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Liver histopathology and biochemical biomarkers in *Gobius niger* and *Zosterisessor ophiocephalus* from polluted and non-polluted Tunisian lagoons (Southern Mediterranean Sea)

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ABSTRACT

The aim of this study was to appraise the response of a multi-marker approach in fish species, *Gobius niger* and *Zosterisessor ophiocephalus*, in a polluted lagoon (Bizerte lagoon: MB and ML sites) and in a reference site (Ghar-El-Melh lagoon entrance) by the analysis of physiological indexes, liver histopathology and some biochemical biomarkers. The results showed liver hypertrophy in fish collected from Bizerte lagoon as well as many non-specific lesions, unlike the reference site. All Bizerte lagoon sites had the same prevalence of histopathological lesions, but the mean intensity (MI) of parasites seemed to be more sensible as an indicator of pollution levels. Indeed, parasite MI was more important in MB site that has a higher pollution level. Also, biochemical biomarkers showed an induction in Bizerte lagoon sites with some differences within sites and species. The impact of the continuous release of pollution on the biomarker's response is discussed.

1. Introduction

Biomarkers in fish are considered relevant tools for providing an integrated measure of exposure and/or effects of pollutants in the aquatic environment (Miranda et al., 2008) and could serve as early warning signals of environmental degradation (Lam and Gray, 2003). It is now widely admitted that a battery of complementary biomarkers at different biological organizational levels (e.g. morphological, histological and biochemical) is necessary to address multi-contamination exposure contexts (Flammarion et al., 2002; Amato et al., 2006; Wang et al., 2010; Pereira et al., 2013). Such a combination of biomarkers provides a toxicologically-relevant integrated assessment of environmental stress-induced alteration at the (sub)individual level (Van Der Oost et al., 2003). However, while many molecular and biochemical biomarkers are more and more developed and applied in the field, correlating their response to an impact at the tissue or individual levels is not systematically reported.

Histological biomarkers inform on alterations on tissue structure or organization, which may have important functional consequences when associated with crucial physiological functions in essential organs. Depending on the intensity of the response, their variation can inform on either an adaptive response to an altered environment or, in the case of severe alteration, on cell damage and toxicity. Histo-cytological biomarkers reflect fish fitness more realistically (Au, 2004) and are thus considered more ecologically relevant, yet less sensitive and specific than molecular biomarkers (Van Der Oost et al., 2003).

In the aquatic environment, histopathological changes have been related to the action of various unfavourable factors that are stress generators such as environmental conditions, microbial pathogens, nutritional factors and anthropogenic pollutants (Fanta et al., 2003; Kurtović et al., 2008; Marchand et al., 2009). The association between specific types of lesions and exposure to chemical pollutants was observed in field studies (Bernet et al., 1999; Feist et al., 2004), and fish liver lesions have been shown reliable and sensitive biomarkers of exposure to marine contaminants (Bernet et al., 1999; Stentiford et al., 2009; Stentiford et al., 2010). Fish liver, as the primary organ responsible for xenobiotic metabolism and detoxification (Oliveira Ribeiro et al., 2013), is a key tissue for studying such alterations by chemical contaminants (Buslé and Anadon, 1996; Fernandes et al., 2008; Gül et al., 2004). The functional complexity of liver depends on

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its structure; thus any change in liver anatomy can be followed by considerable repercussions, which are reflected in fish health status (Grund et al., 2010; Yancheva et al., 2016). The utility of the liver histopathological biomarker has been confirmed in several wild fish species (Rodrigues and Fanta, 1998; Stentiford et al., 2003). The liver histological response to stress has been well described in many fish species (Velkova-Jordanoska and Kostoski, 2005; Liebel et al., 2013), while the data relating to histologic response in gobies fish to stressful conditions are scarce (Louiz et al., 2009).

In lagoon ecosystems, *Gobius niger* (Linnaeus, 1758) and *Zosterisessor ophiocephalus* (Pallas, 1811) are ubiquitous and their sedentary and benthic way of life (Miller, 1986) make them suitable bioindicator species (Jones and Kaly, 1996; Monteiro et al., 2007; Dauvin et al., 2010). Several studies on the impact of chemical exposure on fish have used Gobiidae fish as bioindicator organisms (Corsi et al., 2003a, 2003b; Kwon et al., 2006; Monteiro et al., 2007; Louiz et al., 2016). In previous reports, we showed that, at some polluted sites of Bizerte lagoon, black and grass goby health status were altered as indicated by gonad and vertebral deformities (Louiz et al., 2007; Louiz et al., 2009; Louiz et al., 2018) and seasonal variations of biochemical biomarkers (Louiz et al., 2016). Bizerte lagoon has an important ecological and economic status in Tunisia (Beji, 2000; Diawara et al., 2008) but is threatened by several agronomic industrial and urban activities, which cause a deterioration of environment quality (Cheikh et al., 2002; Yoshida et al., 2002; Hlaili-Sakka et al., 2003; Derouiche et al., 2004; Mzoughi et al., 2005; Louiz et al., 2008; Ben Garali et al., 2010; Bellakhal-Fartouna and Bellakhal, 2013; Barhoumi et al., 2014). The latter endangers the health of organisms inhabiting this ecosystem.

In the present study, we further characterized the impact of anthropogenic contamination on fish in this area by assessing histopathological lesions in the liver of male gobies harvested at two contaminated sites of the Bizerte lagoon and one reference site from the Ghar El Melh lagoon. Histological alterations observed at these sites are discussed in comparison with fish health status, as informed by somatic indexes, and of liver biochemical defences as measured by detoxification enzymes (EROD, GST) and oxidative stress (total GSH, TBARS) biomarkers.

2. Materials and methods

2.1. Study area

The gobies, which were used in this study, were sampled in two lagoons of northeastern Tunisia, namely Bizerte and Ghar El Melh lagoons (Fig. 1). Bizerte lagoon, of an area of approximately 150 km2, is connected to the Mediterranean Sea through a straight channel. A relatively unpolluted site (GH), located at seawards entrance of Ghar El Melh lagoon (37°15′ N 10°22′E,) where marine influence continued to act, was chosen as a reference station, since it is free from chemical contamination (Mahmoudi, 2003; Louiz et al., 2008; Nourisson et al., 2013). For comparison purposes, two sampling sites, subjected to anthropogenic pressures, were chosen in Bizerte lagoon (Fig. 1). Thus, Menzel Bourguiba (MB) (37°14′N 9°8′E) site is located near a heavily industrialised area (metallurgical industry, tyre production factories, boatyard…) whereas the other namely Menzel Abderrahmen (ML)

Table 1
Sediment contamination levels in the prospected sites: Menzel Bourguiba (MB), Menzel Abderrahmane (ML) and Ghar El Melh (GH). PAHs: polycyclic aromatic hydrocarbon; DDT: dichlorodiphenyltrichloroethan; PCBs: polychlorinated biphenyl.

	MB	ML	GH	Références
Heavy metal and metalloid				
Al (ng/g)	13.35	6.67	–	Yoshida et al. (2002)
Sb (μg/g)	1.06	0.33	–	Yoshida et al. (2002)
As (μg/g)	41.29	16.52	–	Yoshida et al. (2002)
Co (μg/g)	7.82	6.92	–	Yoshida et al. (2002)
Cu (μg/g)	68.77	17.19	–	Yoshida et al. (2002)
Fe (ng/g)	51.08	25.54	–	Yoshida et al. (2002)
Mn (μg/g)	568.80	455.01	–	Yoshida et al. (2002)
Ni(μg/g)	22.47	17.97	–	Yoshida et al. (2002)
Se (μg/g)	2.10	0.93	–	Yoshida et al. (2002)
Ag (μg/g)	0.63	0.18	–	Yoshida et al. (2002)
Ti (μg/g)	0.39	0.17	–	Yoshida et al. (2002)
U (μg/g)	1.96	1.09	–	Yoshida et al. (2002)
V (μg/g)	58.50	46.80	–	Yoshida et al. (2002)
Pb (μg/g)	286.80	71.72	0,07	Yoshida et al. (2002) and Mahmoudi (2003)
Cr (μg/g)	> 46.20	36.96	0,05	Yoshida et al. (2002) and Mahmoudi (2003)
Hg (μg/g)	0.42	0.11	$2\ 10^{-4}$	Yoshida et al. (2002) and Mahmoudi (2003)
Zn (ng/g)	1.51	0.24	0.09	Yoshida et al. (2002) and Mahmoudi (2003)
Cd (μg/g)	1.32	0.37	0.50	Yoshida et al. (2002) and Chouba et al. (2007)
PCB (ng/g)	4.77	4.68	1.20	Derouiche et al. (2004) and Ben Ameur et al. (2011)
Organochloride				Cheikh et al. (2002)
DDT (ppb)	1.78	0.48	–	
Pesticides (ppb)	1.83	0.65	–	
Organotin μg (Sn) kg^{-1}	200	117	–	Mzoughi et al. (2005)
PAHs (ng/g)	209.1	48.3	4.4	Louiz et al. (2008)

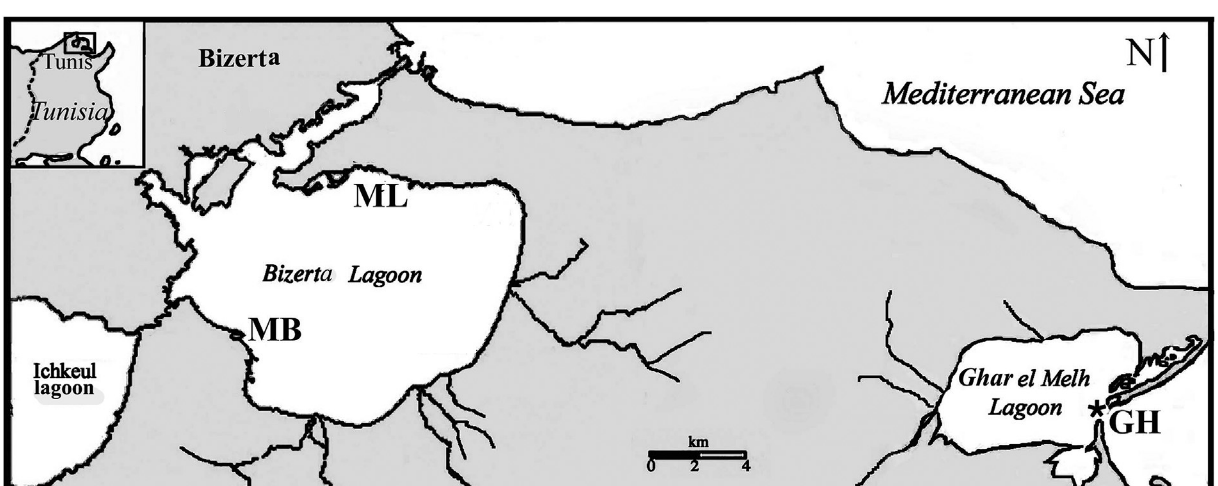

Fig. 1. Map of the study area and localization of sampling sites in Ghar El Melh (GH) and Bizerte lagoons: Menzel Bourguiba (MB) and Menzel Abderrahmen (ML) sites.