Trace metal incorporation in otoliths of a territorial coral reef fish (*Abudefduf saxatilis*) as an environmental monitoring tool

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Abstract. Trace metal levels in the otolith external layer of newly *Abudefduf saxatilis* (Pomacentridae) recruits, a common fish of the Caribbean coral reef, were examined as an indicator of recently occupied habitat from the most important coral reefs of the east of Venezuela (Mochima National Park and La Tortuga Island). These otoliths were analyzed trough an Energy-dispersive X-ray spectroscopy (EDS) fixed to scanning electron microscopy (SEM). The five trace metals analyzed (Cd, Cu, Hg, Pb and Zn) were found at external layer of most evaluated otoliths at all localities, in which %weight of Pb/Ca and Hg/Ca showed the highest values. These results show the bioavailability of evaluated metals at Mochima National Park and La Tortuga Island, and their significant spatial variations on otoliths make evidence of different concentration of Cd, Hg and Pb in water and/or sediments of these locations.

Key Words: Otolith, heavy metals, SEM-EDS, coral reef fish.

Introduction

Otoliths are calcified tissues located in the inner ear of teleost fish, consisting of alternating layers of aragonite and protein deposited continuously through the lifetime of the fish (Degens et al., 1969). These calcified tissues, may offer a permanent record of metal exposure and reflect a trace metal history of the water environment inhabited by the fish due the potential inclusion of trace metals through substitution for Ca in the aragonite crystalline matrix or through co-precipitation of another carbonate (Campana, 1999). The fact that the otolith is acellular and metabolically inert means that any elements or compounds accreted onto its growing surface are permanently retained, whereas the continued growth of the otolith from before the time of hatch to the time of death implies that the entire lifetime of the fish has been recorded (Campana and Neilson, 1985, Mugiya et al., 1991).

Numerous studies have used otolith microchemistry for stock discrimination, migratory studies, and connectivity (see Thorrold and Swearer, 2009), but only a few studies have investigated the ability of otoliths to record exposure to pollution, either directly in terms of potentially toxic elements (Geffen et al., 1998, Milton et al. 2000, Ranaldi and Gagnon 2009), or indirectly as proxies for exposure along a pollution gradient (Hanson and Zdanowicz, 1999, Ranaldi and Gagnon 2008a).

The aim of this study was to quantify the concentrations of Cd, Cu, Hg, Pb and Zn in otoliths of a territorial coral reef fish (*Abudefduf saxatilis*) at the most important coral reefs environments of the east of Venezuela (Mochima National Park and La Tortuga Island), as the first

contribution of this kind of study in Venezuelan marine environments.

Materials and Methods

Abudefduf saxatilis was selected as the study species because it is a common territorial fish on coral reefs. Newly recruits of *A. saxatilis* were collected at seven localities from the most important coral reefs of the east of Venezuela. Of these, three localities were in Mochima National Park (M1, M4 and M6) and four in La Tortuga Island (T1, T2, T4 and T6). These locations are influenced by upwelling waters. Their water and sediments chemistry could be different due to distance to coast and urbanized populations and by then these chemical differences would be reflected in biogenic carbonates, including fish otoliths.

Examined specimens were obtained since 20 September to 20 October 2010. All individuals were frozen immediately after capture. Sagittal otoliths were extracted using plastic tweezers to minimize contamination and were stored dry in plastic vials.

Each otolith were rinsed using ultrapure water (N-pure, resistivity 18 MΩ.cm), soaked in 15% semiconductor-grade H_2O_2, buffered with 0.05 N supra-pure NaOH, in acid-leached trays for 1 hour, and soaked and rinsed and additional five times with N-pure. Otoliths were dried in air and they were placed on carbon conductive tape above aluminum specimen mounts for scanning electronic microscope. The samples were covered with a chrome coating to create a conduct charge surface using a vacuum evaporator HUS-5GB (Hitachi) for 30 minutes. Ca, Cd, Cu, Hg, Pb and Zn were measured on the external layer of each otolith by an Energy-dispersive X-ray spectroscopy (EDS) Thermo Noran System Seven with Si(Li) detector, fixed to scanning electron microscopy (SEM) Hitachi S-800FE. There was used an accelerating voltage of 25 kV during 120 seconds, at several sections of the otolith surface. The elemental quantitative analysis used an automatic background subtraction and a ZAF correction matrix was used to calculate the elemental composition in weight percent. Elemental data were expressed as ratios to Ca as the elements analyzed are likely substituting for Ca in the aragonite matrix. The mean and standard deviation is presented by each element at each locality. Prior to analysis, data were checked for homogeneity of variances and normal data distribution. Afterwards, separate ANOVA for each metal were used with locality as the categorical factor. These tests were followed by post hoc Tukey honest significant test. Statistical analyses were performed using SPSS 16.0 statistical package.

Results and Discussion

The five trace metals analyzed were found at external layer of most otoliths evaluated at all localities, where Pb/Ca and Hg/Ca showed the highest values (Table 1). Variations at locations were detected for metal ratios analyzed (Fig. 1).

Differences in Hg/Ca between localities were founded (ANOVA; F= 7.534; p= 0.0001); nevertheless these differences are between localities of Mochima National Park (Tukey´s post hoc test; p< 0.03) while the values of this element were similar at La Tortuga Island locations. Pb/Ca values are similar at all locations, except T6, which presents the lowest values at La Tortuga Island (ANOVA; F= 4.890; p= 0.0004; Tukey´s post hoc test, p= 0.02). Cd/Ca shows differences between localities (ANOVA; F= 21.189; p= 0.0001), where M4, T1, T2 and T4 have the highest values (Tukey´s post hoc test, p= 0.03).

Table 1. Order of %weight of metal measured relative to Ca at evaluated different localities

Localities	% weight of metal measured relative to Ca
M1	Pb > Hg > Cu > Zn > Cd
M4	Hg > Cu > Pb > Cd > Zn
M6	Pb > Hg > Zn > Cu > Cd
T1	Hg > Pb > Zn > Cd > Cu
T2	Pb > Hg > Cd > Cu > Zn
T4	Pb > Hg > Cd > Cu > Zn
T6	Hg > Pb > Zn > Cu > Cd

Fish have numerous mechanisms for dealing with toxic metals in the environment as Cd, Cu and Hg. Some elements may be metabolized, some sequestered so that they become biologically inactive, and others detoxified by conversion into other phases which are then excreted (Xu and Wang, 2002, Wang and Wong, 2003). Mercury belongs to the group of elements that induces metallothionien production, which detoxifies the metal (Pulsford et al., 1992). On this way, some studies have revealed that high and prolonged exposure can overwhelm the defense mechanisms, and excretion may not keep pace with accumulation (Wang and Wong, 2003); thus mercury remained available for incorporation in the otolith, and its incorporation would reflect both concentration and exposure level, like other authors have reported (Geffen et al., 1998). At the same time, some studies found that the Pb and Cd otoliths content, are more likely related to the fish environment than to physiological processes, and their concentrations are associated with anthropogenic sources and is not correlated with pH, salinity or other environmental factors (Köck et al., 1996; Ranaldi and Gagnon, 2008a).

Pb sources at study localities may include Pb-oxides from combusted fuel of different marine ships, like in other coastal zones of Venezuela (Salazar-Lugo, 2009). In
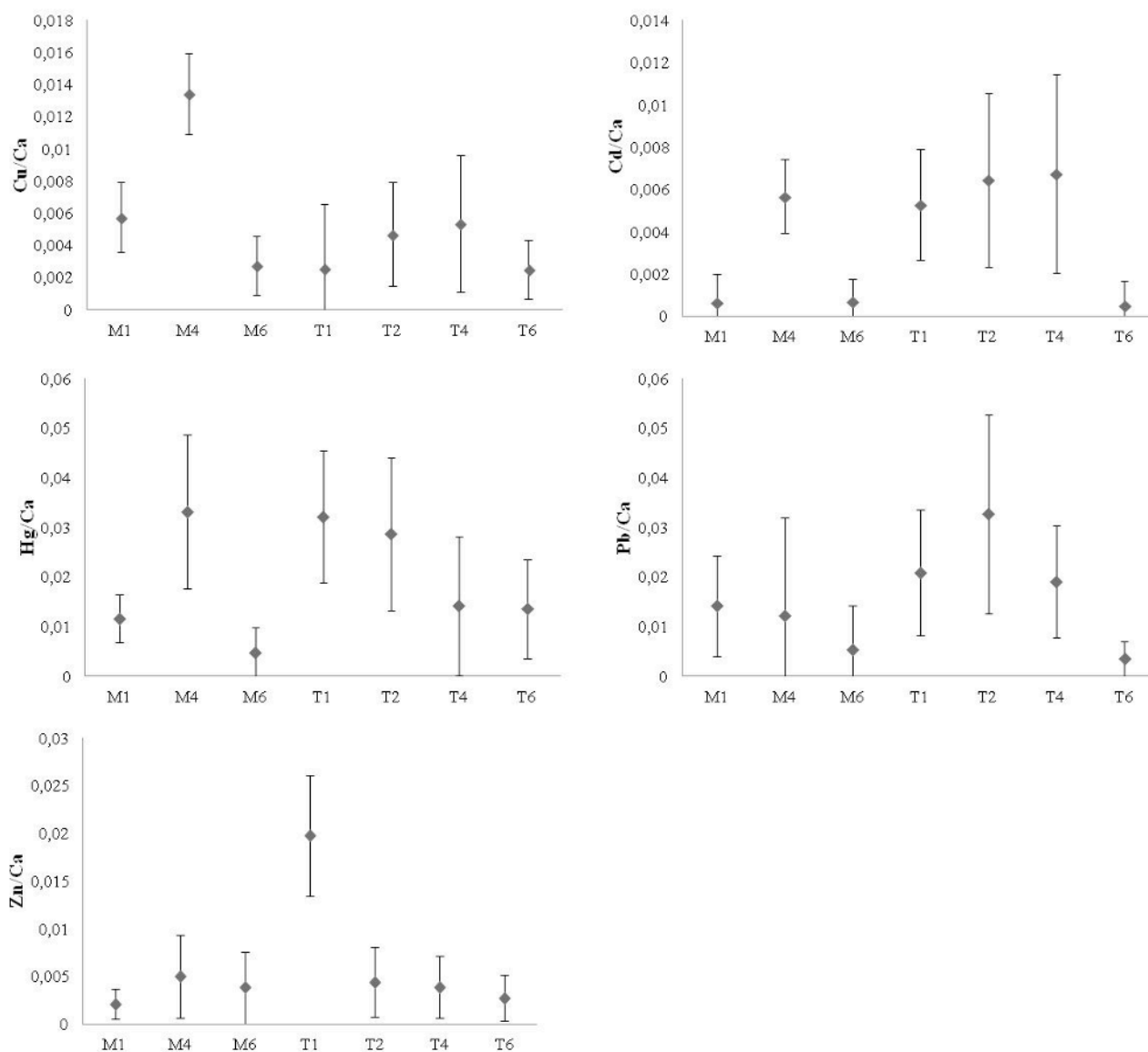
Fig. 1. % Weight of metal measured relative to Ca (mean ± standard deviation) at localities evaluated.

relation to Cd and Hg sources, could come from Orinoco river discharge, which has high levels of these metals (Laya, 1992), and they are being transported along the east coast of Venezuela by the prevailing marine currents in the area (Rojas et al., 2002). Furthermore, these are metals which have biogeochemical behavior similar the ones of nutrients; consequently, upwelling waters may be enriched with some metals, constituting the main source of Hg and Cd to the coastal areas (Cossa et al., 2004, Valdés et al., 2006).

On the other hand, laboratory experiments and field evidences have demonstrated that accumulate Zn and Cu in otoliths is predominantly via dietary exposure rather than through waterborne exposure (Campana 1999, Milton et al., 2000, Ranaldi and Gagnon, 2008b). For this reason, the

highest measured otolith Cu/Ca at M4 (ANOVA; F= 12.472; p= 0.0001; Tukey´s post hoc test, p= 0.0001) and Zn/Ca at T1 (ANOVA; F= 10.502; p= 0.0001; Tukey´s post hoc test, p= 0.0001), may be indicative of the dietary Cu and Zn bioaccumulation in this environment.

Conclusion

The presence of Cd, Cu, Hg, Pb and Zn on the external layers of *A. saxatilis* otoliths shows the bioavailability of these metals at Mochima National Park and La Tortuga Island, where Pb and Hg have the highest concentrations. Spatial variations make evidence of different concentration of Cd, Hg and Pb in the water and/or sediments of these locations, whilst spatial differences of Cu and Zn may be

indicative of the dietary bioaccumulations in some localities.

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