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Biliary and hepatic metallothionein, metals and trace elements in environmentally exposed neotropical cichlids *Geophagus brasiliensis*

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ABSTRACT

One of the metal detoxifying mechanisms that occurs in fish is metallothionein (MT) induction and metal binding. Hepatic MT induction has been well described, but biliary MT metal detoxification has only recently been described in fish. In this scenario, metal-metal interactions have been increasingly evaluated to further understand the behavior of these contaminants regarding homeostasis and biological functions, as well as their toxic effects. Studies, however, have been mainly conducted concerning the elemental pair Se-Hg, and scarce reports are available concerning other metal pairs. Therefore, this study aimed to evaluate biliary and hepatic MT metal detoxification mechanisms in a territorial neotropical cichlid, *Geophagus brasiliensis*. Fish were sampled from the anthropogenically impacted estuarine Rodrigo de Freitas Lagoon, located in Southern Rio de Janeiro, and trace elements and MT were determined by inductively coupled plasma mass spectrometry (ICP-MS) and UV-Vis spectrophotometry, respectively, in fish liver and bile. MT in bile were significantly lower than in liver. Significant differences between bile and liver were observed for many trace elements, and, although most were higher in liver, Cd and Ni were significantly higher in bile, indicating efficient excretion from the body via the biliary route. A significant correlation was observed between MT and Fe in bile, and between MT in liver and Cu and Zn in bile. Molar ratio calculations demonstrated protective elements effects against Al, As, Cd, Hg, Pb and V in both bile and liver, as well as some novel interrelationships, indicating the importance of these investigations regarding the elucidation of element detoxifying mechanisms. Furthermore, investigation of other elemental associations may aid in decision-making processes regarding environmental contamination scenarios linked to public health.

1. Introduction

Aquatic environmental pollution by metals and trace-elements has become a worldwide problem, since these compounds show potential toxic effects and can bioaccumulate in aquatic ecosystems [1]. To counteract the negative effects caused by the presence of toxic elements or essential elements in excess, organisms present certain biochemical defenses, such as increased metallothionein (MT) synthesis, that binds to free elements [2]. MT have been implicated in the homeostasis of essential elements, such as Cu and Zn, as well as in the detoxification of toxic metals, such as Ag, Cd, Pb and Hg [3], although this metalloprotein has also displayed protective free radical scavenging activity, playing an active role in the capture of harmful oxidant species [4], both capabilities due to abundant cysteine residues. Because of these properties, MT are considered adequate biomarkers for metal and trace-element exposure, and have been increasingly applied to detect oxidative stress.

Recently, biliary fish MT have been shown to follow the same trend as hepatic MT, demonstrating an alternative detoxifying mechanism for metals and trace-elements [5]. In addition, fish bile has also been

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validated as a biomarker for metal and trace-element excretion, and certain elements have been reported as excreted preferentially by this route, before reaching a certain threshold and accumulating in the liver [6]. However, studies in this regard are scarce, and many variables in this process are still unknown. Thus, further analyses are required to better investigate this detoxification route.

Elemental interactions in living organisms may lead to different effects, by inducing synergistic (increased), antagonistic (decreased) or additive (independent) behavior [7], depending on element bioavailability, uptake from the environment and different distribution patterns in fish tissues [8]. Because of this, interactive effects of elemental pairs have been increasingly evaluated in both in vivo and in vitro studies, in the laboratory or in the field, and contribute to further understanding of the behavior of these contaminants both in homeostasis and biological functions and concerning toxic effects. Studies in this regard, however, have been mainly conducted concerning the Se-Hg pair, and not many reports are available concerning other elemental pairs. In addition, to the best of our knowledge, no studies in this regard have been conducted in bile.

In this context, the aim of the present study was to characterize biliary and hepatic metallothionein-mediated element detoxification mechanisms and interactions of both essential and non-essential metals and trace-elements by metallothioneins in environmentally exposed territorial neotropical cichlids *Geophagus brasiliensis*.

2. Methodology

2.1. Study area

The estuarine ecosystem of the Rodrigo de Freitas Lagoon is located in the highly urbanized south region of Rio de Janeiro (22°5700200S; 043° 1100900 W), southeastern Brazil (Fig. 1). It is connected to the sea by a narrow channel, although this channel is frequently blocked by sand deposits and, thus, does not allow for free water exchanges, restricting circulation and water renewal [9]. Because of this, water stratification occurs, with the deeper water layer frequently becoming anaerobic and rich in hydrogen sulfide (H_2S) gas, due to oxidation of the organic matter present in the bottom of the lagoon. This, in turn, results in low water quality level, leading to many episodes of low dissolved oxygen levels, anoxic conditions and frequent fish mortality [10]. These characteristics present favorable conditions for pollutant accumulation in the lagoon's substratum. In addition, the lagoon illegally receives untreated domestic sewage enriched in organic matter, detergents, synthetic organic material and metals and trace-elements on a daily basis, and is also exposed to huge hydrocarbon and metal and trace-elements inputs originated from incomplete combustion of fossil fuels, due to the approximately 190 thousand vehicles that pass through the area each day [11], as well as the presence of several surrounding gas stations [9].

Fig. 1. Map of the study area, with the Rodrigo de Freitas Lagoon displayed in the inlay, located in Southeastern Brazil.