Estuarine, Coastal and Shelf Science 155 (2015) 114–125

Contents lists available at ScienceDirect

Estuarine, Coastal and Shelf Science

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

Spatial distribution and bioaccumulation patterns in three clam populations from a low contaminated ecosystem

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ARTICLE INFO

Article history:
Received 23 August 2014
Accepted 2 January 2015
Available online 12 January 2015

Keywords:
metals and metalloids
sediment contamination
bioaccumulation
shellfish consumption
health risk
elements partitioning

ABSTRACT

When consuming bivalves, special concern should be taken to the total element burden. In order to assess this issue the present study aimed to measure the element levels in the sediments of different harvesting areas and relate them with clam accumulation; to assess the elements body burden, their availability for trophic transfer and relate it with total accumulation in clams, comparing the native (*Ruditapes decussatus* and *Venerupis corrugata*) and the invasive (*Ruditapes philippinarum*) species; to evaluate the human risk associated with the consumption of different clam species. The results showed that the element burden in clams does not reflect the sediment contamination and BAF values were higher in the less contaminated areas. Comparison of Maximum Levels (MLs) from international organizations with the concentration of elements in clams showed that As exceeded standard levels. The ingestion of less than 1 Kg per week of clams would result in exceeding the PTWI threshold for As. Furthermore, the results showed that, when comparing to other elements, As and Hg are more easily available to be transferred trophically.

1. Introduction

The introduction and spread of invasive species has been identified as a major ecological threat in coastal marine communities that, in most of the cases, can negatively affect the native species leading, in extreme situations, to their replacement (e.g. Pranovi et al., 2006). Thus, the study of interactions between the non-indigenous and native marine species has become a focus of interest, especially when dealing with economically relevant species. In this regard, different bivalve species have been intentionally introduced in several coastal ecosystems, such as the case of the Manila clam *Ruditapes philippinarum* (Adams and Reeve, 1850). The Manila clam is a native species to the Indo-Pacific region and it was introduced in Europe at the beginning of the 1970s for culture purposes (Flassch and Leborgne, 1992; Jensen et al., 2004), becoming a highly exploited resource (Usero et al., 1997; Allam et al., 2000; Pranovi et al., 2006; Delgado and Pérez-Camacho, 2007; Dang et al., 2010; Figueira et al., 2012; Moschino et al., 2012; Figueira and Freitas, 2013; FAO, 2014a). The capacity of the Manila clam to rapidly adapt to a new environment, its fast growth capacity and important commercial value (Usero et al., 1997) has greatly changed the exploitation of living resources in different aquatic systems. In Portugal, *R. philippinarum* was recently introduced, appearing for the first time in FAO statistics in 2009 (Gaspar, 2010), living in sympatry with the native clams *Ruditapes decussatus* (Linnaeus, 1758) and *Venerupis corrugata* (Gmelin, 1791). The grooved carpet shell clam (*R. decussatus*) is native to Europe, being found along the Atlantic coasts from the British Isles to as far as Senegal and the Mediterranean Sea (Breber, 1985; FAO, 2014b). The pullet carpet shell clam (*V. corrugata*, formerly known as *V. pullastra*) range, extends from North East Atlantic to the Mediterranean Sea, being mainly harvested in Spain, Portugal, France and Italy (Anacleto et al., 2013; FAO, 2014c). For Portugal, recent information shows that the national annual production of clams represents 42% of the total shellfish production (INE, 2013) being fundamental to the national socioeconomic framework, directly or indirectly employing thousands of workers.

In the aquatic environment, metals (e.g. Hg, Pb and Cd) and metalloids (e.g. As) are among the most dangerous groups of pollutants, not only because of their high toxicity but also due to their high accumulation. Due to their filtration nature, bivalves can accumulate high amounts of these pollutants, often having concentrations higher than those of the sediments where they are buried (Karouna-Renier et al., 2007). Metals and metaloids

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http://dx.doi.org/10.1016/j.ecss.2015.01.004
0272-7714/

accumulated by bivalves may be transferred to higher trophic levels, namely to human consumers (Figueira et al., 2011). For this reason, the level of metals in seafood raise public health concerns and international organizations like EFSA, USFDA or FSANZ have set maximum levels (MLs), above which edible seafood cannot be marketed.

Due to their feeding behavior, clams have been recommended as possible indicators of seawater and sediments contamination (Lie et al., 2006; Wang et al., 2012). Furthermore, because alterations of the environmental conditions in aquatic systems may alter the ecosystem equilibrium and may favor some species over others, the comparison of the effects of contaminants in native and invasive clam species inhabiting the same areas is of prime relevance. Earlier data revealed that, when submitted to laboratory conditions, differences in the accumulation and tolerance to pollutants may exist between the clams *R. decussatus* and *R. philippinarum* (Wang, 2001; Wallace et al., 2003; Figueira et al., 2012). Studies comparing native and invasive species under laboratory conditions are becoming increasingly common, although there are few field studies with different species inhabiting the same areas. In this context, the identification of suitable harvestable grounds and a reliable estimation of potential commercial yield are necessary to both guarantee a substantial fishery and the security of the seafood product. Therefore, this work aims to assess the bioaccumulation ability of the three clam populations (*R. decussatus*, *V. corrugata*, *R. philippinarum*), inhabiting the Ria de Aveiro coastal lagoon, to relate their spatial distribution and densities with the contamination levels of different locations where these species are harvested for human consumption. Furthermore, although health authorities use total metal as standards of food safety, total burden in bivalves may not directly relate to the metal amount that is actually transferred to consumers. The elements accumulated by organisms are partitioned in two fractions: soluble and insoluble fraction. In the soluble fraction, elements are present in the cytosol along with proteins for detoxification (metallothionein-like proteins), and it has been shown that elements present in this fraction are more available for assimilation by predators (Wallace and Luoma, 2003; Metian et al., 2009). The absorption of elements present in the insoluble fraction (metal-rich granules, organelles and cellular debris) is mostly dependent on the consumers digestive capacity (Wallace and Luoma, 2003; Geffard et al., 2010). Therefore, the present study will present the partitioning of elements in clams providing valuable information on the metal fraction more trophically available to predators and consumers.

Thus, the present study aims to: (1) measure the element levels in the sediments of sampling areas differing in metal and As contamination and relate these levels to wild clams contamination (bioaccumulation factor); (2) assess clams element body burdens available for assimilation and relate them with total levels in clams, comparing the contamination levels of the native (*Ruditapes decussatus* and *Venerupis corrugata*) and the invasive (*Ruditapes philippinarum*) species; (3) determine the total element concentrations in wild clams and compare with the EFSA (European Food Safety Authority), USFDA (U.S. Food and Drug Administration) and FSANZ (Food Standards Australia and New Zealand) maximum levels (MLs), and determine the amount of clams flesh needed to be consumed to exceed provisional tolerable week intake (PTWI); (4) discuss the human risk associated with clam consumption, evaluating differences between native and invasive species. Since little information is available about *V. corrugata*, the present work will further increase the knowledge on this species that is distributed along the Atlantic coast.

2. Materials and methods

2.1. Study area

The present study was conducted in the Ria de Aveiro (Fig. 1), a shallow vertically homogeneous estuary located on the northwest coast of Portugal (40°38′ N, 8°45′ W). This aquatic system is 45 km long and 10 km wide and covers an area of 83 km^2 at high tide (spring tide), which is reduced to 66 km^2 at low tide. It is characterized by narrow channels and by large areas of mud flats and salt marshes (Picado et al., 2009).

This aquatic system has been submitted to anthropogenic pressure, namely mercury (Hg) contaminated effluent discharges, from the 1950s until 1994 (Pereira et al., 2009). In the last decade, the Hg discharge diminished considerably but the concentrations of this metal in the surface sediments of some areas of this system are still very high, namely in the Laranjo bay and in the surrounding area (Pereira et al., 2009; Figueira et al., 2011).

2.2. Sampling procedure

Along the Ria de Aveiro, 15 areas (named from A to O) were selected, and 3 sites per area were surveyed in September 2012. At each site, all clam individuals present in a square of 50 × 50 cm were collected for clam density (# clams m^{-2}) determination (Elliott, 1977). After sampling, the organisms were transported on ice to the laboratory and preserved at −20 °C until analysis. At the same sampling sites, sediment samples were collected, for sediment grain size analysis, total organic matter (TOM) quantification and determination of elements (chromium, Cr; nickel, Ni; copper, Cu; lead, Pb; cadmium, Cd; mercury, Hg; arsenic, As) concentration. Samples for TOM and contaminant quantification were transported on ice (0 °C) to the laboratory and preserved at −20 °C. In the field pH, salinity and temperature were measured in sediments with specific probes.

2.3. Laboratory procedures

2.3.1. Biometric data

In the laboratory, individuals collected at each site, were weighed and measured. The Condition Index (CI), which gives an indication of the general physiological status of the animals, was calculated individually. Soft tissues were carefully separated from shells and washed with distilled water to remove dirt. Both soft tissues and shells were put in an oven at 60 °C for 48 h and then weighed for CI. CI values were expressed as the ratio between the dry weight of soft tissues and the dry weight of shell × 100 (Matozzo et al., 2011). Individuals collected at each sampling site were weighed and measured (length and width).

2.3.2. Grain size analysis

Sediment grain-size was analyzed by wet and dry weight following the procedure described by Quintino et al. (1989). The silt and clay fraction (fine particles, with diameter <0.063 mm) and the gravel fraction (particles with diameter >2.000 mm) were expressed as a percentage by dry weight of the total sediment. The sand fraction (0.063–2.000 mm) was dry sieved through a battery of sieves spaced at 1 phi (Φ) ($\Phi = -\log_2$ the particle diameter (mm)). Data were used to calculate the median value, P_{50}, expressed in phi (Φ) units (dry weight, DW). The median and the percent content of fines were used to classify the sediment, according to the Wentworth scale: very fine sand (median from 3 to 4 Φ); fine sand (2–3 Φ); medium sand (1–2 Φ); coarse sand (0–1 Φ); very coarse sand (−1 to 0 Φ). All sediment grain-size fractions were expressed as a percentage of the whole sediment, DW. The final