Comparative Biochemistry and Physiology, Part C 156 (2012) 80–86

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Ruditapes decussatus and *Ruditapes philippinarum* exposed to cadmium: Toxicological effects and bioaccumulation patterns

Etelvina Figueira [a,*], Paulo Cardoso [c], Rosa Freitas [b]

[a] CBC, Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[b] CESAM, Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal
[c] Departamento de Biologia, Universidade de Aveiro, 3810-193 Aveiro, Portugal

ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 21 April 2012
Accepted 22 April 2012
Available online 27 April 2012

Keywords:
Clams
Metal contamination
Cd subcellular partitioning
Tolerance mechanisms
Methallothioneins

ABSTRACT

Since differences in metal accumulation may exist between bivalve species, the aim of this study was to assess the impact of cadmium (Cd) on *Ruditapes decussatus* and *Ruditapes philippinarum*. For this, the Cd accumulation, mortality rates and biochemical responses were analysed in the two species after 5 days of exposure, under laboratory-controlled conditions. The concentration of Cd that caused 50% of mortality on clams was two-times higher in *R. decussatus* than in *R. philippinarum*. For both species, higher percentage (84.5–98.2%) of the Cd was in the insoluble fraction, but the Cd concentration in solution was 3 to 8 times higher in *R. decussatus*. Nevertheless, *R. philippinarum* presented higher oxidative stress and higher CAT activity. The paradox observed between the two clams can be explained by the higher capacity of *R. decussatus* to increase the expression of MTs when exposed to Cd.

1. Introduction

Metal contamination has been of great concern in marine and coastal ecosystems, since they cause, even at quite low concentrations, environmental deterioration of natural conditions, affecting the organisms inhabiting these areas. Metals may accumulate in aquatic species, such as bivalves, at concentrations several times higher than concentrations in water and sediment due to their ability to filter large quantities of particles, including contaminants, from the seawater, sediment or food (Metian et al., 2009). In addition, the wide spread distribution, sedentary life style and ease of collection make bivalves good candidates for studying bioaccumulation of contaminants. For these reasons, bivalves are often used as sentinel organisms in monitoring programmes. Thus, *Ruditapes decussatus* and *Ruditapes philippinarum* have been successfully employed throughout the world as biological monitors of anthropogenic pressures, namely metal pollution (e.g. Roméo and Gnassia-Barelli, 1997; Smaoui-Damak et al., 2009; Serafim and Bebiano, 2010; Ramos-Gómez et al., 2011; Wang et al., 2012). *R. decussatus* (Linnaeus, 1758), the European clam, can be found in the Atlantic, from the North of Africa to the South of Scandinavian and in the Mediterranean waters (Flassch and Leborgne, 1992; Gosling, 2002; Jensen et al., 2004). Natural populations of this species can be found in sandy and muddy-sand sediments in bays, estuaries and coastal lagoons (Gosling, 2003). *R. philippinarum* (Adams and Reeve, 1850), commonly known as the Manila clam, is an endemic species in the Indian–Pacific region, but it was introduced in North European Atlantic and Mediterranean coastal waters at the beginning of the 1970s, for commercial cultivation (Gosling, 2002; Jensen et al., 2004; Delgado and Pérez-Camacho, 2007). Because *R. philippinarum* is more resistant to environmental physical stress (Solidoro et al., 2000; FAO, 2005; Pravoni et al., 2006; Tanguy et al., 2008), its immune response to bacteria is faster (Moreira et al., 2012), and its habitat and requirements are similar to *R. decussatus*, they compete not only on aquaculture farms but also in the natural conditions (Usero et al., 1997). Although it has been already described the replacement of *R. decussatus* by *R. philippinarum* in different ecosystems (Solidoro et al., 2000; Gosling, 2003; Pravoni et al., 2006), little information is available regarding the reasons behind this process.

In the aquatic ecosystems, cadmium (Cd) is one of the most abundant, ubiquitously distributed toxic metal (Novelli et al., 2000) and its effects have been well documented, especially in bivalves (e.g. Blasco and Puppo, 1999; Ketata et al., 2007; Martín-Díaz et al., 2007; Haiqing et al., 2009; Smaoui-Damak et al., 2009; Paul-Pont et al., 2010a; Wang et al., 2011). Within the Water Framework Directive, this metal is considered as a priority substance (EC, 2001). Cadmium accumulation in organisms increases the formation of reactive oxygen species (ROS) which can cause protein, DNA and lipid damage (Regoli et al., 2004), enzyme inhibition, cell signalling impairment, and changes in gene regulation (Stohs and Bagchi, 1995; Elbekai and El-Kadi, 2005). In bivalves, the levels of oxidative stress caused by metal have been

* Corresponding author at: CBC (Centre for Cell Biology), Departamento de Biologia, Universidade de Aveiro, Campus Universitario de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 234370782; fax: +351 234372587.
E-mail address: efigueira@ua.pt (E. Figueira).

1532-0456/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2012.04.004

studied in terms of antioxidant defence systems and lipid peroxidation levels. Studies, conducted with *R. decussatus*, demonstrated the effects of Cd exposure in the activity of antioxidant enzymes (Matozzo et al., 2001, 2012) and lipid peroxidation (Roméo and Gnassia-Barelli, 1997; Geret et al., 2002), induction of metallothioneins (Hamza-Chaffai et al., 1999, 2000; Smaoui-Damak et al., 2004, 2009; Serafim and Bebiano, 2010), reproduction (Smaoui-Damak et al., 2006; Ketata et al., 2007), and changes in protein expression profiles in the gills and the digestive gland (Chora et al., 2009). Works performed with *R. philippinarum* exposed to Cd are less common but have showed alterations in metallothionein synthesis (Ng and Wang, 2004; Paul-Pont et al., 2010a, 2010b) and antioxidant enzyme activities (Wang et al., 2011).

Since differences in Cd accumulation may exist between *R. decussatus* and *R. philippinarum*, the objective of the present work was to assess the impact of Cd contamination on both clam species, living in sympatry and exposed simultaneously to the same experimental conditions. Because information on their levels of tolerance and bioaccumulation abilities are scarce, analysis of different bioaccumulation rates and toxicological responses to Cd can be of major value.

2. Material and methods

2.1. Sampling strategy and experimental conditions

For laboratory experiments, specimens of *R. decussatus* and *R. philippinarum* were collected in March, in Ria de Aveiro (a lagoon at the Northwest coast of Portugal). For the selection of the sampling site it was taken into account that both species were living in sympatry and, therefore, under the same environmental conditions. Specimens of similar size (4.1–4.5 mm) and weight (6.7–7.1 g) were selected to minimise differences in the results.

In the laboratory organisms were maintained in seawater, under continuous aeration. Clams were submitted to depuration 48 h prior to the Cd exposure assay that consisted in the submission of 15 organisms/species (3 replicates, 5 organisms/replicate) to 5 different Cd concentrations (10, 18, 32, 56 and 100 μM) and to a control condition (0 μM Cd). The Cd concentrations used in experimental assays were selected to resemble low, medium and high contaminated areas. Also, this concentration range was used to determine the tolerance of organisms. A plastic container, with 1 l of filtered seawater, was used for each replicate. During the experiment, clams were not fed and the water of each container was renewed completely once daily. After 5 days of exposure organisms were frozen at −80 °C. Temperature (20 ± 1 °C) and salinity (32 ± 1.5) were maintained during the depuration and experimental periods and the photoperiod was fixed at 12 h. During the experiments, when identified, dead organisms were removed from the containers. Organisms were considered dead when their shells gaped and failed to shut again after external stimulus.

For metal quantification in sediments and water, at the sampling site, 3 replicates of sediment and water were collected and preserved at −20 °C until analysis.

2.2. Quantification of cadmium

The concentration of Cd was measured in sediments, water (interstitial water and water column) and organisms. All quantifications were done by a certified laboratory at the University of Aveiro. Regarding the quality controls, the calibration of the apparatus was made with IV (Inorganic Venture, Christiansburg, Virginia, USA) standards (IV–ICP–MS 71A) and they were verified with reference material (NIST 1643e). During metal analysis, the accuracy observed ranged between 90 and 110%. All samples below this accuracy level were rejected and the analysis repeated. Determinations were performed using 3 replicates.

Sediments: For Cd quantification, 2 g of homogenised air dried sediment was digested overnight (± 18 h) at 115 °C with 10 ml of 65% HNO_3 (Suprapur, Merck) in digestion Teflon bombs (sealed chambers). To prevent the loss of metals by volatilisation, chambers were only opened when completely cooled. The cooled digest was made up to 25 ml with1M HNO_3 and Cd concentration was determined by ICP–MS (Inductively Coupled Plasma–Mass Spectroscopy).

Water: For quantification of Cd in interstitial water (IW), 70 g of sediment was centrifuged for 5 min at 16,000 *g* and the supernatant collected. Both IW and water from the water column (WC) were acidified with 0.5 ml 65% HNO_3 (Suprapur, Merck). Samples were stored at +4 °C until Cd determination, following the procedure described for sediments.

Organisms: In order to obtain the total concentration of Cd present in both species, organisms were transferred to Teflon bombs. The biological samples (excluding shells) were digested overnight (± 18 h) at 115 °C with 2 ml of 65% HNO_3 (Suprapur, Merck). The cooled digest was made up to 5 ml using 1 M HNO_3, and the concentration of Cd was determined by ICP–MS.

The soluble and insoluble distribution of elements in the organisms was adapted from Wallace et al. (2003). Samples were thawed, wet weighed and homogenised with liquid nitrogen using a mortar and a pestle, and subjected to subcellular fractionation by centrifugation at 100,000 *g*, for 15 min at 4 °C. Fractionation resulted in the isolation of two distinct fractions: soluble and insoluble. In the first, elements are soluble in the cytosol and in the second elements are contained in the organelles, MRG (metal-rich granules) and cellular debris. Once isolated, samples were preserved at 4 °C until Cd present in both fractions was quantified by ICP–MS, as described previously.

2.3. Biochemical parameters

For biochemical measurements, frozen organisms (soft tissues) were thawed, weighted, and pulverised under liquid nitrogen with a mortar and a pestle. Extraction was performed with the specific buffer for each biochemical analysis and the samples centrifuged for 10 min at 10,000 *g* and 4 °C. Supernatants were stored at −80 °C or used immediately.

Total protein was determined by the Biuret method (Robinson and Hogden, 1940), using bovine serum albumin (BSA) as standard.

Lipid peroxidation was determined by measuring the amount of total malondialdehyde (MDA) following the method described by Ohkawa et al. (1979). The calculation of MDA concentration was made using its extinction coefficient ($1.56 \times 10^5\ M^{-1}\ cm^{-1}$).

Antioxidant enzymes: The activity of CAT was measured in cell extracts, homogenised in 50 mM phosphate buffer (pH 7.0), containing 1 mM ethylenediaminetetraacetic acid (EDTA), 1% Triton X-100 (v/v), 1% PVP (w/v) and 1 mM dithiothreitol (DTT) in the proportion of 1:5 (w/v). CAT activity was determined by the reaction of the enzyme with methanol in the presence of H_2O_2 (Lars et al., 1988). One unit was defined as the amount of enzyme that caused the formation of 1.0 nmol formaldehyde under the assay conditions. The standard curve was determined using formaldehyde standards. For SOD activity, cell extracts were homogenised in 100 mM Tris–HCl buffer (pH 8.0) containing 0.1 mM EDTA, 0.5% Triton X-100 (v/v) and 1% PVP (w/v) in the proportion of 1:2 (w/v). The method of Beauchamp and Fridovich (1971) was followed with slight modifications (Freitas et al., 2012). One unit of enzyme activity corresponds to a reduction of 50% of nitroblue tetrazolium (NBT). The standard curve was performed with SOD standards.

For the activity of GSTs, cell extracts were homogenised in 0.1 M phosphate buffer (pH 6.8) containing 1 mM EDTA in the proportion of (1:2 w/v). The activity of GSTs was determined following the method described by Habig et al. (1974). The activity of GSTs was determined using extinction coefficient of 9.6 $mM^{-1}\ cm^{-1}$ for CDNB.