Marine Pollution Bulletin xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Baseline

A baseline study of the metallothioneins induction and its reversibility in *Neohelice granulata* from the Bahía Blanca Estuary (Argentina)

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ARTICLE INFO

Article history:
Received 2 October 2015
Received in revised form 4 July 2016
Accepted 6 July 2016
Available online xxxx

Keywords:
Heavy metals
Sediments
Metallothioneins
Neohelice granulata
Depuration

ABSTRACT

Contamination by heavy metals causes serious effects in marine systems. Nowadays, the combination of chemical and biological data is recommended in monitoring programs. Metallothioneins (MT) are early-warning signals of metal exposure and are widely used in biomonitoring. The present research evaluates the heavy metals levels in sediments and the MT synthesis in the crab *Neohelice granulata* from the Bahía Blanca Estuary (BBE). Then, the recovery capabilities of *N. granulata* followed by a depuration phase are assessed. Results demonstrate a slow decline in the level of metals in the study area. In relation to MT, female crabs showed elevated MT when compared to males. Furthermore, MT synthesis diminished after the depuration phase. These data constitute the first MT information in organisms from the BBE and may be considered as baseline for future studies in this matter. Although pollution level found was low, further biomonitoring considering both types of data is recommended.

Contamination by heavy metals has received much attention in the last decades, since these pollutants depending on their oxidation state, can be highly reactive and toxic to most organisms (Pinto et al., 2003). The main concerns exist in estuarine and harbor areas, where point and non-point metal sources are predominant, especially in intertidal zones, which receive chemical input from a variety of anthropogenic sources (Banni et al., 2005; Tlili et al., 2010). In this regard, evaluation strategies have been historically developed aiming at determining the presence, concentration and distribution of contaminants in abiotic and biotic compartments. Although the exclusive use of these traditional methods of assessment of metals is a useful guide to evaluate environmental contamination, it has a limited application. Since the main purpose of environmental monitoring is to protect the biological/ecological systems, it is necessary to study the biological effects of exposure to substances present in the environment (Langston et al., 2003). In this way, to improve the efficacy of monitoring programs, the combination of chemical data and biological responses is strongly recommended (Clements, 2000; van der Oost et al., 2003; Laffon et al., 2006). Among the wide range of biological responses, biochemical markers have played a singular role, representing early-warning signals whose detection can prevent adverse effects at hierarchical levels (van der Oost et al., 2003).

Metallothioneins (MT) are small non-enzymatic metalloproteins that bind to metals through cysteine bonds (Amiard et al., 2006). These proteins act in the cellular regulation of metabolically important metals, such as Cu and Zn, as well as in reducing the toxicity of non-essential metals, *i.e.* Cd and Pb (Livingstone, 1993; Viarengo et al., 1997). The induction of MT synthesis in response to metal contaminants in many marine species has been demonstrated. The relationship between metal levels in the environment and MT levels in animal tissues has led to the use of these proteins as biomarkers for monitoring the biological effects resulting from exposure to metals (Hylland et al., 1992; Livingstone, 1993). In aquatic invertebrates it has been demonstrated that MT also acts against cellular oxidative stress, being directly connected to the cellular antioxidant defense system (Viarengo et al., 2000; Cavaletto et al., 2002).

According to their toxicity, impacts by metals have been reported for various species of organisms. However, little is known about the recovery capabilities of aquatic organisms after exposure to trace metals (Baudrimont et al., 2003; Hédouin et al., 2007; Reichmuth et al., 2010; Mouchet et al., 2015). Moreover, there is little research concerning the reversibility or irreversibility of metal impacts in aquatic organisms at the biochemical, genetic and physiological scale after decrease or disappearance of metal contamination. Assessing the responses generated by metal exposure and the persistence of disturbances after contamination ends would provide a better understanding of the metal depuration

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http://dx.doi.org/10.1016/j.marpolbul.2016.07.007
0025-326X/

process in aquatic organisms since the way that different species respond to depuration is also poorly understood. In addition, this phase of decontamination/purification aims to study whether the altered biomarkers during exposure are resilient.

The Bahía Blanca Estuary (BBE) is located in the SW area of Buenos Aires province, Argentina (38°45′–39°25′ S and 61°45′–62°30′ W) (Fig. 1). This estuary extends over about 2300 km^2 and comprises several tidal channels, islands and extensive tidal flats with patches of low salt marshes and crab caves or “cangrejales” from populations of the crab *Neohelice granulata* (Piccolo et al., 2008). At the northern boundaries of the estuary various cities (Bahía Blanca, Punta Alta, Gral. Cerri) and industries (oil, chemical, and plastic factories) are located. The great majority of these industries discharge their effluents within the estuary. In this area is located the only deep water harbor system of Argentina, consisting of various harbors: Galván, Ing. White, Rosales and Naval Base Pto. Belgrano. Thus, the Main Channel is navigated consistently by fishing boats, cargo ships and fuel and cereal transports. For this reason the Main Channel is subjected to periodic dredging, affecting contaminant transport (Marcovecchio, 2000). At this site, there are good chances to introduce pollutants into the environment through man-activities. The main tributaries are the Sauce Chico River (drainage area of 1600 km^2) in the head of the estuary and the Napostá Grande Creak (drainage area of 920 km^2) upward Bahía Blanca city on the northern margin (Perillo et al., 2001).

Since 1978, studies related to the distribution and concentration of heavy metals in different compartments were carried out in this estuary; but so far nothing is known about the induction or expression of biomarkers in organisms from this area. In this context the main goal of the present study was to evaluate for the first time the levels of MTs in the hepatopancreas of the crab *Neohelice granulata* from the BBE, as well as, the potential reversibility of these sequestration proteins synthesis after the depuration phase. Additionally, we analyzed the possible relationships among MTs and heavy metals concentration in sediments.

Neohelice granulata is a benthic and abundant crab that lives intimately associated with sediments, and is considered of ecological importance as an structuring agent of the coastal ecosystem. It is an important member of the estuarine food chain due to its high abundance and its multiple roles as a scavenger, predator and prey. As a key species within this ecosystem, *N. granulata* could play a major role in the transference of pollutants to higher trophic levels. Given *N. granulata*'s ecological role and the extensive knowledge about their biology and maintenance conditions in captivity, it is an excellent model for ecotoxicological studies.

Specimens of *N. granulata* of both sexes were collected in April 2014 at Cuatreros Harbor (innermost area of the BBE) adjacent to the town of Gral. Cerri (Fig. 1). This site is characterized by a water salinity range of 20.8–31.9 psu, a water temperature range of 8.5–23.3 °C, and a sediment composition of mainly silt–clay (96.3%) (Botté, 2005; Angeletti et al., 2014). Sluggish crabs or those lacking one or more appendixes were discarded. Thirty eight mature crabs of each sex in the intermolt stage with a carapace width (maximum distance between the two prominent lateral spines) between 20 and 30 mm for females and between 25 and 35 mm for males were selected. Crabs were transported to the laboratory in thermally isolated boxes with *in situ* collected water.

Complementing sediment samples (approximately 10 to 15 cm thickness of the surface sediment) were also extracted for analysis of heavy metals. Sediment samples were immediately transported to the laboratory in refrigerated boxes and stored at 4 °C. Simultaneously, temperature, conductivity/salinity, pH, turbidity, and dissolved oxygen (DO) concentration were *in situ* measured using a Horiba U-10 multisensor device.

Once in the laboratory, one group of males and females crabs was anesthetized by freezing, weighed and their carapace width was

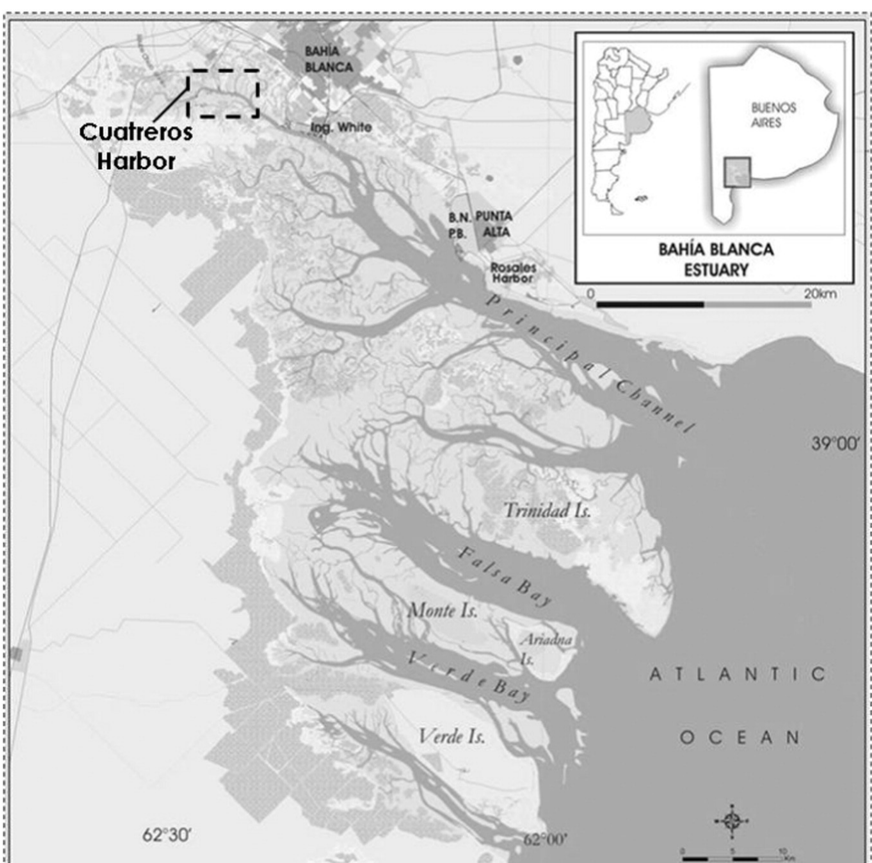

Fig. 1. Location of the study area in the Bahía Blanca Estuary.

Please cite this article as: Buzzi, N.S., Marcovecchio, J.E., A baseline study of the metallothioneins induction and its reversibility in *Neohelice granulata* from the Bahía Blanca Estuary (Argentina)..., Marine Pollution Bulletin (2016), http://dx.doi.org/10.1016/j.marpolbul.2016.07.007