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Biochemical performance of native and introduced clam species living in sympatry: The role of elements accumulation and partitioning

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The present study reports metal and arsenic contamination in sediments, as well as element accumulation and partitioning in native (*Ruditapes decussatus* and *Venerupis corrugata*) and introduced (*Ruditapes philippinarum*) clam species living in sympatry at the Óbidos lagoon (Portugal). The biochemical performance and the human health risks derived from the consumption of these species are also discussed. The results obtained showed that *R. decussatus* was the most abundant species in all the sampling sites, revealing that the introduced clam has not yet supplanted the native species. The concentration of elements was higher in areas with higher Total Organic Matter (TOM) and fines content, being Chromium (Cr), Copper (Cu) and Lead (Pb) the most abundant metals. Clams from these areas showed the highest concentration of elements but the lowest bioaccumulation levels. Furthermore, except for As, higher concentration of elements was found in clams insoluble fraction, the less toxic fraction to the organisms. Due to the low contamination levels and because elements, except As, were mainly allocated to the insoluble fraction, clams presented similar biochemical parameters among distinct areas, with no significant oxidative stress induced. Furthermore, clams from the Óbidos lagoon represent a low health risk to human consumption since, except for As, their contamination levels were below the maximum permissible limits defined by international organizations.

1. Introduction

Coastal lagoons are among the most productive ecosystems supporting high biodiversity and important ecological role (Costanza et al., 1997; Beltrame et al., 2009). Nevertheless, these environments are subjected to different sources of contamination including domestic, agricultural and industrial discharges (Kjerfve and Magill, 1989; Correia et al., 2012). Among different contaminants, metals and metalloids are a worldwide problem (Acton, 2011; ATSDR, 2014).

Due to their persistence and toxicity, particular attention has been given to identify the key factors which control the concentration and spatial distribution of metals and metalloids in aquatic systems (Chakraborty et al., 2012). It is known that environmental factors, such as organic matter, pH, salinity, temperature, and fine particles content may strongly affect the bioavailability of metals and metalloids in the marine environment and, consequently, their toxicity (Eggleton and Thomas, 2004; Allen and Janssen, 2006). For this reason ecotoxicological effects of a given element are often poorly correlated with its concentration in the environment, namely with sediments contamination (Eggleton and Thomas, 2004; Allen and Janssen, 2006). Nevertheless, several studies concerning metal and metalloids contamination in aquatic systems have focused on the use of bioindicator species, organisms with the capacity to bioaccumulate contaminants and to reveal their effects (e.g. Mora et al., 2004; Sfriso et al., 2008; Bergayou et al., 2009; Jena et al., 2009; Pellerin and Amiard, 2009; Velez et al., 2015a). However, although metals and metalloids accumulation in bivalves, namely clams, has been widely studied (e.g. Alkarkhi et al., 2008; Nilin et al., 2012; Chandurvelan et al., 2015), as well as the risks associated with their consumption (Sfriso et al., 2008; Figueira et al., 2011), less information is available regarding organisms biochemical alterations induced by contaminants (e.g. Leiniö and Lehtonen, 2005; Bergayou et al., 2009; Pellerin and Amiard,

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2009; Velez et al., 2015a; Chandurvelan et al., 2015). Furthermore, while studies at the community level use different species to assess the impacts of a given contamination source, comparing species richness and abundance within the same area and among different areas, when evaluating the biochemical alterations induced by contaminants few studies compared the responses of different species coexisting in the same area (Leiniö and Lehtonen, 2005; Pellerin and Amiard, 2009; Freitas et al., 2012a; b; Velez et al., 2015a).

Among the most common bivalve species used as bioindicators are clams, namely *Ruditapes decussatus* (Linnaeus, 1758) (e.g. Matozzo et al., 2012; El Nemr et al., 2012), *Ruditapes philippinarum* (Adams and Reeve, 1850) (Sfriso et al., 2008; Matozzo et al., 2012; Wang et al., 2012) and *Venerupis corrugata* (Adams and Reeve, 1850) (Velez et al., 2014). These species have a wide distribution, present high abundance, sessile nature and filter-feeding capacity (Beeby, 2001; Kucuksezgin et al., 2010). In addition to their ecological importance these clams represent an important source of income to coastal populations (Costanza et al., 1997).

The species *R. philippinarum*, native from Indo-Pacific waters, has been introduced in the Atlantic and Mediterranean, for culture purposes, in early 70's: first, in France and later in England, Spain, Italy and recently in Portugal (Moschino et al., 2012; FAO, 2015a). The grooved carpet shell clam (*R. decussatus*) and the pullet carpet shell clam (*V. corrugata*, formerly known as *Venerupis pullastra*) are native from Europe, being the most cultivated species in Atlantic coast of France, Spain, Portugal and in the Mediterranean basin (FAO, 2015b). The habitat requirements of the native and introduced species are similar, living buried in sand, gravel or mud bottoms (FAO, 2015a,b,c; Juanes et al., 2012; Bidegain and Juanes, 2013; Bidegain et al., 2015; Velez et al., 2015b), leading to clams competition in their natural habitat (Juanes et al., 2012; Giani et al., 2012; Bidegain and Juanes, 2013). In fact, recent studies performed by Velez et al. (2015b) revealed that, in Portugal, the recently introduced clam *R. philippinarum* is already spread along the Ria de Aveiro but this clam has not replaced the native species *R. decussatus* and *V. corrugata* in this aquatic system. In other aquatic systems, such as Arcachon (France), this replacement already happened (Blanchet et al., 2004; Dang et al., 2010; Bidegain and Juanes, 2013).

Thus, the objectives of the present work were: a) determine the abundance of *R. decussatus*, *R. philippinarum* and *V. corrugata* in different sampling areas in a moderate contaminated system; b) quantify elements concentration in the sediments and the bioaccumulation pattern of the three clam species, identifying if sediments or clam species from different areas presented significantly different contamination levels and/or if different clam species from the same area presented similar accumulation values; c) compare sediment and organisms contamination, evaluating the Biota-Sediment Accumulation factor (BSAF) in each of the areas and compare the bioaccumulation capacity among clams; d) assess if clams elements concentration may cause any human health risks due to these species consumption and e) identify the biochemical responses of the three species, assessing the differences among areas and comparing species performance at each area.

2. Material and methods

2.1. Field sampling

The Óbidos Lagoon is a small and shallow coastal lagoon located in the west Portuguese coast with a mean depth of 1 m, covering a wet area of approximately 7 km^2 (Pereira et al., 2009a) (Fig. 1). Being permanently connected to the sea by a narrow inlet (on the order of 100 m) (Oliveira et al., 2006), this lagoon is characterized by extensive intertidal sandbanks partially separated by deep channels and small freshwater contributions (Carvalho et al., 2005). The freshwater inflow enters the lagoon by the Cal River at the Barrosa arm, Vala do Ameal at the Bom Sucesso arm and by the Arnóia River discharge (between both lagoon arms) (Oliveira et al., 2006). The Arnóia River contributes about 90% of freshwater fluxes into the lagoon, being the major source of sediments, whose deposition has created an extensive sand bank (Oliveira et al., 2006). This lagoon has been classified as a moderately contaminated system (Pereira et al., 2009a, 2009c; Carvalho et al., 2011). Previous studies showed that the Barrosa arm receives agriculture and urban effluents from Caldas da Rainha City, resulting in an area with high nutrient availability, being previously classified as eutrophic (Pereira et al., 2009b). Concerning metals and As contamination Oliveira et al. (2006) and Pereira et al. (2008) showed that the major source of these contaminants was related to wastewater discharges in Cal River (Barrosa arm), with maximum concentrations of Ni, Cu and Cd in periods of higher inflow and remobilization from sediments in summer months (Pereira et al., 2009b). The Bom Sucesso arm is also a confined area but receives a smaller freshwater flow (Vala do Ameal) with better water quality than the Cal River, according to the Portuguese categorization of freshwater systems. In this area, metal remobilization from sediments is less likely due to the greater depth (Pereira et al., 2009).

According to Carvalho et al. (2005), in this lagoon three different macrobenthic communities are identified, corresponding to the inlet, central and inner parts of the system which seem to reflect the adaptations of the communities to the environmental gradients established within the lagoon due to tidal currents and fluvial discharge. The bivalves, *Cerastoderma edule*, *R. decussatus* and *V. corrugata* were among the most characteristic species of the intermediate area of the lagoon (Rodrigues et al., 2012). To our knowledge no works are known where *R. philippinarum* has been described for this lagoon.

2.2. Sampling procedure

R. decussatus, *V. corrugata* and *R. philippinarum* specimens were collected in June 2014 from 5 different areas at the Óbidos Lagoon, representing areas with different contamination levels and different physico-chemical characteristics (Fig. 1). In each area 3 sites were selected and in each site all clams present in rectangle (45 cm × 25 cm) were collected, being determined the relative abundance (the percentage of each species had in the total number of organisms). Also, at each area, three replicates of sediments were collected (one per site) for sediment grain size analysis, total organic matter (TOM) content determination, and quantification of elements concentration.

The environmental variables pH, salinity and temperature were measured with specific probes at each sampling site.

After sampling, specimens and sediments were transported on ice (0 °C) to the laboratory. In the laboratory, 12 individuals of each species, collected in each area were weighted, measured and used to determine the Condition Index (CI). The remaining organisms were preserved at −80 °C until analysis.

For biochemical analysis and elements quantification 6 individuals per species and from each site were used. For both analysis organisms (whole soft tissues) were, individually, pulverized with liquid nitrogen.

2.3. Laboratory analysis

2.3.1. Sediment grain size and organic matter content determination

Sediment grain-size was analyzed by wet and dry sieving