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Assessment of Oxidative Stress and Histopathological Biomarkers in the Parablennius Incognitus Fish as Potential Contamination Indicators of the Bay of Sousse (Tunisia)

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Abstract

Oxidative stress and histopathological biomarkers were investigated in the gills and liver of *Parablennius incognitus* fishes. Individuals sampled in the bay of Sousse were compared to fishes from a reference site, i.e. Ghdamsi Island (Tunisia). Severe biochemical and histological alterations were observed in fishes from Sousse bay and associated with urban discharges contamination. Several tissue alterations were also observed in gills, particularly lamellar epithelium detachment, disorganization of pillar cells, and hypertrophy of chloride cells. In the liver we noted particularly hypertrophy of hepatocytes, congestion and dilation of the central vein and sinusoid capillaries, pyknotic nucleus, and hepatic steatosis. The high reduction of superoxide dismutase and catalase activities in gills and of superoxide dismutase and glutathione peroxidase activities in the liver of contaminated fishes may explain the increase of lipoperoxidation in both organs of *P. incognitus*. The integrated biomarker response values found in individuals from the contaminated site were in good agreement with alteration of physico-chemical parameters and with the high level of Cd concentrations detected in water of the stressful place, i.e. the bay of Sousse. Oxidative stress and histopathological alterations were sensitive biomarkers to discriminate between fishes from the polluted Sousse bay site and those from the reference site, suggesting their potential utility in bio monitoring.

Keywords: *Parablennius incognitus*; Histology; Superoxide dismutase; Catalase; Glutathione peroxidase; Malondialdehyde; Oxidative stress; Pollution

Introduction

The marine littoral ecosystem and estuaries are threatened by increasing levels of various pollutants originating from human activities, urban, agricultural and industrial discharges. A considerable number of chemicals have already been released into the environment and persist in sediment, water and biota. Organic and inorganic chemicals are a major factor of aquatic organisms' poisoning [1]. In the recent years, environmental biomarkers have quickly evolved and have been extensively used for the assessment of the potential adverse effects of pollutants on the environment [2]. The assessment of biological effects of chemicals, from molecular to tissue levels, has been considered as an effective biological tool in the bio monitoring of marine ecosystem contamination [3]. Blenny fishes are interesting sentinel organisms for contaminants exposure in bio monitoring programs, since their diversity, their ubiquity, and their position in the trophic chain make them particularly exposed to sediment-associated contamination. In the Tunisian coast, fourteen Blenniidae species have been identified [4,5]. The use of Blennies for toxicity assays has been the subject of international recommendations [6-10]. However, only a few ecotoxicological studies have been conducted to date on Blenniidae species. For example, the works of Barhoumi et al. using *Salaria basilisca*, reported first the bioaccumulation of cadmium in the liver and gills of this Blennie fish, [11] and then the spatial and seasonal variability of some biomarkers, i.e. metallothioneins with their reduced and oxidized forms, labile zinc, 7-ethoxyresorufin-O-deethylase, DNA strand breaks and lipid peroxidation [12]. Beside, Messaoudi et al. [13] studied diverse antioxidant enzyme activities under laboratory controlled conditions. Thus, to the best of our knowledge, the present work is the first study that aims to assess the biochemical and histological biomarkers responses in the Blenniidae family in field conditions and particularly in the *P. incognitus* species from the Tunisian coast. Biochemical biomarkers are extensively used in bio monitoring of aquatic ecosystems using bivalves and fishes, as well as indicators of chemical complex pollution. They have been validated in crustaceans, bivalves and fishes from the Tunisian coast area [14-19]. The biomarker approach, coupled with a chemical analysis, was used to examine the environmental status of several sites in the Tunisian coast area, and to assess the relationships between pollutants and biological responses. The antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxydase (GPx) are the primary enzymes of the antioxidant defence against oxidative damages generated by multiple environmental stressors and have been studied in fish of Tunisian marine environments [3,19]. Their induction reflects a specific response to variable pollutants including hydrocarbons and metals [20]. In addition, malonedialdehyde (MDA) is a biomarker of oxidative damage that reflects the state of lipid peroxidation of the membranes [21]. Finally, the integrated biomarker response (IBR) was computed from the biochemical biomarker measurements to assess the

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ecological risk of the polluted area [in the Tunisian coast] and establish a possible correlation between metals, physico-chemical parameters and their biological effects. Altogether, when used with the Mediterranean fish *P.incognitus,* oxidative stress indicators and histopathological alterations were sensitive biomarkers to discriminate between fishes from the polluted Sousse bay site and those from the reference site, suggesting their potential utility in biomonitoring.

Materials and Methods

Studied area and fish sampling

P. incognitus was chosen because of its abundance on the Tunisian coast, which allowed spatial and seasonally comparisons. Specimens of *P. incognitus* (0.3-2.8 g weigh and 4-6 cm length) were manually collected in winter 2011, and immediately carried to the laboratory within 30 min in a 20 L tank of seawater. Twenty fishes per site were collected from Ghdamsi Island and Sousse bay (Tunisia). Thus, ten fishes per site were used for the biochemical analyses and the other ten served for the histopathological study. The two sites were selected in the east coast of Tunisia. The Sousse bay site, more precisely Sidi Abdelhamid (35°47' N, 10°40'E) is an industrial area located in the city of Sousse. This site is characterized by chronic discharges of urban and industrial pollutants by Hamdoun drain [22]. As a reference site, we selected Ghdamsi Island (Monastir, Tunisia) (36°02'N, 10°29' E), which is a relatively clean area, without industrial or urban influences (Figure 1). The reproductive season of *P.incognitus* is generally in summer (data under publication) and the sampling was carried out during the non-reproductive period to avoid interference of reproduction with the oxidative stress.

Physico-chemical analysis of water

Water quality was assessed at each fish sampling time. One surface water sample per site was taken out and analysed in situ for the following parameters: pH, dissolved oxygen (mg/L), turbidity (NTU: Nephelometric Turbidity Unit) and temperature (°C). All determinations were carried out in triplicate and the variation coefficient was usually less than 10%. For the Cadmium and Lead metal determinations, seawater samples were filtered at the time of collection through a 0.45 µm membrane and acidified for the determination of dissolved elements. All water samples were analysed by inductively coupled plasma/atomic emission spectrometry using OPTIMA 3300 RL apparatus and according to the standard NF EN ISO 11885.

Biochemical analysis

Tissues (liver and gills) of *P. incognitus* were homogenized in phosphate buffer (0.1 M; pH7.4) in a ratio of 1g of tissue per 3 ml buffer. This homogenate was centrifuged at 9500 rpm for 30 min at 4°C. Then, the recovered supernatant was conserved at-80°C until oxidative stress biomarkers measurements. The obtained supernatant was used for total protein determination, superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) activities, lipid peroxidation (MDA) level, and protein determination.

Total protein determinations: Protein contents in the individual supernatant tissues were determined according to Bradford's method (1976) by using bovine serum albumin (BSA) as a standard. 50 µL of supernatant was added to 150 µL of distilled water and 2 mL of Bradford's reagent. After 5 min of incubation in the darkness, the absorbance was determined at λ=595 nm.

Superoxide dismutase (SOD) determination: SOD activity was determined using pyrogallol as a substrate by the method of Marklund and Marklund [23]. This method is based on pyrogallol oxidation by the superoxide anion and its dismutation by SOD. One unit (U) of total SOD is defined as the amount of enzyme required to inhibit the rate of pyrogallol autooxidation by 50%.

Catalase (CAT) determination: Catalase activity was determined by the method of Claiborne [24] measuring the rate of enzymatic decomposition of hydrogen peroxide determined as absorbance decrements at 240 nm. Reaction mixture (final volume of 1.78 mL) contained 1.56 mL of 100 mM phosphate buffer (pH 7.5) and 200 mL of 500 mM hydrogen peroxide After a 30 sec pre-incubation time, the reaction was started by the addition of 20 µL of cytosolic fraction. CAT activity was evaluated by kinetic measurement at 25°C. Results were expressed as µmol hydrogen peroxide consumed per min and per mg of protein.

Glutathione peroxidase (GPx) determination: GPx activity was performed according to the method described by Günzler et al. [25]. In

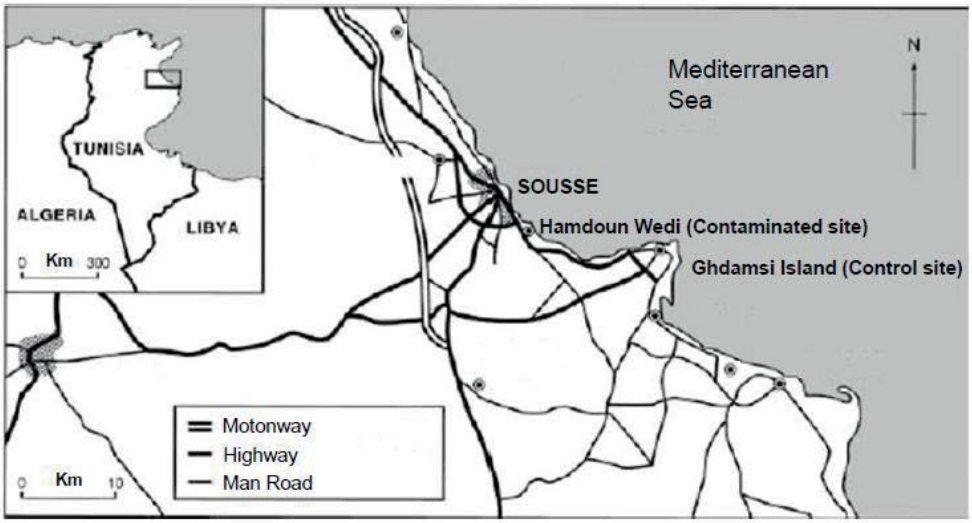

Figure 1: Map of Tunisia indicating the location of sampling sites in the bay of Sousse (Hamdoun Wadi) as a contaminated site and Ghdamsi Island (Monasti) as the reference site.

the typical assay, we used t-butylhydroxyde (t-BOOH) as oxidant and glutathionereductase (GR) allowing the reduction of GSSG to GSH by oxidizing NADPH to NADP. The activity was determined by measuring the decrement of NADPH at λ=340 nm. The activity is expressed as µmol of NADPH per min per mg of protein.

Lipid peroxidation (MDA) determination: Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species production using 1,1,3,3-tetraethoxypropane as a standard. The reaction was determined at 532 nm using thiobarbituric acid reagent according to the method of Buege and Aust (1978). Malondialdehyde (MDA) content was expressed as nmol equivalent MDA per mg of protein.

Histopathological lesion determinations

Histopathological analysis was performed on the gills and liver of 10 specimens of *P. incognitus*. Sample tissues were fixed in Bouin's solution for 24 h, dehydrated with ethyl alcohol 95% and 100%, and then transferred into paraffin. The longitudinal sections of 4 to 5 µm were stained with Masson's trichrome color and observed with optical microscope.

Statistical analysis

The biochemical biomarkers data were expressed as mean ± SD (n=10). The biochemical data were first tested for normality and homogeneity of variance to meet statistical demands. The data were analyzed by the Student t-Test using the SPSS Statistical Program (Version 19.0); significance is noted as *, $p<0.05$ or **, $p< 0.01$.

Integrated Biomarkers Response determination (IBR)

The responses of the biochemical biomarkers were integrated based on the mathematic method developed by Beliaeff and Burgeot [26]. For each biomarker: (1) Calculation of mean and SD for each site. (2) Standardization of the data for each site, i.e. Xi'=(Xi-x)/S), where Xi' is the standardized value of the biomarker, Xi is the mean value of a biomarker from each site, x is the mean of the biomarker calculated for all sites, and S is the standard deviation calculated for the site-specific values of each biomarker. (3) Using standardized data, we add the value obtained for each site Xi' to the absolute (non-negative) value of the minimum value: $B=Xi'+ | Xmin |$, where B is the score of each biomarker and for each site. Then, we adjusted the lowest value in the set to zero for all the biomarkers. (4) Calculation of star plot areas by multiplication of the obtained value of each biomarker (Bi) with the value of the next biomarker, arranged as a set. (5) Summing-up of all values. The corresponding IBR value (average of different arrangements of biomarkers in the set) is: $\{[(B_1 \times B_2)/2]+[(B_2 \times B_3)/2] + \cdots + [(B_{n-1} \times B_n)/2]\}$ where B_{n-1} and B_n are two scores of two successive biomarkers.

	Reference site	Contaminated site	Tunisian norms
Temperature (°C)	27 ± 1[a]	32 ± 2	35
pH	7.29 ± 0.06	8.83 ± 0.08	6.6-8.5
Dissolved O_2 (mg/L)	9.6 ± 0.47	4.5 ± 0.45	>6
Turbidity (NTU)	1.08 ± 0.05	2.57 ± 0.08	N.A
Total nitrogen (mg/L)	10.5 ± 2	245 ± 11.53	30
Lead (mg/L)	0.277 ± 0.01	0.277 ± 0.02	0.5
Cadmium (mg/L)	0.0038 ± 0.001	0.0198 ± 0.003	0.005

[a]Data are reported as mean ± SD of three measurements of the same sample per site. N.A: not available.

Table 1: Physico-chemical analysis of water samples from the Sousse bay polluted site and from Ghdamsi Island (Monastir) reference site.

Results

Routine physico-chemical parameters differed between reference and contaminated sites. In the Sousse bay polluted site, temperature, turbidity, total nitrogen and pH were high; and the dissolved oxygen was reduced when compared to the reference site values (Table1). In addition, a high Cd level was also noted in the seawater of the contaminated site that exceeds the Tunisian authorized level.

Biochemical biomarkers responses

Reduced SOD and CAT activities and increased MDA level in gills: The activity of antioxidative enzymes SOD and CAT was significantly reduced ($p <0.05$) in fishes from the Sousse bay contaminated site, being 72% and 48% of the reference values, respectively (Table 2). However, no significant change ($p>0.05$) in GPx activity was observed. In contrast to the reduction of antioxidant enzymes, MDA level was significantly increased by 70% with respect to the reference value (Table2), indicating a high lipid peroxidation level in fishes from the polluted site compared to the reference individuals.

Reduced SOD and GPx activities and increased MDA level in liver: In the liver, the CAT activity, which is higher than in gills, showed no significant differences between fishes from the reference site and those from the contaminated site (Table 2). By contrast, the GPx activity, unchanged in gills, was reduced to 48% of the reference value in fishes from Sousse bay (Table 2).In addition, the SOD activity was decreased to 76% of the reference value. In the same way than in gills, the reduction of antioxidant enzyme activities (i.e. GPx and SOD in liver) was associated with a significant increase in lipid peroxidation, MDA level being greater of 27.5% relative to the reference value (Table 2).

Histopathological biomarkers

Gill histopathological lesions in contaminated fishes: Figure 2A showed the typical structure of the gills of *P. incognitus* from the reference site. The primary lamellae are lined by a thick stratified epithelium containing chloride cells. In addition a cartilaginous support composed of chondrocytes could be found in the centre of each primary lamella. The secondary lamellae, located on the coast side of the primary lamellae, contain erythrocytes and pillar cells (Figure 2A) as usual. When comparing histological sections of fishes of both contaminated and reference sites, an unusual tissue aspect appears in the gill of specimens from the contaminated site. The cartilage lost its chondrocytes in 40% of the samples. Limited hyperplasia was noted in individual fishes from the contaminated site, resulting in the fusion of secondary lamellae in 40% of the samples. These lamellae were narrowed with the development of telangiectasia in 60% of the samples. Similarly, the epithelium of secondary lamellae remained a single stratum and erythrocytes formed together an epithelioid line in 60% of the samples (Figure 2B).

Liver histopathology lesions in contaminated fishes: In the liver of fishes from the reference site, the tissue sections showed clearly the homogeneous structure of the parenchyma (Figure 2C). The central vein, the sinusoids and polygonal hepatocytes could be observed in the

Biomarker	Reference Site	Contaminated site	%
Gills			
Superoxide dismutase (SOD)	41.925 ± 8.985[a,b]	32.526 ± 11.780[a,b]	76%[f]
Catalase (CAT)	3.106 ± 1.058[a,c]	1.390 ± 0.512[a,c*]	45%
Glutathione peroxidase (GPx)	1195.311 ± 520.825[a,d]	1154.000 ± 173.30[a,d]	N.A.
Lipid peroxidation (MDA)	0.040 ± 0.005[a,e]	0.0510 ± 0.005[a,e**]	127.50%
Integrated Biomarker Response (IBR)	0.812	1.364	
Liver			
Superoxide dismutase (SOD)	36.923 ± 11.845 [a,b]	26.937 ± 10.495[a,b*]	72%
Catalase (CAT)	15.294 ± 11.888 [a,c]	79.745 ± 40.780[a,c]	N.A.
Glutathione peroxidase (GPx)	973.373 ± 405.460[a,d]	470.466 ± 282.908[a,d*]	48%
Lipid peroxidation (MDA)	0.037 ± 0.007 [a,e]	0.063 ± 0.007[a,e**]	170%
Integrated Biomarker Response (IBR)	0	7.025	

[a]Data are represented as the means ± SD; n = 10. Statistical differences between contaminated and reference groups were made at 0.05 confidence level. Statistical significance of the results is indicated as *, p<0.05; **, p<0.01. Enzymatic activity units are bμmol/min/mg protein for SOD, cμmol H2O2 consumed/min/mg protein for CAT, dμmol NADPH/min/mg protein for GPx, and e nmol TBARS/mg protein for MDA level.f For the oxidative stress biomarkers, the value measured in samples from the contaminated site is given as the % of the reference value when a significant difference was observed. N.A., not applicable.

Table2: Oxidative stress biomarkers and Integrated Biomarker Response (IBR) in gills and liver of P.incognitusfishes from reference and contaminated sites.

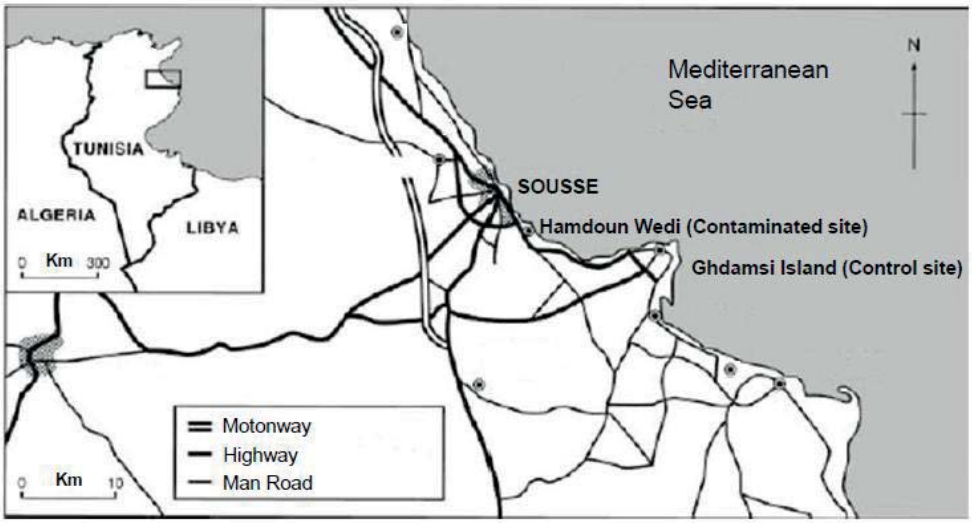

Figure 1: Map of Tunisia indicating the location of sampling sites in the bay of Sousse (Hamdoun Wadi) as a contaminated site and Ghdamsi Island (Monasti) as the reference site.

parenchyma (Figure 2C). Each hepatocyte contained a central spherical nucleus with concentrated chromatin near the nuclear membrane. In addition, the hepatocytes of reference fishes stained with Masson's trichrome showed a good storage of glycogene. The cytoplasm is homogeneous and composed of basophilic granulations. The nucleus is round with dense chromatin and individual nucleoli (Figure 2E). However, the microscopic observation of *P. incognitus* hepatic tissue of fishes from the contaminated site showed various tissue alterations. Hypertrophy of hepatocytes was seen in 40% of the samples, congestion and dilation of the central vein and sinusoid capillaries in 70% of them (Figure 2D, compared to Figure 2C). Associated to these pathological changes, a hepatic steatosis was also reported in 70% of the samples. Moreover, in 40% of the samples, the nucleolus has disappeared and the nucleus became pyknotic, displaying a dense chromatin, characteristic of necrotic cells (Figure 2F, compared to Figure 2E).

Discussion

Oxidative stress biomarkers responses

The present study investigated the oxidative stress and histopathological biomarkers responses in *P.incognitus* fishes from the bay of Sousse, an area of the Tunisian coast impacted by different anthropogenic activities. In the present work, we showed that the decrease in gills SOD, CAT, and hepatic SOD and GPx activities was highly associated with a significant increase of the lipid peroxidation in both organs of the *P. Incognitus* from Sousse bay. Various environmental pollutants may exert a negative effect on the antioxidative enzymes in particular metals, organic compounds such as polychlorobiphenyls (PCBs), and polyaromatics hydrocarbons (PAHs). We suspected that a mixture of chemicals might be present in the chronic discharges of Hamdoun Wadi site in the bay of Sousse. These environmental pollutants deeply affect the antioxidant/proxidant balance leading to the accumulation reactive oxygen species (ROS) and affecting the antioxidative enzymes [27,28]. Moreover, Vutukuru et al. [29] and Ates et al. [30] showed a decrease in SOD and CAT activity in the gills of *Oncorhynchus mykiss* and *Esomus danricus* treated with lead and copper. A recent ecotoxicological study on the *Solea solea* fishes from Sousse bay collected at the discharges point of Hamdoun Wadi, showed a high induction of hepatic and gills CAT activity [31]. The differences in CAT responses may be related to different fish species sensitivity

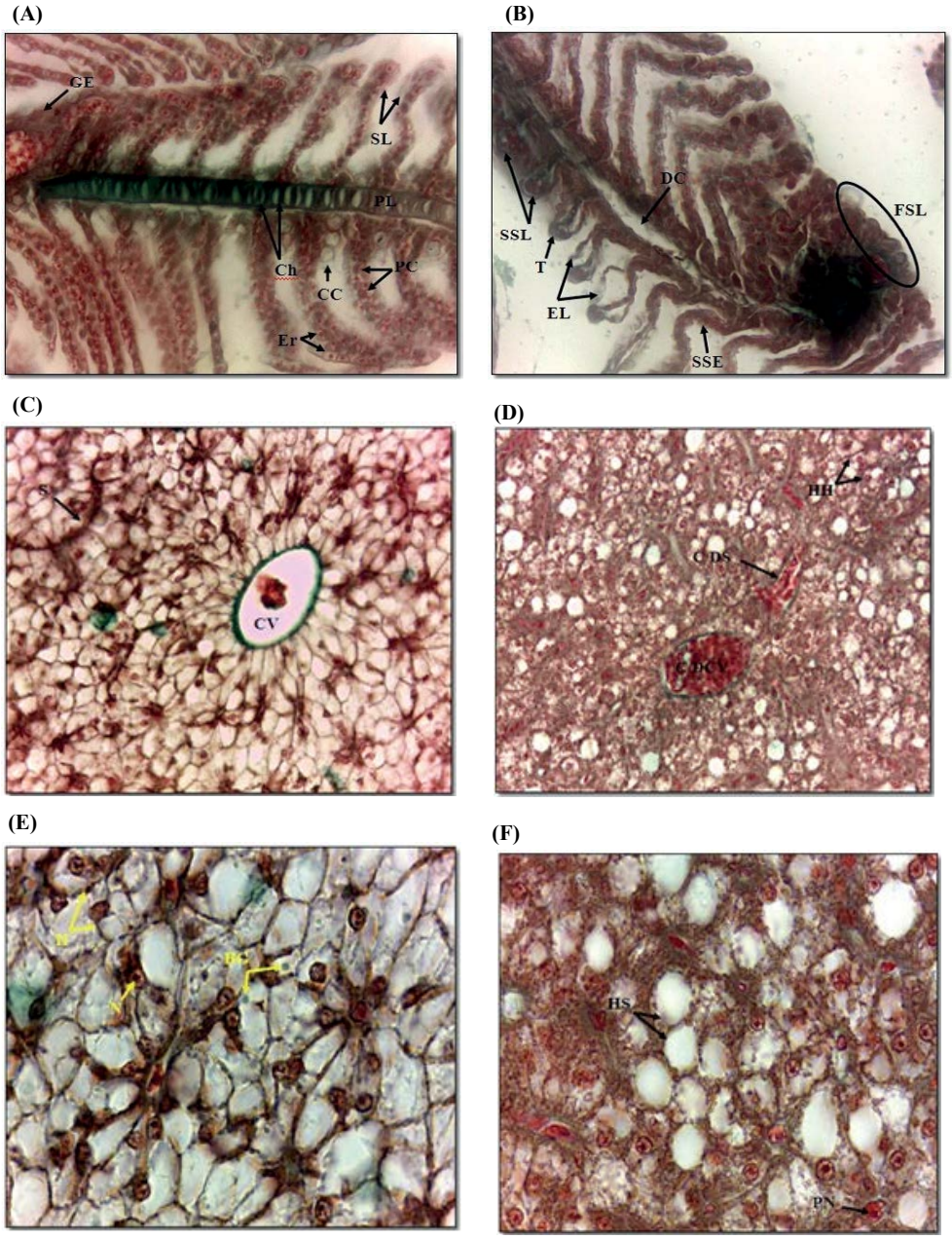

Figure 2: Histological analysis of *P. incognitus*gills (A,B) and liver (C-F) from reference site (A,C,E) and polluted Sousse bay site (B,D,E) colored by Masson trichrome. **(A)** Reference gills section showing the filament (GF), primary lamellae (PL), secondary lamellae (SL), pillar cell (PC), chondrocytes (Ch), gill epithelium (GE), chloride cells (CC) and erythrocytes (Er) (X40). **(B)** Gills section from fish of the contaminated sites howing a single stratum epithelium (SSE), fusion of secondary lamellae (FSL), telangiectasis (T), epithelium lifting (EL), shorting of secondary lamellae (SSL) and disappearance of chondrocytes DC (X40). **(C)** Liver section in reference *P. incognitus* showing sinusoids (S) and central vein (CV) (X40). **(D)** Liver section in *P. incognitus* from the contaminated site showing hypertrophy of hepatocytes (HH) congested and dilated sinusoids (C/DS) and congested and dilated central vein (C/DCV) (X40). **(E)** Liver section in reference *P. incognitus* showing hepatocytes (H) with basophilic. granulation (BG) and central and round nucleus (N) (X100). **(F)** Liver section in *P. incognitus* from the contaminated site showing pyknotic nucleus (PN) and hepatic steatosis (HS) (X40)..

to pollutants. Fishes under contamination stress are susceptible to the effects of ROS and have developed effective antioxidant defenses, including antioxidant substances (vitamin E, reduced glutathione and carotenoids) and enzymes (CAT, GR, GPx) [20]. Oxidative stress occurs when the rate of ROS generation exceeds the antioxidant defense system. Its deleterious effects include oxidation of proteins, DNA, and carbohydrate components, as well as peroxidation of unsaturated lipids in cell membranes [20]. CAT is known to protect the cell by reducing H_2O_2 to H_2O. CAT responds to a wide range of contaminants capable of ROS production such as PAHs, PCBs, heavy metals and pesticides by increase of enzyme activities. Thus, the anti-oxidative stress defenses were found altered in many field studies using crustaceans or fishes as bio indicators [31-34]. The results revealed that the activity of SOD and CAT in gills, and SOD and GPx in liver was highly repressed in the specimen collected from Sousse bay compared to those from the reference site. Such trends in antioxidant defense activities can be found in others

native organisms at polluted sites according to the levels and duration of pollutant exposure. Such a repression antioxidant defense activities is similar to that reported by Ghedira et al. [34] in gills and digestive gland of the crab *Carcinus maenas* collected from the contaminated area of Bizerte lagoon (Tunisia). In this work, some differences in physico-chemical parameters (temperature, pH, dissolved oxygen, turbidity, total nitrogen and Cd) between reference and polluted sites were noted. These abiotic stresses highly altered the biochemical responses of fishes from the polluted site. The recent ecotoxicology study of Ben Khedher et al. [18] showed that the alteration of biochemical biomarkers of crabs from polluted area of Tunisian coast was associated with metals contamination and also with seawater physico-chemical parameters such as temperature and dissolved oxygen. The same authors suggested that the elevated temperature and the decrease of dissolved oxygen may increase in return the concentrations of dissolved metals and, indeed, higher concentrations of metals have been accumulated in the Mediterranean crab (*C. maenas*) tissues [35]. In the present work, IBR values found in the contaminated site is in good agreement with the alteration of physico-chemical parameters. Thus the high value of IBR found in the gills and in the liver was associated with the low level of the dissolved oxygen and the increase of temperature, pH, turbidity, and total nitrogen and Cd concentrations detected in water. The higher IBR values suggest that Sousse bay is a stressful place for fish life. Similarly, Damiens et al. [36] found that IBR values calculated from AChE, GST and CAT activities and TBARS concentrations in the three successive experiments were in good agreement with copper and PCB concentrations in transplanted mussels but not with PAH concentrations.

Histopathological biomarkers

Gills are considered an important route for uptake, bioconcentration and excretion of chemical compounds and a prime target to contaminants, due to the wide surface area in contact with the external medium and reduced distance between internal and external medium. In the present study, severe tissular lesions were noted in gills and liver of fishes from Sousse bay. We observed, particularly, the absence of chondrocytes in primary lamellae. The appearance of a single stratum epithelium, the fusion, the telangiectasia, the hyperplasia and the narrowing are also reported in the secondary lamellae. These results are consistent with the research of Koca et al. (2008) who investigated the tissue lesions of *Capito pectoralis* and *Chondrostoma nasus* after exposure to polluted water. The authors reported a passage from the double stratum to the single stratum epithelium in gills, causing a modification in capillary circulation due to the erythrocyte expansion. However, hyperplasia and fusion of secondary lamellae are defence mechanisms for species because they increase the distance through which the water pollutants are able to reach the blood circulation [37,38]. These effects slow free gas exchange [39,40], since they are probably related to the decrease in ATPase activity and/or the declining of sodium and chloride concentrations in the blood [41]. These lesions are increasing the barrier thickness between the water and the blood and therefore they decrease the oxygen uptake [42]. The liver plays key roles in the endogen molecule metabolism and detoxification of xenobiotics. Several molecular, biochemical and physiological processes may intercept the toxic effects of pollutants. The chronic exposure of fishes to contaminants may cause cellular process dysfunctions and tissue lesions. We have demonstrated a necrosis of hepatocytes and hepatic steatosis in fishes from the contaminated site. Others severe lesions such as congestion and dilation of the central vein and the sinusoid capillaries were also observed. Koca et al. [43] reported that congestion of the central vein, steatosis, nuclear pyknosis and hepatocyte necrosis, are the results of the exposure of *Barbus capito pectoralis* and *Chondrostoma nasus* to various types of pollutants. Otherwise, Arellano et al. [41] showed a hepatocellular necrosis, an increase in fat vacuolization and the congestion of central vein and sinusoid capillaries, in *Solea senegalensis* treated with copper. Moreover Braunbeck et al. [44] have demonstrated that alterations of hepatocytes are due to the increase of metabolic activity. The hepatocyte hypertrophy, the nuclear pyknosis and the dilatation of sinusoid capillaries has been shown to result from the inhibition of Na^+/H^+ transport by cadmium and from the depression of osmoregulation process. Hepatic steatisis is one of the detoxification reactions by Kupffer cells [43,45]. It results from the exhaustion of the rough endoplasmic reticulum and disruption of protein synthesis [46]. The observed vacuolization lesions may be due to the accumulation of glycogen particles in the hepatocytes [47].

Conclusions

Oxidative stress and histopathological biomarkers responses, representing different biological endpoints in the *P.incognitus* Blennie fishes, allowed to distinguish between sites receiving chronic domestic discharge and reference site in the Tunisian coastal area, and are thus useful for the assessment of environmental pollution effects. The multiple biomarker responses measured in two distinct tissues provided discrimination between sites with different levels of contamination. Therefore, it is important to note that the responses of histopathological biomarkers depend on the sampling site, and that they are sensitive tissue biomarkers appropriated to be integrated as a complementary biological tool to the biochemical biomarkers in the bio monitoring of marine ecosystems.

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References

1. Van der Oost R, Beyer J, Vermeulen NPE (2003) Fish bioaccumulation and biomarkers in environmental risk assessment: A review. Environ Toxicol Pharmacol 13: 57-149.
2. Viarengo A, Lowe D, Bolognesi C, Fabbri E, Koehler A (2007) The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. Comparative Biochemistry and Physiology Part C 146: 281-300.
3. Jebali J, Banni M, Boussetta H (2012) Biochemical biomarkers in aquatic ecotoxicology: fundamental mechanisms, application and perspectives. 143-168 In Advance in environmental research, Daniel JA, Nova Science Publisher, New York 23: 323p.
4. GharredT, Ktari MH, Ben Salem M (1998) Systematic inventory of Bleaniidae of Tunisian coasts. Cybium 22: 99-105.
5. Gharred T, Ktari MH (2001) polymorphisme interspécifique des Blenniidea (poissons téléostéens) des côtes Tunisiennes. Bull Inst Natn Scien Tech Mer de Salammbo 28: 1-19.
6. Jacobsson A, Neuman E, Thoresson G (1986) The viviparous blenny as an indicator of environmental effects of harmful substances. Ambio 15: 236-238.
7. Jacobsson A, Neuman E, Olsson M (1993) The viviparous blenny as an indicator of effects of toxic substances. Fiskeriverket Kustlaboratoriet 538: 1-25.
8. Schladot JD, Backhaus F, Ostapczuk P, Emons H (1997) Eel-pout (Zoarces viviparus) as a marine bioindicator. Chemosphere 34: 2133-2142.
9. Noaksson E, Tjärnlund U, Ericson G, Balk L (1998) Biological effects on viviparous blenny exposed to Chrysene and held in synthetic as well as in natural brackish water. Mar Environ Res 46: 81-85.

10. Lyons BP, Bignell J, Stentiford GD, Feist SW (2004) The viviparous blenny (Zoarces viviparus) as a bioindicator of contaminant exposure: application of biomarkers of apoptosis and DNA damage. Mar Environ Res 58: 757-761.

11. Barhoumi S, Messaoudi I, Tmim D, Said K, Kerkeni A (2009) Cadmium bioaccumulation in three benthic fish species, *Salaria basilisca*, *Zosterisessor ophiocephalus* and *Solea vulgaris* collected from the Gulf of Gabes in Tunisia. Journal of Environmental Sciences 21: 980-984.

12. Barhoumi S, Messaoudi I, Gagné F, Kerkeni A (2012) Spatial and seasonal variability of some biomarkers in *Salaria basilisca* (Pisces:Blennidae): Implication for biomonitoring in Tunisian coasts. Ecological Indicators 14: 222-228.

13. Messaoudi I, Deli T, Kessabi K, Barhoumi S, Kerkeni A, et al. (2009) Association of spinal deformities with heavy metal bioaccumulation in natural populations of grass goby, Zosterisessor ophiocephalus Pallas, 1811 from the Gulf of Gabès (Tunisia). Environ Monit Assess 156: 551-560.

14. Banni M, Jebali J, Daubeze M, Clerandau C, Guerbej H, et al. (2005) Monitoring pollution in Tunisian coasts: application of a classification scale based on biochemical markers. Biomarkers 10: 105-116.

15. Messaoudi I, Barhoumi S, Said K, Kerkeni A (2009) Study on the sensitivity to cadmium of marine fish *Salaria basilisca* (Pisces: Blennidae). Journal of Environmental Sciences 21: 1620-1624.

16. Kessabi K, Kerkeni A, Saïd K, Messaoudi I (2009) Involvement of cd bioaccumulation in spinal deformities occurrence in natural populations of Mediterranean killifish. Biol Trace Elem Res 128: 72-81.

17. Bouraoui Z, Banni M, Chouba L, Ghedira J, Clerandeau C, et al. (2010) Monitoring pollution in Tunisian coasts using a scale of classification based on biochemical markers in worms Nereis (Hediste) diversicolor. Environ Monit Assess 164: 691-700.

18. Ben-Khedher S, Jebali J, Kamel N, Banni M, Rameh M, et al. (2013) Biochemical effects in crabs (Carcinus maenas) and contamination levels in the Bizerta Lagoon: an integrated approach in biomonitoring of marine complex pollution. Environ Sci Poll Res 20: 2616-2631.

19. Jebali J, Sabbagh M, Banni M, Kamel N, Ben-Khedher S, et al. (2013) Multiple biomarkers of pollution effects in Solea solea fish on the Tunisia coastline. Environ Sci Pollut Res 20: 3812-3821.

20. Ferreira M, Moradas-Ferreira P, Reis-Henriques MA (2005) Oxidative stress biomarkers in two resident species, mullet (Mugil cephalus) and flounder (Platichthys flesus), from a polluted site in River Douro Estuary, Portugal. Aquatic Toxicology 71: 39-48.

21. Almeida EA, Dias Bainy AC, Melo Loureiro AP, Martinez Regina R, Miyamoto S, et al. (2007) Oxidative stress in Perna perna and other bivalves as indicators of environmental stress in the Brazilian marine environment: Antioxidants, lipid peroxidation and DNA damage. Comparative Biochemistry and Physiology Part A 146: 588-600.

22. Annabi A, Kessabi K, Navarro A, Saïd K, Messaoudi I, et al. (2012) Assessment of reproductive stress in natural populations of the fish Aphanius fasciatus using quantitative mRNA markers. Aquatic Biology 17: 285-293.

23. Marklund S, Marklund G (1974) Involment of anion radical in the auto oxidation of pyrogallol and convenient assay for superoxide dismutase. Eur Biochem 47: 469-474.

24. Claiborne A (1985) Catalase activity. In: Greenwald R A (ed) CRC Handbook of Methods in Oxygen Radical Research CRC, Florida. 283-284.

25. Gunzler WA, Kremers H, Flohae L (1974) An improved coupled test procedure for glutathione peroxidase in blood. Z Klin Biochem 12: 444-448.

26. Beliaeff B, Burgeot T (2002) Integrated biomarker response (IBR): a useful graphical tool for ecological risk assessment. Environ Toxicol Chem 21: 1316-1322.

27. Padmini E, Rani MU (2009) Evaluation of oxidative stress biomarkers in hepatocytes of grey mullet inhabiting natural and polluted estuaries. Sci Total Environ 407: 4533-4541.

28. Toni C, Ferreira D, Kreutz LC, Loro VL, Barcellos LJG (2010) Assessment of oxidative stress and metabolic changes in common carp (Cyprinus carpio) acutely exposed to different concentrations of the fungicide tebuconazole. Chemosphere, in Press.

29. Vutukuru SS, Chintada S, Madhavi KR, Venkateswara Rao J, Anjaneyulu Y (2006) Acute effects of copper on superoxide dismutase, catalase and lipid peroxidation in the freshwater teleost fish, *Esomus danricus*. Fish Physiol Biochem 32: 221-229.

30. Ates B, Orun I, Talas ZS, Durmaz G, Yilmaz A (2008) Effects of sodium selenite on some biochemical and hematological parameters of rainbow trout (Oncorhynchus mykiss Walbaum, 1972) exposed to Pb2+and Cu2+Fish Physiol Biochem 34: 53-59.

31. Jebali J, Chicano-Galvez E, Banni M, Guerbej H, Boussetta H, et al. (2013) Biochemical responses in seabream (Sparus aurata) caged in-field or exposed to benzo(a)pyrene and paraquat. Characterization of glutathione S-transferases. Ecotoxicology and Environmental Safety 88: 169-177.

32. Fernandes D, Andreu-Sánchez O, Bebianno MJ, Porte C (2008) Assessment of pollution along the Northern Iberian shelf by the combined use of chemical and biochemical markers in two representative fish species. Environ Pollut 155: 327-335.

33. Pereira P, de Pablo H, Pacheco M, Vale C (2010) The relevance of temporal and organ specific factors on metals accumulation and biochemical effects in feral fish (Liza aurata) under a moderate contamination scenario. Ecotoxicol Environ Saf (73): 805–816.

34. Ghedira J, Jebali J, Banni M, Chouba L, Boussetta H, et al. (2011) Use of oxidative stress biomarkers in Carcinus maenas to assess littoral zone contamination in Tunisia. Aquat Biol 14:87-98.

35. Ben-Khedher S, Jebali J, Houas Z, Hmida N, Jrad A, et al. (2014) Metals bioaccumulation and histopathological biomarkers in Carcinus maenas crab from Bizerta lagoon, Tunisia. Environ Sci Pollut Res 21: 4343-4357.

36. Damiens G, Gnassia-Barelli M, Loquès F, Roméo M, Salbert V (2007) Integrated biomarker response index as a useful tool for environmental assessment evaluated using transplanted mussels. Chemosphere 66: 574-583.

37. Van Heerden D, Tiedt LR, Vosloo A (2004) Gill damage in Oreochromis mossambicus and Tilapia sparrmanii after short-term copper exposure. Int Congr Ser 1275: 195-200.

38. Figueiredo-Fernandes A, Ferreira-Cardoso JV, Garcia-Santos S, Monteiro SM, Carrola J, et al. (2007) Histopathological changes in liver and gill epithelium of Nile tilapia, *Oreochrmis niloticus*, exposed to waterborne copper. Pesq Vet Bras 27: 103-109.

39. Skidmore JF, Tovell PWA (1972) Toxic effect of zinc sulphate on the gills of rainbow trout. Water Res 6: 217-230.

40. Olson KR, Fromm PO (1973) A scanning electron microscopic study of secondary lamellae and chloride cells of rainbow trout (Salmo gairdneri). Z Zellforsch 143: 439-449.

41. Arellano JM, Storch V, Sarasquete C (1999) Histological changes and copper accumulation in liver and gills of the Senegales Sole, Solea senegalensis. Ecotoxicol Environ Saf 44: 62-72.

42. Nowak B (1992) Histological changes in gills induced by residues of endosulfan. Aquat Toxicol 23:65-83.

43. Koca S, Koca YB, Yildiz Ş Gürcü B (2008) Genotoxic and Histopathological Effects of Water Pollution on Two Fish Species, Barbus capito pectoralis and Chondrostoma nasus in the Büyük Menderes River, Turkey. Biol Trace Elem Res 122: 276-291.

44. Braunbeck T, Storch V, Hresch H (1990) Species-specific reaction of liver ultrastructure in zebrafish (Brachydarios rerio) and trout (Salmo gairdneri) after prolonged exposure to 4-chloroaniline. Arch Environ Contam Toxicol 19: 405-418.

45. Malik RN, Zeb N (2009) Assessment of environmental contamination using feathers of Bubulcus ibis L., as a biomonitor of heavy metal pollution, Pakistan. Ecotox 18: 522-536.

46. Oulmes Y, Negele RD, Braunbeck T (1995) Cytopathology of liver and kidney in rainbow trout (*Oncorhynchus mykiss*) after long-term exposure to sublethal concentrations of linuron. Dis Aquat Org 21: 35-52.

47. Wester PW, Canton JH (1986) Histopathological study of *Oryzias latipex* (medaka) after long-term β-hexachlorocyclohexane exposure. Aquat Toxicol 9: 21-45.