Ecotoxicology and Environmental Safety 155 (2018) 152–161

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

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

Trace metals and oxidative status in soft tissues of caged mussels (*Aulacomya atra*) on the North Patagonian coastline

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ARTICLE INFO

Keywords:
Transplanted mussels
Metal bioaccumulation
Lipid peroxidation
ROS
TOSC
Antioxidant system

ABSTRACT

This study investigated metal accumulation and oxidative effects in mantle, gill and digestive gland of the ribbed mussel *Aulacomya atra* from the Argentinean North Patagonian coastline. Mussels were transplanted over an 18-month period from a site with low anthropogenic impact to a harbor site with higher seawater concentration of aluminum, chromium, copper, manganese, nickel and zinc. Total trace metal concentration in seawater did not change throughout the 18-month transplant in either site. *A. atra* bioaccumulated metals in digestive gland, gills and mantle at different levels. Digestive gland had the highest concentration of metals, especially towards the end of the transplant experiment in the harbor area. Mussels transplanted to the harbor site experienced an upregulation in their antioxidant system, which likely explains the lack of oxidative damage to lipids despite higher metal accumulation. These results demonstrate that *A. atra* selectively accumulates metals from the water column and their prooxidant effects depend on the tissue antioxidant defenses and the exposure time.

1. Introduction

Changes in physicochemical variables and biological parameters due to pollution have been recorded in most of the world's coastal zones. High concentrations of pollutants in closed seas and coastal waters are a major environmental concern because these areas have high biological productivity and human activities (Cohen et al., 1997; Muniz et al., 2015). Metal pollution is one of the most severe anthropogenic disturbances affecting marine organisms because these are unable to degrade metals. As a consequence, metals may be bioaccumulated and biomagnificated throughout food chains and result in several toxic effects (Macfarlane and Burchett, 2000; Miller et al., 2002; Censi et al., 2006). Metal uptake into the body of aquatic animals occurs through the permeable surfaces of the body, mainly the gills and mantle, or through injection of contaminated food particles (Wang and Fisher, 1996). Determination of pollutants in tissues of aquatic organisms is a suitable indicator of its presence in the marine environment (Baqueiro-Cárdenas et al., 2007), especially for those xenobiotics that

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https://doi.org/10.1016/j.ecoenv.2018.02.064
Received 29 December 2017; Received in revised form 20 February 2018; Accepted 22 February 2018

are not metabolized, like metals and metalloids (Luoma and Rainbow, 2005).

Mussels filter relatively high volumes of water for feeding, and as a result typically take up large amounts of trace metals from the surrounding environment, which they accumulate in their soft tissues (Andral et al., 2004; Attig et al., 2010; Ciacci et al., 2012). Transplanting caged mussels' from a site with low anthropogenic impact to polluted sites is widely applied in ecotoxicological studies for monitoring coastal environments (Box et al., 2007; Fasulo et al., 2012; Cappello et al., 2015). The caging approach allows analyzing the harmful effects of aquatic pollutants for a certain time period reducing the effects on the measured biomarkers due to the genetic variability and physiological status (growth and reproduction, between others) of the studied population (Cappello et al., 2013; Marigómez et al., 2013). *Aulacomya atra* is an epifaunal bivalve mollusk that inhabits rocky or mixed bottoms in temperate-cold coastal waters, from shallow depth up to 40–50 m, on hard and soft substrates (Guzmán et al., 1998; Zaixso, 1999). This species is a resource exploited by multispecies artisanal fisheries in several rural and urban locations in Argentinean Patagonia and it constitutes an important economic support for family and regional economies (Narvarte et al., 2007; Orensanz et al., 2007).

Metals can cause oxidative damage to cellular components by increasing the levels of reactive oxygen species (ROS) (Livingstone, 2001; Lesser, 2006; Sheehan and McDonagh, 2008; Tsangaris et al., 2010; Jaishankar et al., 2014) through Haber-Weiss and Fenton-like reactions (Lloyd and Phillips, 1999; Eberhardt, 2001). Aquatic organisms have a ROS scavenging antioxidant defense system that protects against oxidative damage (Gorinstein et al., 2003; Valavanidis et al., 2006; Troschinski et al., 2014; Banni et al., 2015). The antioxidant system comprises enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, and non-enzymatic agents like reduced glutathione and vitamin E, among others. Oxidative stress parameters such as changes in antioxidant levels and oxidative damage to different cell components are frequently used as biomarkers to quantify effects induced by trace metals, test their toxicity and assess the health of aquatic life (de Almeida et al., 2004; Führer et al., 2012; Kumari et al., 2014).

Along the ~3000 km long Argentinean Patagonian coastline, trace metals toxicity is associated to harbor areas, which generally have the highest pollutantion levels (Esteves, 2008). The Almirante Storni pier (Puerto Madryn, North Patagonia) was used by the aluminum industry for importing raw materials (aluminum oxide, aluminum fluoride, silicon metal and steel) and for exporting manufactured aluminum products for approximately 50 years, with a maximum metal production capacity of 460,000 t of metal per year (Di Salvatore et al., 2013). Previous studies reveal higher levels of metal and polycyclic aromatic hydrocarbons (PAHs) concentrations in the Almirante Storni pier (Gil et al., 1999; Commendatore and Esteves, 2007; Massara Paletto et al., 2008; Di Salvatore et al., 2013).

The aim of this study was to investigate metal accumulation and their oxidative effects on soft tissues of the native ribbed mussel *A. atra*. To achieve this goal, mussels were transplanted from a site with low anthropogenic impact to a site near the Almirante Storni pier, and oxidative stress parameters were measured over an 18-month period.

2. Materials and methods

2.1. Sampling area and experimental design

96 mussels of similar size (7.75 ± 0.48 cm shell length) were sampled at Punta Cuevas (PC) (42° 46 ´28 ´´S; 64° 56 ´54 ´´W) (Fig. 1) by diving at a depth of 9–10 m during March (summer) 2012, on the southern edge of Puerto Madryn city. This site experiences low anthropogenic impact (Di Salvatore et al., 2013). After collection, mussels were randomly sorted and placed inside 16 plastic (PVS) cages with mortar bottoms as substrate (6 mussels per cage, similar density per square meter that registered in the sampling site). The cages were placed in the collection site for a two-week acclimation period. After the acclimation period, all cages were recovered by diving and mussels from four cages were sampled (PC, initial time: it), while the others 12 cages were transported to the harbor site at a depth of 9–10 m (Almirante Storni pier, AS) (42° 44 ´14 ´´S; 65° 1 ´43 ´´W). Four cages were collected at random after 6, 12 and 18 months. After collection, mussels were immediately anesthetized by placing on ice before they were killed. The digestive gland, gills and mantle were dissected and weighted and immediately frozen at − 80 °C during three days. After that, samples were transported frozen (− 4 °C) to the University of Buenos Aires where metals levels and oxidative stress biomarkers were measured. In addition, 500 mL seawater samples were collected at the beginning and the end of the experiment to determine the total concentration of metals in each sampling site. Water samples were collected and stored in sterile plastic bottles previously washed with 2 M nitric acid, and acidified to pH < 2 with (1:1) nitric acid (Martin et al., 1991),

2.2. Metals measurements

Aluminum (Al), chromium (Cr), copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) concentrations were measured by ICP-OES (Perkin Elmer, Optima DV 2000, USA) equipped with a sample introduction unit consisting of a Scott chamber and a flow GemCone™ nebulizer. Perkin Elmer quality control standards N9300281 and N9300280 were used as the stock standards for preparing working solutions of Al, Cr, Cu, Mn, Ni and Zn. In order to validate the method for accuracy and precision, certified reference materials ERMCE278K (SIGMA-ALDRICH) and the Fish Protein DORM-4 NRC were analyzed. In whole set of measurements recovery (%) were around 85–110% (data not shown).

Water samples were analyzed directly, while mussels' soft tissues were weighed, washed in ice-cold saline, homogenized individually in 0.134 M KCl (1:5, w/v) as described by Türkmen and Ciminli (2007) and Di Salvatore et al. (2013). Solid particles were removed using a cellulose nitrate filter (0.45 μm) coupled to a syringe prior to measuring metal concentrations.

Each sample was analyzed by triplicate (standard deviation less than 4%) and a blank was run to correct the intensity emission values. Additionally, for every ten water or tissue samples, a procedure blank and a spike sample containing all reagents were run to check for interference and cross-contamination. The water used throughout the present study was obtained from a Milli-Q water purification system (Millipore GmbH, France) with a resistivity of 18.2 MOhm cm^{-1}.

Data is expressed as μg metal per L water and μg metal per g wet tissue, respectively.

2.3. Sample preparation for biochemical measurements

Digestive gland, gills and mantle tissues from each animal were homogenized on ice with 0.134 M KCl (1:5, w/v) containing protease inhibitors (phenylmethylsulfonil fluoride 0.5 mM and benzamidine 10 mM). The homogenates were centrifuged 15 min at 11,000 × *g* and the resulting supernatants were used for the assays described below.

2.4. Protein content

Total soluble protein content was measured by the method of Bradford (1976), using bovine serum albumin as standard. Results are expressed as mg protein per mL.

2.5. Reduced glutathione (GSH) content

Reduced glutathione (GSH) content was determined in the supernatants as described by Anderson (1985) in presence of 5,5-dithiobis-(2- nitrobenzoic) acid (DTNB). A freshly prepared solution of