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ScienceDirect
Journal of Environmental Sciences 21(2009) 1620–1624
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Study on the sensitivity to cadmium of marine fish *Salaria basilisca* (Pisces: Blennidae)

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Received 26 November 2008; revised 04 January 2009; accepted 23 April 2009

Abstract

The present study tested the sensitivity of *Salaria basilisca* to water-cadmium (Cd) contamination. For this purpose, liver somatic index (LSI), Cd concentrations and the activities of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were measured in the liver of *S. basilisca* exposed to Cd-contaminated water (2 mg Cd/L as $CdCl_2$) for 14 and 28 d. The results showed that the LSI decreased significantly after 14 and 28 d of Cd-exposure. Cd bioaccumulation in the liver resulted in an increasing uptake up to 42 μg/g dry weight after 28 d of exposure. Activities of CAT and SOD were significantly increased with increasing exposure time. A significant increase in GSH-Px activity, under Cd influence, was observed during 14-day exposure period ($p < 0.0001$). However, a significant decrease ($p < 0.05$) in this activity with respect to control fish was registered after 28 d of Cd-exposure. These results showed that Cd accumulation in the liver of *S. basilisca* could induce oxidative stress as demonstrated by changes in the antioxidant enzyme activities. Results also emphasized that *S. basilisca* may considered as a sensitive species to Cd exposure.

Key words: cadmium; *Salaria basilisca*; oxidative stress; antioxidant enzymes
DOI: 10.1016/S1001-0742(08)62464-X

Introduction

Cadmium (Cd) as a widespread environmental pollutant is highly toxic and considered to have no biological function. Due to the deleterious effects of Cd on aquatic ecosystems, it is necessary to monitor its bioaccumulation and toxicity in key species, and thus give an indication of the temporal and spatial extent of the process, as well as an assessment of the potential impact on organism health. It is known that in the interaction with living organisms one of the first effects of Cd is alteration of enzyme activities and membrane transport mechanisms (Viarengo, 1989), which in turn are responsible for physiological and metabolic alterations in the whole organism. Cd accumulation causes an increase in highly reactive oxygen species leading to an oxidative stress in aquatic organisms (Roche and Boge, 1993; Atli *et al*., 2006). It has been suggested that oxidative stress biomarkers could be employed in environmental monitoring programs (McCarthy and Shugart, 1990). Activities of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) have been successfully employed in aquatic biomonitoring studies (Filho *et al*., 1996).

Most of the articles published on organisms as pollution bioindicators have concentrated on invertebrates, mainly molluscs and crustaceans. The use of fish as indicators for marine pollution monitoring is just at present widely recognised (Marcovecchio, 2004). Moreover, most data on Cd-induced oxidative stress in aquatic organisms originate are from research on numerous invertebrate and freshwater fish species (Bacha and Rani, 2003; Gül *et al*., 2004; Atli *et al*., 2006), whereas oxidative stress under Cd influence in marine fish is poorly documented (Roméo *et al*., 2000; Speers-Roesch and Ballantyne, 2005).

Several studies conducted in the Gulf of Gabes located in the southeastern coast of Tunisia, showed that the industrial activities are associated to Cd pollution that touched terrestrial (Messaoudi and Ben Chaouacha-Chekir, 2002) and aquatic (Banni *et al*., 2007; Messaoudi *et al*., 2008a) fauna and flora. In recent studies, we have reported an association of spinal deformities with Cd bioaccumulation in natural populations of fish collected from the Gulf of Gabes in Tunisia (Messaoudi *et al*., 2008a, 2008b; Kessabi *et al*., 2009). Therefore, it is important to select a marine teleost fish which can be used as a bioindicator of Cd pollution in this region. Some requirements are necessary to select suitable bioindicators, particularly their sensitivity and their capability to accumulate the pollutant of interest (Phillips and Segar, 1986; Marcovecchio, 2004).

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Salaria basilisca is very common benthic fish (Blennidae) in Tunisian coasts. This species is abundant in the Gulf of Gabes. In our previous findings, *S. basilisca* liver showed the highest ability to accumulate Cd from sediment when compared to other benthic fish species collected from some selected sites with different degrees of Cd contamination in the Gulf of Gabes (Barhoumi *et al*., 2009). Keeping in view these findings, current study was conducted to test the sensitivity of *S. basilisca* to Cd by measuring Cd concentrations and the activities of antioxidant enzymes such as CAT, SOD and GSH-Px in the liver after 14 and 28 d of exposure to Cd-contaminated water.

1 Materials and methods

1.1 Test animals

Fish samples were collected by professional fishermen with a 4-m beam trawl during January 2006 from the coast of Luza located ca. 50 km north of Sfax (Tunisia). This site is unaffected by heavy metal pollution (Banni *et al*., 2007; Messaoudi *et al*., 2008a). Fish were kept in 30 L maintenance plastic tanks and immediately transported alive to the laboratory.

Fish were acclimatized for two weeks in well aerated holding glass aquaria (300 L), containing natural seawater with a salinity of 30 g/L, under a natural photoperiod 12 h:12 h (light:dark) cycle and temperature of (24 ± 1)°C. Fish were fed twice daily with commercially balanced fish food sticks (Tetramine, Hagen, France). The medium was renewed every two days. During the acclimatization period, inspections were conducted twice a day to discard wounded, diseased and dead fish.

1.2 Experimental design

The experiment was conducted over a period of four weeks. It was performed on 24 sexually immature *S. basilisca* whose weight reaches (30.60 ± 5.70) g and length (15.72 ± 0.90) cm. Fish were equally divided into two groups and transferred in separate glass aquaria (100 L each). Animals in the first group were maintained in Cd-free water to serve as control, whereas, those of the second group were maintained in natural sea water supplemented with 2 mg Cd/L (as $CdCl_2$). Based on the available published data (Tophon *et al*., 2003; Annabi *et al*., 2008), the dose chosen was 10% of the 96-h LC_{50} value from the acute toxicity test, which was 20 mg/L (data not shown). In each aquarium, water was pumped continuously over a biofilter column at the rate of 4 L/min. The water was continuously aerated throughout the experiment. Fish were fed twice daily and uneaten food was quickly removed from the system. The experimental water (50%) was changed every two weeks to keep the water quality in acceptable limit according to APHA (1995). Six fish from each aquarium were sampled at 14 and 28 days post-exposure. Fish samples were weighed (W_{total}) and dissected and their livers were removed for weight (W_{liver}) determination after rinsing in physiological salt water. Liver somatic index (LSI) was calculated according to the following formula: $LSI = W_{liver}/W_{total}$ (Tejeda-Vera *et al*., 2007).

1.3 Cadmium analysis

Liver samples were dried to constant weight for 48 h at 60°C in Pyrex test tubes. Dried tissues were weighed and digested with concentrated HNO_3 at 120°C. When fumes were white and the solution was completely clear, the samples were cooled to room temperature and the tubes were filled to 5 mL with ultra pure water. Cd concentrations were determined using an atomic absorption spectrophotometer (AAS) model ZEEnit 700-Analytik-Jena (Germany) by flameless AAS with electrothermal atomization in a graphite furnace. Samples were analyzed in triplicates. The variation coefficient was usually less than 10%.

1.4 Antioxidant enzymes activities assays

About 0.1 g of fish liver was sliced and homogenized in cold sodium phosphate buffer (pH 7.4) containing 1 mmol/L EDTA. The homogenates were then centrifuged at 4000 r/min for 15 min at 4°C. The supernatants were separated and used for enzyme assay and protein determination. The SOD activity was determined using pyrogallol as a substrate by the method of Marklund and Marklund (1974). This method is based on pyrogallol oxidation by the superoxide anion ($O_2^{\bullet -}$) and its dismutation by SOD. The CAT activity was determined using the method described by Beers and Sizers (1952) through measuring hydrogen peroxide decomposition at 240 nm, while the GSH-Px activity was assayed by the subsequent oxidation of NADPH at 240 nm with *t*-buthyl-hydroperoxide as substrate (Gunzler *et al*., 1974). The concentration of total protein was estimated according to the Biuret method (Gornall *et al*., 1949) using serum albumin as standard.

1.5 Statistics

All obtained data were expressed as mean ± SE. Differences among the control and Cd-exposed groups were assessed by one-way ANOVA followed by Least Significant Difference test (PLSD Fisher). Values were considered statistically significant when $p < 0.05$.

2 Results

2.1 Liver somatic index

As shown in Fig. 1, LSI was significantly ($p < 0.01$) decreased after 14 d exposure compared to correspondent control group. This decrease was more evident ($p < 0.001$) at the end of the experiment. In fact, the LSI values were significantly lower ($p < 0.01$) in fish exposed in Cd for 28 d than those in fish exposed for 14 d.

2.2 Cadmium concentrations

The hepatic Cd concentrations in *S. basilisca* are reported in Table 1. The result showed an uptake up to 17 μg Cd/g dry weight (dw) after 14 d of exposure. This amount increases significantly over time to reach 42 μg/g dw at the

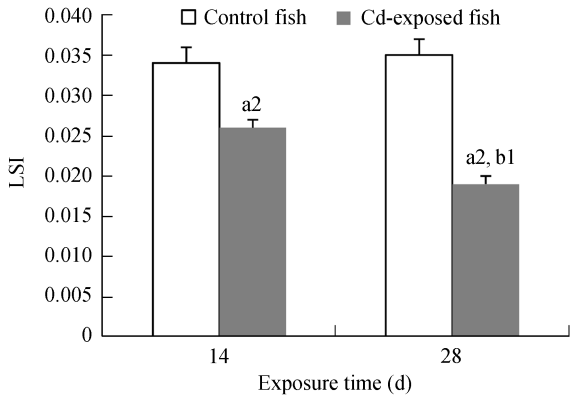

Fig. 1 Liver somatic index (LSI) of control and fish exposed to cadmium. Means ± SE from 6 animals in each group. Significance from the correspondent control group: [a2] $p < 0.001$; significance from Cd-exposed fish for 14 d: [b1] $p < 0.01$.

end of the experiment.

2.3 Antioxidant enzyme activities

As shown in Figs. 2a and 2b, exposure to Cd for 14 d resulted in a significant increase in both CAT ($p < 0.001$) and SOD ($p < 0.001$) activities compared to the correspondent control group. After 28 d Cd-exposure this increase is much higher ($p < 0.001$) for both CAT and SOD activities with respect to control fish.

Compared with the controls, significant increase in GSH-Px activity was observed during 14-d exposure ($p < 0.001$). However, a significant decrease ($p < 0.05$) in this activity with respect to control fish was registered after 28 d of Cd-exposure (Fig. 2c).

3 Discussion

The damaging action of Cd on the antioxidant defense system in various tissues during Cd-exposure has been extensively studied in aquatic organisms, mainly in numerous invertebrate and freshwater fish species. The present study was conducted to test the sensitivity of *S. basilisca*, to Cd by measuring Cd concentrations and the activities of antioxidant enzymes such as CAT, SOD and GSH-Px in the liver after 14 and 28 d exposure to Cd-contaminated water. To our knowledge, Cd-induced oxidative stress had not been documented before in this fish species.

Table 1 Cadmium concentrations in liver fish exposed to Cd

Exposure time (d)	Cd in liver (μg/g dw)		Cd in contaminated water (μg/L)
	Control fish	Cd-exposed fish	
14	0.030 ± 0.003	17.810 ± 0.822[a2]	1.920 ± 0.087
28	0.030 ± 0.003	42.738 ± 2.126[a2,b2]	1.870 ± 0.055

Data are presented as means ± SE from 6 samples in each case. Significance from the correspondent control group: [a2] $p < 0.001$, significance from Cd-exposed fish for 14 d: [b2] $p < 0.001$.

The concentrations of metals in the liver represent the storage of metals from the water where the fish species live (Karadede *et al*., 2004). The liver is known to be one of the major organs that accumulates Cd. It has been reported that fish liver accumulated substantial amounts of Cd after both acute and chronic exposures (Bouraoui *et al*., 2008). Consistent with these reports, we have noticed that Cd accumulation in the liver of *S. basilisca* increases significantly over time to reach 42 μg/g dw at the end of the experiment. Our results showed that Cd storage in the liver is linked to a significant decrease in liver somatic index. These results were similar to those of other investigators who studied the chronic effects of Cd in *Oncorhynchus mykiss* (Richard *et al*., 1998) and in *Oreochromis mossambicus* (Van Dyk *et al*., 2007).

Liver not only acts as a storage organ, but also a primary site for detoxification mechanisms. Fish liver is endowed with antioxidant defense systems, such as CAT, SOD and GSH-Px, to protect tissues from oxidative stress caused by metals (Basha and Rani, 2003). In previous studies, liver was found to be stronger into the face of oxidative stress than the other tissues and a uniform organ with the highest antioxidant enzyme activities. This could be related to the fact that liver is the site of multiple oxidative reactions and maximal free radical generation (Gül *et al*., 2004; Avci *et al*., 2005).

The SOD-CAT system provides the first defense against oxygen toxicity. SOD catalyzes the dismutation of the superoxide anion radical to water and hydrogen peroxide, which detoxified by the CAT activity. Various responses of SOD and CAT activities have been observed in animals exposed to organic or metallic contaminants in both field and laboratory experiments. These enzymes have been shown to be either induced or inhibited by metals depending on the dose, the species or the route of exposure (Roméo *et al*., 2000; Atli *et al*., 2006). Usually, a simultaneous induction response in the activities of SOD and CAT is observed

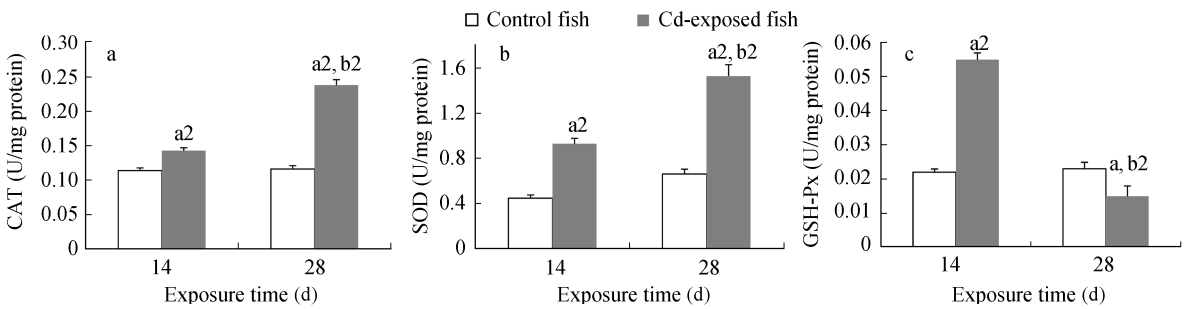

Fig. 2 Activities of catalase (CAT) (a), superoxide dismutase (SOD) (b), glutathione peroxidase (GSH-Px) (c) in liver tissues of control and fish exposed to cadmium. Means ± SE from 6 animals in each group. Significance from the correspondent control group: [a] $p < 0.05$; [a2] $p < 0.001$; significance from Cd-exposed fish for 14 d: [b2] $p < 0.001$.

when exposed to pollutants (Dimitrova *et al*., 1994). In agreement, our results showed that the exposure to Cd for 14 and 28 d resulted in significant increase in both SOD and CAT activities with increasing exposure time. Increases in antioxidant enzyme activities may be associated with increased oxidative stress under Cd influence (Atli *et al*., 2006). The high activity of SOD may indicate a high production of superoxide anion radical, while the high activity of CAT should indicate the presence of a large amount of hydrogen peroxide in the system.

The liver is a major site of detoxification, the first target of ingested oxidants, and a very important tissue in the study of the role of GSH-Px in protection against oxidative stress. GSH-Px catalyzes the reduction of both hydrogen peroxide and lipid peroxides and is considered an efficient protective enzyme against lipid peroxidation (Winston and Di Giulio, 1991). In our study, the high GSH-Px activity recorded in fish exposed during 14 d could provide evidence of a good capability to counterbalance the oxidative damage that may be induced by Cd recorded in this accumulative species. Then, a reduction in GSH-Px activity with increasing exposure time was noticed. GSH-Px activity was inhibited, which suggested that the oxidative damage induced by the high hepatic concentration of Cd is enough to cause GSH-Px to be poisoned.

The choice of *S. basilisca* for use as test organism in our study is based on its widespread occurrence in Tunisian coasts (Gharred, 1999), facility of sampling all year round as well as its easy maintenance in laboratory conditions. In a field study performed by our group, the liver of *S. basilisca* showed the highest ability to accumulate Cd from sediment when compared to other benthic fish species, such as *Zosterisessor ophiocephalus* and *Solea vulgaris*, collected from some selected sites with different degrees of Cd contamination in the Gulf of Gabes (Barhoumi *et al*., 2009). In the present study we have noticed that the Cd concentration in the liver of *S. basilisca* increased with increasing exposure time. In addition, *S. basilisca* was showed to be sensitive to Cd. This is supported by the fact that the metal is able to decrease the LSI and to induce antioxidant enzyme activities in the liver. On the basis of the obtained results, we propose to use *S. basilisca* as an environmental indicator species for future monitoring programs to evaluate the evolution of Cd pollution in Tunisian coasts. However, before *S. basilisca* can be used as bioindicator, further studies on its ecology and biology are critical. More detailed experiments will also be required for better understanding the response mechanism of antioxidant enzymes to Cd exposure in this species.

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