07^{a}
	liver	0.14 ± 0.02^{a}	$0.19 \pm 0.03^{a,b}$	0.26 ± 0.14^{b}
Chrysene	sediments	0.06 ± 0.01^{a}	0.07 ± 0.02^{a}	0.05 ± 0.02^{a}
	gills	ND	0.08 ± 0.01^{a}	1.17 ± 0.07^{b}
	liver	ND	0.04 ± 0.01^{a}	0.05 ± 0.04^{a}
ΣPAHs	sediments	1.82 ± 0.25^{a}	3.83 ± 0.19^{b}	7.33 ± 2.42^{c}
	gills	0.93 ± 0.14^{a}	13.73 ± 1.65^{b}	15.53 ± 0.56^{b}
	liver	10.83 ± 0.31^{a}	$11.27 \pm 0.52^{a,b}$	14.43 ± 0.31^{b}
ΣPAH_{CARC}	sediments	1.33 ± 0.02^{a}	2.88 ± 0.32^{b}	2.51 ± 0.24^{b}
	gills	0.59 ± 0.08^{a}	2.05 ± 0.03^{b}	2.09 ± 1.03^{b}
	liver	0.75 ± 0.15^{a}	1.27 ± 0.26^{a}	2.08 ± 1.63^{b}

Values with different superscripts are significantly different (LSD multiple range test, $P<0.05$).
N D = not detected
ALIP = aliphatic hydrocarbons; PAHs = Polycyclic aromatic hydrocarbons; PAH_{CARC} = carcinogenic PAHs
Each value represents the mean of 7 samples ± SE.

Table 2. Effect of fractionated hydrocarbons on some biochemical parameters in gills and liver of *Siganus canaliculatus* caught from the three studied locations along the Saudi coast of the Arabian gulf and results of one-way ANOVA assessing the effects of location on these variables.

Biochemical Parameters (units)•		Al-Dammam	Dareen	Maniefa
Glycogen (mg /100 mg tissue)	gills	0.80 ± 0.07^{a}	0.64 ± 0.04^{b}	0.45 ± 0.03^{c}
	liver	0.91 ± 0.05^{d}	0.58 ± 0.04^{e}	0.25 ± 0.03^{f}
Total Lipids (mg /100 mg tissue)	gills	0.72 ± 0.02^{a}	0.53 ± 0.02^{b}	0.51 ± 0.04^{b}
	liver	0.96 ± 0.05^{d}	0.72 ± 0.03^{e}	0.53 ± 0.02^{f}
Total Protein (mg /100 mg tissue)	gills	18.23 ± 2.11^{a}	16.17 ± 1.47^{b}	14.21 ± 1.83^{c}
	liver	20.48 ± 2.72^{d}	18.44 ± 1.63^{e}	13.47 ± 1.24^{f}

-Each value is expressed as the mean of 9 samples ± SE.
Values with different superscripts are significantly different (LSD multiple range test, $P<0.05$). Series a-c is used for gills; series d-f is used for liver.

Table 3. Enzymatic activities in gills and liver of *Siganus canaliculatus* caught from the studied locations along the Saudi coast of the Arabian gulf and the results of one-way ANOVA assessing the effects of location on these variables.

Enzymes (units)•		Al-Dammam	Dareen	Maniefa
AST	gills	7.43 ± 0.83^{a}	7.63 ± 0.75^{a}	7.81 ± 0.66^{a}
(µ moles pyruvate/mg protein/h)	liver	9.62 ± 1.03^{d}	12.83 ± 1.32^{e}	16.44 ± 2.36^{f}
ALT	gills	6.33 ± 0.82^{a}	7.10 ± 0.61^{a}	9.63 ± 1.04^{b}
(µ moles pyruvate/mg protein/h)	liver	10.83 ± 1.52^{d}	12.88 ± 1.36^{e}	15.52 ± 1.64^{f}
ALP	gills	2.71 ± 0.22^{a}	2.63 ± 0.35^{a}	2.26 ± 0.14^{b}
(ρ-nitro phenol formed/30 min/mg protein)	liver	3.77 ± 0.62^{d}	2.82 ± 0.42^{e}	2.14 ± 0.41^{f}
ACP	gills	3.73 ± 0.25^{a}	3.15 ± 0.27^{b}	2.97 ± 0.18^{b}
(ρ-nitro phenol formed/30 min/mg protein)	liver	2.85 ± 0.12^{d}	2.16 ± 0.24^{e}	1.74 ± 0.05^{f}
AChE	gills	27.63 ± 2.42^{a}	24.27 ± 1.24^{b}	21.80 ± 1.06^{c}
(nmole thiocholine /min/mg protein)	liver	32.85 ± 3.85^{d}	29.17 ± 1.73^{e}	25.82 ± 1.52^{f}

-Each value is expressed as the mean of 9 samples ± SE.
AST = Aspartate aminotransferase; ALT = Alanine aminotransferase; ALP = Alkaline phosphatase; ACP = Acid phosphatase; AChE = Acetylcholinesterase.
Values with different superscripts are significantly different (LSD multiple range test, $P<0.05$). Series a-c is used for gills; series d-f is used for liver.

As it is well known that these pollutants are characterized by their hydrophobic behaviour, enter the marine environment mostly in absorption on particles suspended along the water column (Scarpato et al., 2010). Sediments act as a trap for these pollutants and represent a natural matrix to be considered in order to assess chemical contamination (Bozcaarmutlu et al., 2009). In the current study, gill and liver tissues of Rabbitfish caught from Dareen and Maniefa showed higher concentrations of ALIP and PAHs compared to fish from a reference location (Al-Dammam) indicating the presence of specific pollution with hydrocarbons and industrial waste discharge in these investigated locations. These results showed that these investigated locations represent a large enough spatial variability concerning the concentrations of checked pollutants and usefulness of the Rabbitfish *S. canaliculatus* as pollution sentinels for these tested hydrocarbons. The present study declared that the sum of aliphatic fractions was higher in Dareen and Maniefa coast, indicating the presence of fresh petroleum source as stated by Sakuragui et al. (2013). Pristane and Phytane are common isoprenoids detected in coastal marine sediments were also detected in

the investigated polluted locations. They are present in most oil products, usually as the main component parts within a much wider range of isoprenoid alkanes and usually considered as good indicators of oil pollution (Readman et al., 2002). The occurrence of PAHs in fish gills and liver is an indication of the contamination of coastal waters with these compounds. The major inputs of hydrocarbon pollutants in the Saudi coast of the Arabian Gulf are from anthropogenic industrial activity, natural fires and/or combustion of organic matter (Youssef et al., 2016). The general distribution of the PAHs in the majority of samples reflects the high contribution of pyrolytic sources because of the prevalence of parent PAHs over their alkylated derivatives (Garrigues et al., 1995). Exposure pathways of PAHs to fish include bioconcentration from the water across their gills and skin (Gobas et al., 1999) and ingestion of PAHs-contaminated particulate matter along with food (Mohammadi et al., 2013), as PAHs readily adsorb onto particulate organic matter (Raoux et al., 1999). PAHs tend to accumulate in the fatty tissues of fish following their uptake (Bouloubassi et al., 2001). Fish are also likely to be contaminated with PAHs as they come into contact with fishing nets contaminated with oil from oil slicks, and PAHs-contaminated plastics dumped into the sea, which is usually caught with the fish (Nyarko et al., 2011). Pimentel et al. (2014) found that different pollution sources give rise to different PAHs assemblages, thus, the findings from the present study suggest a common source of PAHs in the present three studied locations. The concentrations of contaminants in fish reflect the state of contamination of the environment (Lanfranchi et al., 2006), accordingly, the observed levels of total PAHs in Rabbitfish caught from Dareen and Maniefa indicate high contamination of the environment with these pollutants. These results are in accordance with that reported by Baumard et al. (1998). The concentration range of total PAHs was lower than the acceptable level (100 µg/g) on the basis of wet weight in edible fish tissue as suggested by Paulino et al. (2014).

Table 4. Oxidative stress biomarkers in gills and liver of *Siganus canaliculatus* caught from the three studied locations along the Saudi coast of the Arabian gulf and results of one-way ANOVA assessing the effects of location on these variables.

Parameter (units)▪		Al-Dammam	Dareen	Maniefa
SOD	gills	88.53 ± 4.36^{a}	79.25 ± 3.58^{b}	56.35 ± 5.24^{c}
(nmol NADPH oxidized/min/mg protein)	liver	116.36 ± 8.66^{d}	102.55 ± 8.54^{e}	80.25 ± 5.2^{f}
CAT	gills	136.82 ± 11.37^{a}	153.28 ± 12.58^{b}	182.06 ± 11.84^{c}
(nmol H_2O_2 consumed/min/mg protein)	liver	145.78 ± 13.31^{d}	168.22 ± 13.55^{e}	203.51 ± 13.52^{f}
GPx	gills	274.83 ± 14.98^{a}	255.02 ± 11.25^{b}	221.86 ± 9.34^{c}
(nmol NADPH oxidized/min/mg protein)	liver	316.75 ± 12.55^{d}	302.22 ± 18.28^{e}	177.54 ± 11.82^{f}
GST	gills	137.22 ± 9.64^{a}	161.66 ± 14.56^{b}	205.33 ± 13.82^{c}
(nmol CDNB oxidized/min/mg protein)	liver	153.26 ± 8.91^{d}	182.47 ± 11.25^{e}	224.35 ± 13.55^{f}
GR	gills	73.86 ± 3.17^{a}	64.28 ± 6.11^{b}	51.02 ± 3.14^{c}
(nmol NADPH oxidized/min/mg protein)	liver	84.93 ± 5.85^{d}	68.24 ± 3.55^{e}	56.08 ± 4.88^{f}
GSH	gills	0.15 ± 0.03^{a}	0.14 ± 0.02^{a}	0.12 ± 0.03^{b}
(nmol GSH/g tissue)	liver	0.27 ± 0.08^{d}	0.18 ± 0.05^{e}	0.21 ± 0.04^{e}
TBARs	gills	44.84 ± 2.83^{a}	48.23 ± 1.62^{a}	63.58 ± 3.52^{b}
(nmol TBARS formed/h/mg protein)	liver	67.73 ± 3.89^{d}	85.26 ± 4.14^{e}	113.28 ± 11.22^{f}

▪ Each value is expressed as the mean of 8 samples ± SE.
SOD = Superoxide dismutase; CAT = Catalase; GPx = Glutathione peroxidase; GST = Glutathione s-transferase; GR = Glutathione reductase; GSH = Reduced glutathione; TBARs = Thiobarbituric acid reactive substances

Surveys of sediment and fish have shown the marine environment around the Arabian Gulf to be polluted with a range of ALIP, PAHs and organochlorinated xenobiotics (Beg et al., 2009; de Mora et al., 2010). PAHs are known to cause growth reduction (Christiansen and George, 1995), endocrine alteration (Meador et al., 2006), malformations of embryos and larvae (Carls et al., 2008), DNA damage in fish, as well as adverse impacts on marine life (Caliani et al., 2009).

Fish are particularly sensitive to water pollution and contaminants may impair many physiological processes when metabolised by fish tissue (Durmaz et al., 2006). The present study indicated significant decrease in the levels of glycogen, total lipids and total proteins in tissues of Rabbitfish caught from the Dareen and Maniefa as compared to that of Al-Dammam but this decreasing is more obvious in liver than that in the gill tissues. Decrement in tissue glycogen and lipids in polluted fish may be explained on the basis that glycogen and lipids reserves are being used to meet the stress (Sobha et al., 2007). They also reported that fall in the glycogen content in the liver clearly indicates its fast utilization to meet the enhanced energy demands in fish exposed to the pollutants through hexose monophosphate pathway or glycolysis. It is assumed that the reduction in glycogen level may be due to the suppression of hormones which contribute to glycogen synthesis. In addition the results obtained by Sobha et al (2007) are in agreement with the results of the present study in which the total protein content was found to be reduced in tissues of the fish exposed to pollution. They attributed the decrease in the protein content observed in most of the polluted fish tissues to metabolic utilization of the ketoacids to gluconeogenesis pathway for the synthesis of glucose, or for the maintenance of osmo and ionic regulation and the production of heat shock proteins or destructive free radicals or could be a part of pollutants induced apoptosis (Al-Ghais, 2013).

The significant high activities of AST and ALT in livers of study Rabbitfish collected from the Dareen and Maniefa are considered as a functional response that deals with the extra energy requirements and the increasing rate of metabolism to cope with hydrocarbons stress (Giannini et al., 2005; Kavitha et al., 2010). In addition, high level of aminotransferases may result from damage in liver tissues by the action of the recorded bioaccumulated xenobiotics which may finally become associated with pathological alterations (Omar et al., 2014). The metabolic activity of the hepatic cells has been considered as an important protective mechanism against toxicants and the transformations involved have been referred to as detoxification. In the current study, the decrease of ALP and ACP activities observed in the fish caught from the polluted locations is in accordance with the results of Yacoub and Gad (2012) in which these enzymes were significantly lower in the gills and liver of the studied fish from polluted sites in the River Nile which indicated disturbance in the structure and integrity of cell organelles, like endoplasmic reticulum and membrane transport system. Also, Gabriel et al. (2012) pointed out that enzyme activity of ALP in gills of *C. gariepinus* were inhibited by different levels of PAHs concentrations; which indicated that the PAHs interfered with the metabolic process and these could affect the physiological functions of the fish in an aquatic environment. The average gill and hepatic AChE activity in *S. canaliculatus* caught from Maniefa was significantly lower than that found in the reference fish caught from Al-Dammam. These findings are in line with an inhibition of AChE activity in the liver and gill of *G. viviparous* caught from a lake in Mexico receiving untreated domestic wastewater and agricultural runoff (Lopez-Lopez et al., 2006). Likewise, low hepatic AChE activity was observed in *M. cephalus* and *Z. ophiocephalus* collected from a highly eutrophic Orbetello Lagoon receiving town pollutant effluent in Italy (Corsi et al., 2003). As suggested in earlier studies (Lama and Gray, 2003; Stefano et al., 2008; Al-Ghais, 2013), the present results indicated that the AChE inhibition was related to neurotoxic chemicals like PAHs. These observations strongly support the importance of Rabbitfish tissue AChE activity as a biomarker for the assessment of physiological changes in fish caused by fractionated hydrocarbons pollution.

The present study showed overall lower SOD, GPx and GR activities in the liver and gills of *S. canaliculatus* caught from Dareen and Maniefa coast compared with fish from the reference site, indicating antioxidant enzyme inhibition. Low SOD activity has also been reported in *M. cephalus* from a polluted estuary in India (Padmini et al., 2009) and in *D. labrax* from Aveiro Lagoon in Portugal (Maria et al., 2009). In contrast, previous studies in sardine showed higher SOD activities in fish from polluted areas (Peters et al., 1994). Accordingly, the low enzyme activities in fish from Maniefa can be associated with deficiency to compensate for oxidative stress, possibly due to high levels of pollutant exposure. SOD is the enzyme that catalyzes the dismutation of the superoxide anion to O_2 and H_2O_2 (Livingstone, 2001). Although the elevation in ROS productivity is expected to enhance antioxidant defenses, the enzymes concerning with the antioxidant protective process may be suppressed by the surplus of oxidants that are their own substrate. Thus, SOD can be suppressed by extreme manufacture of superoxide anion during the detoxification process, which causes the oxidation of the cysteine in SOD and subsequently the SOD deactivation and/or H_2O_2 accumulation (Bagnyukova et al., 2006; Sakuragui et al., 2013). Earlier studies indicated SOD suppession by transitory H_2O_2 accumulation in the liver after fish were exposed to pesticides (Modesto and Martinez, 2010). The current results suggest the possibility of SOD decline due increase of superoxide anion in the gills and H_2O_2 accumulation in the liver of *S. canaliculatus* from the Maniefa.

GSH, a major cytosolic non-protein thiol, is involved in the cellular defense against the toxic action of xenobiotics and oxyradicals (Van der Oost et al., 2003). The results reported in this study showed that Rabbitfish caught from Maniefa and Dareen exhibited a significant

decrease in levels of GSH in the liver of fish indicating that change in ALIP and PAHs concentration affect glutathione synthesis. The down-regulation of GSH level is a signal indicating the inability of liver to successfully scavenge oxyradicals. Exposure to hydrocarbons has been shown to cause a time and dose-dependent increase or decrease of GSH concentrations in various fish species (Brito et al., 2012). Because GPx is a GSH-dependent enzyme, changes in GSH level may affect the activity of GPx. Therefore, in the present study, it seems that the decrease in GSH level led to inactivated GPx activity in most polluted location (Maniefa coast). GPx inhibition was also reported in liver of various fish after exposure to organic compounds or hydrocarbons (Sakuragui et al., 2013). Earlier researches demonstrated that GPx activity can be decreased by negative feedback either from excess of substrate or damage resulted from oxidative modification (Fatima et al., 2000), and a reduced GPx activity in a given tissue could indicate that its antioxidant capacity was exceeded by the accumulation of hydroperoxide products. GR, other biomarker enzyme measured in this study, maintains GSH/Glutathione disulfide homeostasis under oxidative stress conditions. Inhibition of GR activity has been reported in various field studies in fish exposed to organic pollutants such as PAHs, PCBs and halogenated xenobiotics (Gabriel et al., 2012). Significantly low GR activities have been found in the gills and liver of Rabbitfish caught from polluted Dareen and Maniefa, suggesting the presence of hydrocarbons in these sampling locations. In the present study, the significant decrease of GR activities observed in Rabbitfish from the polluted areas may reflect signs of effective toxicity and not an adaptation to chronic exposure to contaminants.

H_2O_2 accumulation due to GPx inhibition in the liver tissue contributed to the increasing CAT activity. CAT is more active at high H_2O_2 concentrations and plays a minor role in the catabolism of H_2O_2 at low production rates (Van der Oost et al., 2003). Furthermore, the increased CAT activity in the gills and liver of fish collected from Dareen and Maniefa indicates high H_2O_2 and ROS generating pollutants; this finding is in agreement with the claim that hydrocarbons stimulate ROS generation. High CAT activity was also found in the liver of *P. maculatus* collected in the Santa Branca reservoir, Brazil after an accidental spill of endosulfan, which is considered a valuable biomarker of contamination (Brito et al., 2012). The use of GST as a biomarker of exposure to organic toxicants, earned credibility in aquatic pollution biomonitoring (Simonato et al., 2011). GST catalyzes the transformation of a broad variety of electrophilic compounds to less toxic substances by conjugating them to GSH (Van der Oost et al., 2003). In the current study, GST activities, in both investigated tissues, were higher in Rabbitfish caught from polluted locations. The increased GST activities indicate the activation of the phase II biotransformation metabolism and of the antioxidant defense systems (Paulino et al., 2012). This may correspond to a gill response to eliminate contaminants and ROS by GSH conjugation in order to prevent lipid peroxidation effects (Ezemonye and Ikpesu, 2011). Induction of GST has been reported in a number of field studies in fish exposed to organic pollutants (Pereira et al., 2013). However, the antioxidant enzyme response to the gills and liver contamination did not prevent the elevation in TBARs levels in Rabbitfish from polluted locations especially Maniefa coast. These fish showed oxidative stress, which, according to Monteiro et al. (2010) is the outcome of the imbalance between pro- and antioxidant molecules favoring the pro-oxidants. The continuous generation of ROS by xenobiotics and/or by the fish detoxification processes overcomes the antioxidant defenses and results in an increase of lipid peroxidation (LPO). Elevation of LPO indicate lipid alterations in cellular membranes, DNA and protein damage, which lead to cellular dysfunction (Carvalho et al., 2012). In the current survey, the TBARs levels in the livers were higher than in the gills. Although the gills are in direct contact with surrounding water and are the principle organs that uptake pollutants due to the large surface area and the thin membrane separating water and blood, these organs seem to be less sensitive to many pollutants and transfer the pollutants quickly to the blood stream (Paulino et al., 2012).

In conclusion, the present work reinforces the suitability of oxidative stress and enzymatic biomarkers as tools for assessing hydrocarbon contamination impacts in the investigated sampling locations with several anthropogenic impacts. These biomarkers represented sensitive and efficient tools for reflecting adverse environmental conditions for Rabbitfish health. Recommendations are given for continued biomonitoring of the pollution load in the aquatic environment in Saudi eastern region coast to highlight the need of remedial action and for early warning of potential adverse effects before population and community level changes occur.

Conflict of interest

The author declared no conflicts of interest.

Acknowledgments

The Author would like to acknowledge gratefully the General Director of Fishery Research Centre in Qatief; and Mr. Fouad Abdel Kareem, the Fishery Specialist from Saudi Ministry of Agriculture and Water for their help in selecting sampling sites, providing and identification of studied fish samples.

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