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Oxidative stress, genotoxicity and histopathology biomarker responses in *Mugil cephalus* and *Dicentrarchus labrax* gill exposed to persistent pollutants. A field study in the Bizerte Lagoon: Tunisia

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HIGHLIGHTS

- Biomarkers were evaluated in gill of two fish species from Bizerte Lagoon.
- Enzyme activities were lower in fish from the polluted site.
- DNA damage was higher in both fish species from the contaminated site.
- Gill histopathological analysis revealed alterations in fish from Bizerte Lagoon.

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ABSTRACT

The use of biomarkers has become an important tool for modern environmental assessment as they can help to predict pollutants involved in the monitoring program. Despite the importance of fish gill in several functions (gaseous exchange, osmotic and ionic regulation, acid-base balance and nitrogenous waste) its use in coastal water biomonitoring focusing on protection and damage is scarce.

This field study investigates biochemical (catalase, superoxide dismutase, lipid peroxidation), molecular (DNA integrity) and morphological (histology) parameters in gill of mullet (*Mugil cephalus*) and sea bass (*Dicentrarchus labrax*) and originating from Bizerte lagoon (a coastal lagoon impacted by different anthropogenic activities) and from the Mediterranean Sea (a reference site). Remarkable alterations in the activities of oxidative stress enzymes and DNA integrity in the tissue of the two studied fish species were detected in Bizerte Lagoon. The study of histopathological alterations of gills in both two fish species from Bizerte Lagoon suggest thickening of primary lamellae, cellular hyperplasia, aneurism, curving, shortening and fusion of secondary lamellae.

The adopted approach, considering simultaneously protection responses and damaging effects, revealed its usefulness on the pollution assessment.

1. Introduction

Due to urban, industrial and agricultural activities, aquatic ecosystems are released with different kinds of chemicals that affect the inhabiting biota. In order to evaluate the adverse effects of these pollutants on aquatic organism, there is a worldwide trend to complement chemical and physical parameters with biomarkers in aquatic pollution monitoring (van der Oost et al., 2003). In natural environments, contaminants usually are present as complex mixtures furthermore there is no single biomarker that can give a complete diagnosis of environmental degradation. To overcome this difficulty, the use of a set of complementary biomarkers may be useful to evaluate the various responses to mixtures of pollutants in organisms under stress. Such biomarkers act as an early warning of a specific detrimental biological endpoint.

With the view of biomarker application in environmental monitoring programmes, the evaluation and validation of biomarkers in sentinel species under different field conditions is crucial. Biomarkers of oxidative stress and genotoxicity have been applied

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in several fish species for pollution assessment in harbors and coastal areas influenced by industrial discharges (Oliveira et al., 2010; Ben Ameur et al., 2012).

The suitability of fish gills for environmental biomonitoring was recommended by several works (Fernandes et al., 2008; Oliveira et al., 2009).

In Tunisia, the Bizerte Lagoon, located near some industrial units and agricultural area, exploited in conchyliculture since 1964 (Beji, 2000), represents a receptor of several industrial wastes, pesticides and chemical fertilizers through soil erosion and runoff, leading to a decrease in bivalves and fish production (ANPE, 1990). In this aquatic area, many studies have investigated the application of biochemical tools, but these biomarker approaches have been restricted to certain species such as Mediterranean clam *Ruditapes decussatus* (Dellali et al., 2001, 2004), mussels *Mytilus galloprovincialis* (Dellali et al., 2001; Barhoumi et al., 2014a), *Hexaplex trunculus* (Roméo et al., 2006), *Fulvia fragilis* (Mahmoud et al., 2010), worms *Nereis diversicolor* (Bouraoui et al., 2010) and crab *Carcinus maenas* (Ben-khedher et al., 2013). Only three studies have used fish as sentinel species and tool for the biomonitoring of this lagoon (Louiz et al., 2009; Ben Ameur et al., 2012; Barhoumi et al., 2014b). However, no studies have assessed the use of fish gill in the Bizerte Lagoon, as tools for biomonitoring this coastal ecosystem. However, no studies have assessed the use of fish gill in the Bizerte Lagoon, as tools for biomonitoring this coastal ecosystem.

The aim of the present study was to assess oxidative stress, genotoxic and histopathologic biomarkers in mullet and sea bass gill from animals obtained in the Bizerte Lagoon influenced by multiple pollution sources to evaluate their use for pollution assessment.

2. Materials and methods

2.1. Study area and sampling

Bizerte Lagoon, a Mediterranean lagoon covering roughly 15 km^2 represents an economically important body of water due to a variety of fishing and aquaculture activities. Fifteen animals from the Bizerte Lagoon and five animals from the Mediterranean Sea 7 km northwards from the lagoon (reference site) for each species were caught alive using a net, in June 2010 (Fig. 1). The Mediterranean Sea was chosen to serve as reference site based on previous chemical analysis of polybrominated diphenyl ethers and their methoxylated analogs, as well as of organochorine pesticides and polychlorinated biphenyls (Ben Ameur et al., 2011, 2013), which indicated that the levels of contaminants were low. Immediately after sampling, fish were sacrificed via exsanguination, weighed and measured. Gills were removed and a portion fixed in 10% formaldehyde for histopathological analysis. The remaining gill was divided in two sets of tissue for oxidative stress and DNA integrity measurements and both frozen in liquid nitrogen and stored at −80 °C until further analysis.

2.2. Factor condition

Fish were weighed individually and their total lengths were measured. The Condition Factor (CF) was calculated as CF = [body weight (g)/(length $(mm)^3$] × 100 (Slooff et al., 1983).

2.3. Catalase

Catalase activity was measured in gills as described by Ben Ameur et al. (2012). Gills were homogenized in ice-cold 50 mM sodium phosphate buffer pH 7, containing 1 mM EDTA. Homogenates were centrifuged at 10000*g* for 15 min. All preparation procedures were performed at 4 °C. Catalase enzyme activity was assayed in the 10000*g* supernatant. Catalase activity was measured using a commercial kit (Cayman Chemicals). The Catalase Assay Kit utilizes the peroxidative function of CAT for determination of enzyme activity. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H2O2. The generated formaldehyde is allowed to react with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (as the chromogen); the aldehydes, upon oxidation, change from colorless to a purple color that is measured at 540 nm using a microplate reader. One unit (U) of CAT activity is defined as the amount of enzyme that causes the formation of 1 nmol of formaldehyde per minute at 25 °C.

2.4. SOD

SOD activity was measured in gills as described by Ben Ameur et al. (2012). Gills were homogenized in ice-cold 20 mM HEPES buffer, pH 7.2, containing 1 mM EGTA, 210 mM mannitol, and 70 mM sucrose. Homogenates were centrifuged at 1500*g* for 5 min at 4 °C. The resulting supernatant was centrifuged at 10000*g* for 15 min at 4 °C. The resulting 10000*g* supernatant contained the cytosolic SOD and the pellet contained the mitochondrial fraction of SOD. SOD enzyme activity was measured in the cytosolic fraction.

SOD activity was measured using a commercial kit (Cayman Chemicals). The Superoxide Dismutase Assay Kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine measured at 540 nm using a microplate reader. One unit (U) of SOD activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

2.5. MDA

MDA measurement was carried out using the method described by Ben Ameur et al. (2012). MDA measure was a modification of the methods described by Candan and Tuzmen (2008) and Del Rio et al. (2005). Gills were homogenized in ice-cold 50 mM sodium phosphate buffer pH 7, containing 1 mM EDTA. Homogenates were centrifuged at 10000*g* for 15 min. All preparation procedures were performed at 4 °C. MDA level was assayed in the 10000*g* supernatant. MDA level was measured after incubation at 95 °C with thiobarbituric acid (TBA) in acidic conditions. The pink color produced by these reactions was measured spectrophotometrically at 532 nm. MDA level was expressed as μM g^{-1} tissue.

2.6. Evaluation of DNA integrity by comet assay

The alkaline single cell gel electrophoresis (SCGE) or comet assay was performed as described by Ben Ameur et al. (2012). About 50 mg of frozen pieces of gill were washed three times with chilled phosphate-buffered saline (PBS; Ca++ Mg++ free) to remove most of the red blood cells, then transferred to an ice-cold homogenization buffer (1-X HBSS, 20 mM EDTA, 10% DMSO, pH, 7.0–7.5). The tissues were cut into small pieces with a scissors and finally homogenized to single-cell suspension. The suspension then was centrifuged at 3000 rpm for 5 min at 4 °C, after which the cell pellet was resuspended in PBS. Seventy microlitres of cell suspension were added to an eppendorf tube containing 160 μL of 1% low melting point agarose (37 °C). 110 μL of that suspension were added to an agarose precoated slide and gently covered with a cover slide to make a micro gel. The slides were immersed in lysing solution (2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris and freshly added 1% Triton X-100, pH 10) for 1 h. The slides were then placed in an horizontal gel electrophoresis unit, immersed in cold alkaline