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RESEARCH ARTICLE

Consumption of *Ruditapes philippinarum* and *Ruditapes decussatus*: comparison of element accumulation and health risk

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Abstract *Ruditapes philippinarum*, a species native from the Indo-Pacific region, was introduced in Europe at the beginning of the 1970s for culture purposes, leading to a massive decrease of the native species *Ruditapes decussatus* and a high increase of *R. philippinarum* yields in Europe. Bivalves can accumulate high amounts of metals and thus easily reach concentrations that are toxic not only to themselves but also to consumers. Since differences in the accumulation of pollutants may exist between bivalve species, different health risks may be overcome. For this reason, the level of metals in seafood raises public health concerns, and international organisations like European Food Safety Authority, United States Food and Drug Administration, and Food Standards Australia and New Zealand (FSANZ) set maximum levels (MLs), above which edible seafood cannot be marketed. In order to evaluate the risk associated with the consumption of *R. philippinarum* and *R. decussatus*, both clam species were collected in the same site in Ria de Aveiro and the concentration of eight elements determined in organisms before and after a 48-h depuration period. Results evidence that even at low contaminated areas, the MLs for some elements can easily be achieved. The concentrations of As were above the reference values for FSANZ, and the provisional tolerable weekly intake (PTWI) is exceeded for As when more than 0.5 kg of *R. decussatus* and 0.9 kg of *R. philippinarum* clam flesh is consumed, in 1 week, by an adult (70 kg). When comparing with other systems worldwide, consumers of depurated clams from this coastal system have a similar or lower risk of exceeding the PTWI for Cd, As, Pb, and Hg. The recently introduced clam, *R. philippinarum*, accumulates lower amounts of the most health-threatening elements (less than 71 % of Cd, 40 % of As, and 20 % of Hg) than the native *R. decussatus*, except for Pb. *R. philippinarum* also reduces more the element burden when subjected to depuration than *R. decussatus*. Moreover, *R. philippinarum* allocates a lower proportion of the accumulated elements in the soluble fraction, where they are readily available. Thus, it is safer to consume *R. philippinarum* than *R. decussatus*, except when clams come from areas heavily polluted by Pb.

Keywords Clams · Marine food · Metal and As accumulation · PTWI · Maximum levels (MLs) · Coastal lagoon · Ria de Aveiro

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Introduction

World bivalve production and consumption has increased significantly during the last years, going from a combined total for wild catch and aquaculture of approximately 10.7 million tonnes in 1999 to 14 million tonnes in 2006 (Lee et al. 2008). Although bivalves constitute an economically relevant activity for populations neighboring coastal systems, this income is often limited by the productivity and contamination of coastal systems. One way to overcome these limitations and increase production is introducing species economically more competitive, such as the Manila clam *Ruditapes philippinarum*. By having high reproductive and growth rates and ability to tolerate a wide range of environmental conditions, *R. philippinarum*, a species native from the Indo-Pacific region, was introduced in Europe at the beginning of the 1970s for culture purposes, initially in France (1972) and later in England, Spain, and Italy (Flassch and Leborgne 1992), occupying a habitat that overlaps that of the native species *Ruditapes decussatus*. *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). *R. philippinarum* successfully competes with *R. decussatus* (Bautista-Parejo 1989) not only in aquaculture farms but also on natural conditions (Usero et al. 1997). This led to a massive decrease of the native species, and recently, *R. philippinarum* constituted 91 % of European yields of the two species (Donaghy et al. 2009).

The two clam species are morphologically similar, making it possible to harvest and commercialize both indiscriminately. However, since differences in the accumulation of pollutants may exist between bivalve species (Baudrimont et al. 2005; Figueira et al. 2012; Wallace et al. 2003; Wang 2001), different risks to public health may be overcome. Thus, it becomes urgent to investigate differences among bivalve species in the bioaccumulation of agents which may affect public health.

Bivalves for human consumption require a depuration period (European Regulation, Reg. CE 853 and 854/2004), aiming to reduce the content in potential pathogenic microorganisms. Studies revealed that a depuration period of 48 h is enough to eliminate 100 % of *Escherichia coli* (Bernard 1989). However, the effect of depuration on the chemical load, such as persistent elements, of bivalves has been neglected, and the way that different species respond to depuration is also poorly studied (Freitas et al. 2012a).

Metals are one of the most dangerous groups of pollutants for human health, not only because of their high toxicity but also due to high accumulation and low metabolization. Due to their filtration nature, bivalves can accumulate high amounts of metals, often having concentrations higher than in the sediment where they are buried (Karouna-Renier et al. 2007) and, thus, easily reaching concentrations that are toxic not only to themselves but also to consumers (Figueira et al. 2011). For this reason, the level of metals in seafood raise public health concerns, and international organisations like European Food Safety Authority (EFSA), United States Food and Drug Administration (USFDA), or Food Standards Australia and New Zealand (FSANZ) set maximum levels (MLs), above which edible seafood cannot be marketed. Metals accumulated in organisms are not equally available for uptake (Wallace and Luoma 2003; Metian et al. 2009). While elements in the soluble fraction are readily available to be transferred to consumers, the elements associated with the insoluble fraction are dependent on the digestive capacity of consumers (Rainbow and Smith 2010; Metian et al. 2009). Hence, different patterns of metal distribution between soluble and insoluble fractions imply different amounts available for absorption, which may be species-specific (Freitas et al. 2012b).

Therefore, the main goal of this work was to assess the inorganic element accumulation ability of the clams *R. philippinarum* and *R. decussatus*, directly collected from the environment and after a 48-h depuration period. Evaluating the partitioning of the accumulated elements in soluble and insoluble fractions in both depurated and non-depurated clams was also a goal of the present study. This information allowed us to determine the effect of depuration on the element load in organisms and to compare the risk associated with the consumption of each species. To achieve this goal, the two clam species, collected from Ria de Aveiro (Portugal) where both species are periodically harvested for human consumption, were: (1) used to determine the total levels of Zn, Cu, Cr, Ni, Cd, Hg, Pb, and As, as well as the element distribution between soluble and insoluble fractions; (2) submitted to depuration (48 h), and the total concentration of elements and their distribution through soluble and insoluble fractions was determined. Results of these two tasks were used to: (3) assess if element concentrations of environment and depurated *R. decussatus* and *R. philippinarum* exceeded the EFSA, USFDA, and FSANZ MLs; (4) determine the amount of clam flesh needed to be consumed to exceed provisional tolerable week intake (PTWI); and (5) discuss and compare the health risk linked to *R. decussatus* and *R. philippinarum* consumption.

Materials and methods

Study area

For the present study, *R. decussatus* and *R. philippinarum* were collected in Ria de Aveiro, a shallow coastal system located in the northwest of Portugal. Although *R. philippinarum* was recently introduced in this coastal system, it was possible to find both species living in sympatry in a specific area and, therefore, under the same environmental conditions.

Sampling

In the selected area, 20 organisms of *R. decussatus* and *R. philippinarum* were collected in March. Specimens of similar size (41–45 mm) and weight (6.7–7.1 g) were selected to minimize differences in the results. After sampling, organisms were transported in ice-cold containers to the laboratory, and, upon arrival, ten individuals per species were frozen (−20 °C) for analysis. These organisms were considered as representing field/environmental conditions, further identified as Env. The other ten organisms, of each species, were placed in plastic tanks with seawater under continuous aeration at 20±1 °C and 12-h photoperiod, during 48 h, for depuration. After this period, organisms were stored at −20 °C, until analysis. These organisms represented depuration effects, being designated as Dep.

Simultaneously with clams, sediment samples were collected in the same study area, using a corer with 20-cm diameter, which were transported on ice (0 °C) to the laboratory and preserved at −20 °C until analysis. These sediments were used for element quantification (both in sediment and interstitial water). Additionally, at the each sampling site, water samples from the water column were obtained for element quantification.

Elements quantification

Sediment and water

The concentration of eight elements, zinc (Zn), chromium (Cr), nickel (Ni), copper (Cu), lead (Pb), cadmium (Cd) mercury (Hg), and arsenic (As), was measured in sediments and water (interstitial water and water column). All the element 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 standards, and they were verified with standard reference material (National Institute of Standards and Technology, NIST SRM 1643e). During element analysis, the accuracy observed ranged between 90 and 110 % (information given by the laboratory). All samples below this accuracy level were rejected and the analysis repeated.

For sediment element quantification, 2 g of homogenized sediment was digested overnight at 115 °C with 10 ml of concentrated HNO_3 in Teflon bombs (sealed chambers). To prevent the loss of elements by volatilization, chambers were only open when completely cooled. The cooled digest was made up to 25 ml with 1 M HNO_3, and metals were determined by inductively coupled plasma–mass spectrometry (ICP-MS) using an ICP-MS Thermo X Series.

For quantification of elements 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 element determination, following the procedure described for sediments.

Organisms: R. decussatus and R. philippinarum

In order to obtain the total concentration of metals and As present in both species, five organisms per species and condition were transferred to Teflon chambers. The soft tissues (excluding bivalve shells) were digested overnight at 115 °C with 10 ml of concentrated HNO_3 and allowed to cool before open the chambers. The cooled digest was made up to 25 ml using 1 M HNO_3, and the concentration of metals was determined by ICP-MS model Thermo X Series.

Soft tissues from five organisms were subjected to fractionation, following the method of Wallace et al. (2003) with some modifications (Freitas et al. 2012a). Briefly, samples were thawed, wet-weighed, and homogenized with liquid nitrogen using a mortar and a pestle and subjected to subcellular fractionation by centrifugation at 80,000×*g*, for 1 h at 4 °C. Fractionation resulted in two fractions, defined by Wallace and Luoma (2003) as the cytosol and the organelles plus the debris (containing metal-rich granules and cellular debris), corresponding to element soluble and insoluble fractions, respectively. Once separated, samples were preserved at 4 °C until the concentration of metals and As in both fractions were quantified by ICP-MS, as described previously.

Data analysis

Data obtained from Env and Dep organisms (elements concentrations and partitioning) were submitted to hypothesis testing using permutation multivariate analysis of variance, with the PERMANOVA+ add-on in PRIMER v6 (Anderson et al. 2008), following the calculation of Euclidean distance matrices among samples. The pseudo-F values in the PERMANOVA main tests were evaluated in terms of significance. When the main test revealed statistical significant differences ($p<0.05$), pairwise comparisons between species and conditions (Env and Dep) were performed. The *t*-statistic in the pairwise comparisons was evaluated in terms of significance. Values lower than 0.05 revealed that areas differed significantly. Distinct letters (a–d) were used to indicate differences between species and conditions (Env and Dep).

The null hypotheses tested were: (a) for each element (soluble and insoluble fractions), no significant differences exist between species and conditions; (b) for each element (bioaccumulation and partitioning factors), no significant differences exist between species and conditions; (c) for each element (total concentration), no significant differences exist between species; (d) for each element (PTWI), no significant differences exist between species.

Results

Sediment contamination

The concentration of the elements (metals and As) in the WC, IW, and sediment of the sampling area are presented in Table 1. All elements in the WC and most in the IW are below the detection limits; the only exception is As in the IW. When analyzing the elements present in the sediment, it is noticeable that Zn is the most abundant metal, followed by Pb and Cr. The levels of Ni, Cu, and As are similar; Hg is in much lower concentration, and Cd is below the detection limit (0.013 mg/kg). Table 1 also presents the levels of metals and As in Antarctic sediments, generally described as a pristine area (Ahn et al. 1996), revealing that the

Table 1 Environmental contamination: element concentrations in sediment (milligrams per kilogram dry weight) and water (water column and interstitial water, micrograms per liter), from the sampling site

Sample	Cr	Ni	Cu	Zn	As	Cd	Pb	Hg
Sediment	2.4 (0.2)	1.6 (0.2)	1.1 (0.0)	7.3 (0.9)	1.7 (0.3)	< 0.01	5.2 (1.0)	0.01 (0.00)
Water column	<10	<10	<10	<20	<10	<1	<10	<0.50
Interstitial water	<10	<10	<10	<20	28.2 (6.4)	<1	<10	<0.50
Antarctic nearshore	7.6[a]	15.4[b]	11[b]	32[b]	6.2[c]	0.2[b]	7.0[b]	0.01[d]

Values are the mean of three replicates, with standard deviation in brackets

[a] Ahn et al. 1996

[b] Leniban et al. 1990

[c] Xie and Sun 2008

[d] Bargagli et al. 1998

sediments of the study area present similar (Hg and Pb) or lower element concentrations than Antarctic sediments.

Elements in environmentally exposed and depurated clams

The element concentration in the insoluble and soluble fractions present in *R. philippinarum* and *R. decussatus*, collected from the environment or submitted to depuration, are shown in Figs. 1 and 2, respectively. In clams from the environment, both species presented similar concentrations of Cr, Ni, and Cd in the insoluble fraction (Fig. 1). Although differences were not significant, *R decussatus* accumulated more Zn, Cu, As, and Hg in the insoluble fraction, whereas *R. philippinarum* accumulated significantly more Pb. Generally, in both species, a depuration period of 48 h reduced the concentration of elements in the insoluble fraction. Nevertheless, in this fraction, the levels of Ni in both species, As, Cu, Zn, and Hg in *R. philippinarum* and Cd in *R. decussatus* were not significantly different ($p<0.05$) between environmental and depurated clams (Fig. 1). The concentration of elements in the soluble fraction of *R. decussatus* and *R. philippinarum* from the environment were similar for Cr, Cd, Zn, and Hg (Fig. 2). However *R philippinarum* accumulated more Cu, As, and Ni, although differences were not statistically different, whereas *R. decussatus* accumulated more Pb. In the soluble fraction, depuration reduced the concentration of Cu, As, and Hg and increased Pb in *R. philippinarum*, whereas in *R.*

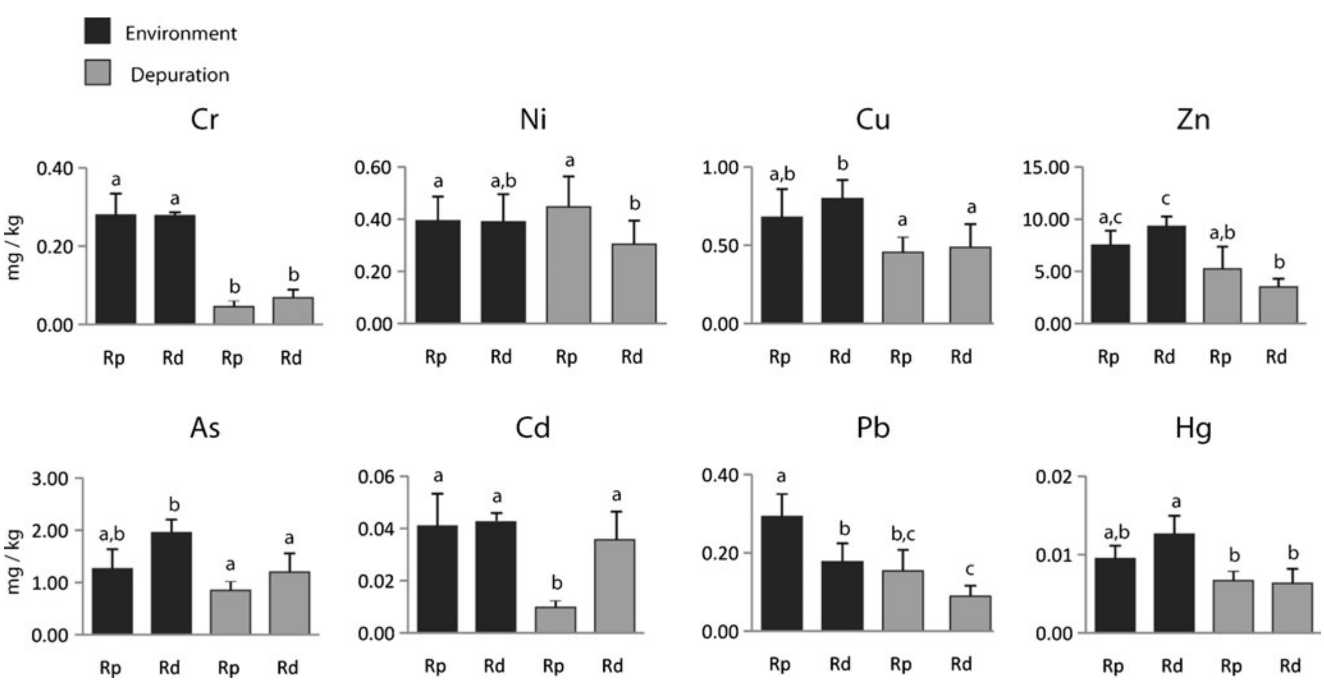

Fig. 1 Element concentrations (milligrams per kilogram wet weight) in the insoluble fraction of *R. decussatus* (Rd) and *R. philippinarum* (Rp). Values are the mean of five replicates±standard deviation. Different letters (*a*–*d*) represent significant differences ($p<0.05$) between columns of the same chart

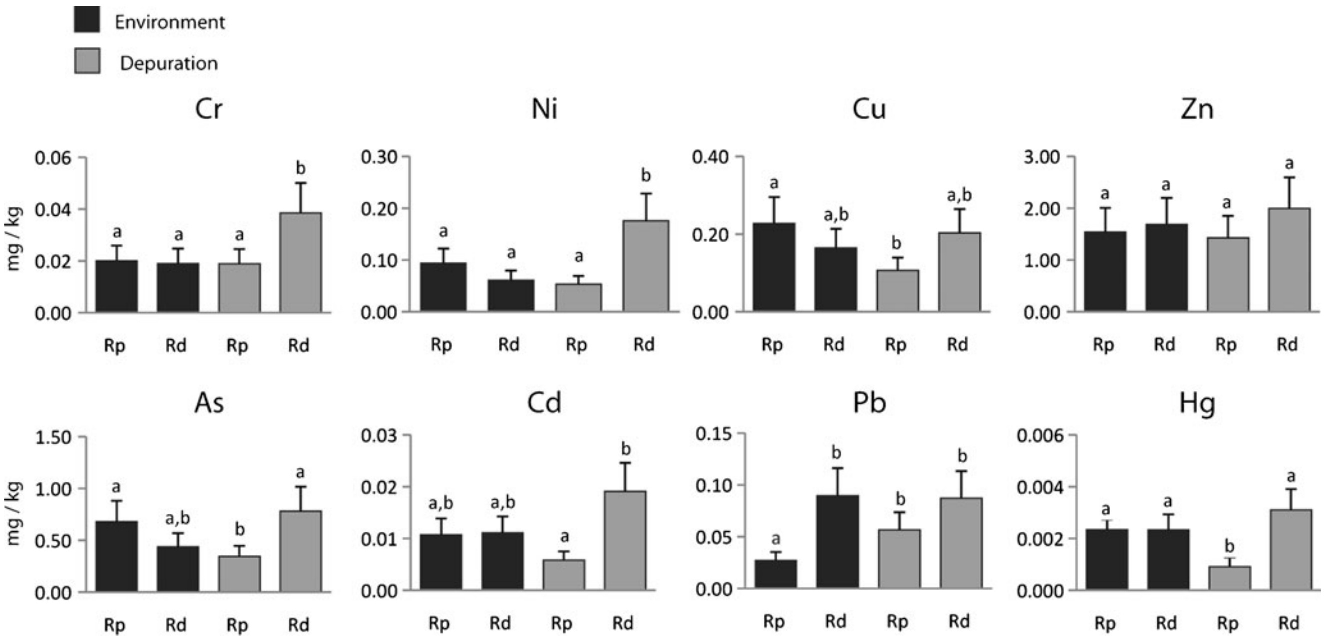

Fig. 2 Element concentrations (milligrams per kilogram wet weight) in the soluble fraction of *R. decussatus* (Rd) and *R. philippinarum* (Rp). Values are the mean of five replicates±standard deviation. Different letters (*a*–*d*) represent significant differences ($p<0.05$) between columns of the same chart

decussatus the concentration of the eight elements, especially for Cr (2 times) and Ni (2.9 times) increased after depuration (Fig. 2).

Comparing the concentration of the elements in clams with sediments (bioaccumulation factor (BAF)), it is evident that both clam species had lower concentrations of Cr (7.9–8.9×), Ni (3.2–3.5×), Cu (1.2×), and specially Pb (16.4–19.6×) than in the sediment (Table 2). Reversely, the concentrations of Zn, As, Cd, and Hg were higher in both clam species than in sediments (cf. Table 2), with *R. decussatus* presenting higher BAF values than *R. philippinarum* for these elements. A depuration period of 48 h reduced the bioaccumulation factors for most elements, although significant differences were only determined for Cr in both species, Zn in *R. decussatus*, and Cd and Hg in *R. philippinarum* (cf. Table 2).

Partitioning factor evidences that the majority of accumulated elements are in the insoluble fraction (cf. Table 2). Differences between elements and species are noticeable. Cr is the element with the lowest partitioning factor, while Pb, Cd, and As are presented as the highest. In organisms from the environment except for Pb, the partitioning factor is generally higher in *R. philippinarum* than in *R. decussatus*. After 48 h of depuration, the element distribution changes. In *R philippinarum*, with the exception of Zn, Cd, and Pb, the partitioning factor significantly decreases, revealing a lower amount of elements in the soluble fraction while, in *R. decussatus*, the partitioning factor increases significantly for the all elements analyzed, showing that the amount of elements in the soluble fraction is higher after depuration.

Dietary risk assessment

Both *R. philippinarum* and *R. decussatus* are commonly fished and the whole flesh consumed. Clams, collected in the Ria de Aveiro, where both species are harvested for human consumption, were analyzed for toxic elements in order to assess the potential health risks. The concentrations of most of the elements tested in both clam species after depuration, a procedure required for commercialization in EU, were below the EFSA, USFDA, and FSANZ maximum levels (Table 3), and only As exceeded these standards (FSANZ). The element content in the whole soft parts of the clams allowed the computation of the mass of bivalves to be eaten by a 70-kg adult to reach the PTWI (cf. Table 3). Overall, As appears to be the main element of concern regarding both species consumption. The ingestion of less than 0.9 kg of *R. philippinarum* and 0.5 kg of *R. decussatus* per week would result in exceeding the PTWI threshold. For other elements, large amounts (>8.3 Kg) of clam flesh would need to be consumed each week in order to exceed the PTWI standard values (cf. Table 3). Nevertheless, for most elements, it is necessary to consume 14 % (Hg) to 71 % (Cd) more *R. philippinarum* than *R. decussatus* flesh to exceed PTWI. Exceptions are made for Zn and Pb, where the consumption of more 18 % and 16 % respectively of *R. decussatus* than *R. philippinarum* flesh is needed to exceed PTWI.

Table 2 Bioaccumulation and partitioning factors of the clams *R. philippinarum* (Rp) and *R. decussatus* (Rd) for different elements and at distinct conditions

Element	Bioaccumulation factor				Partitioning factor			
	Rp-Env	Rd-Env	Rp-Dep	Rd-Dep	Rp-Env	Rd-Env	Rp-Dep	Rd-Dep
Cr	0.13 (0.03) a	0.13 (0.01) a	0.03 (0.01) b	0.05 (0.01) b	0.07 (0.01) a	0.06 (0.02) a	0.29 (0.03) b	0.36 (0.01) c
Ni	0.31 (0.08) a	0.29 (0.08) a	0.41 (0.09) a	0.40 (0.09) a	0.19 (0.01) a	0.14 (0.00) b	0.11 (0.01) c	0.37 (0.00) d
Cu	0.83 (0.23) a'b	0.88 (0.16) b	0.52 (0.11) a	0.63 (0.19) a'b	0.25 (0.01) a	0.17 (0.02) b	0.19 (0.02) b	0.29 (0.05) a
Zn	1.23 (0.25) a	1.50 (0.20) a	0.91 (0.17) a'b	0.75 (0.19) b	0.17 (0.02) a	0.15 (0.03) a	0.21 (0.02) b	0.36 (0.02) c
As	1.11 (0.33) a'b	1.37 (0.22) b	0.68 (0.15) a	1.14 (0.34) a'b	0.35 (0.01) a	0.18 (0.03) b	0.29 (0.02) c	0.40 (0.00) d
Cd	>3.97 (>1.15) a	>4.15 (>0.46) a	>1,20 (0.27) b	>4.22 (>1.23) a	0.21 (0.03) a	0.20 (0.03) a	0.37 (0.05) b	0.35 (0.01) b
Pb	0.06 (0.02) a	0.05 (0.01) a	0.04 (0.20) a	0.03 (0.01) a	0.09 (0.00) a	0.34 (0.01) b	0.27 (0.12) b	0.49 (0.00) c
Hg	1.20 (0.20) a	1.50 (0.30) a	0.76 (0.15) b	0.95 (0.30) a'b	0.20 (0.04) a	0.15 (0.04) a'b	0.12 (0.03) b	0.33 (0.00) c

Bioaccumulation factor: ratio between the total element concentration in the organism and the element concentration in the sediment. Partitioning factor: ratio between element concentration in the soluble fraction and the total element in the organism. Values are the mean of five replicates, with standard deviation in brackets. For each element, different letters (a–d) represent significant differences ($p<0.05$) between species and conditions

Env environment, *Dep* depuration

Discussion

The risk associated with the consumption of *R. philippinarum* and *R. decussatus* was evaluated by determining the concentration of eight elements in both species inhabiting the same site in Ria de Aveiro, before and after a 48-h depuration period. In the environment, both species accumulated low amounts of elements, except for As. Kucuksezgin et al. (2010) reported for the same elements (except for As) concentrations in *R. decussatus* 1.6× (Cd) to 34× (Cr) higher of those found in the present work. The concentrations of Cd, Cr, Zn, Pb, Ni, and Cu found in *R. philippinarum* from seven sites in Maluan Bay (Wang et al. 2012) were always much higher than those reported in the present study for the same species, but As concentrations were only 1.8 to 6.3× higher. In other clam species (*Mercenaria mercenaria*), the concentration of the same elements from several sites in the Indian River Lagoon were similar to or higher than the values determined in our work (Trocine and Trefry 1996). The low concentrations obtained in the present work, for most of the elements analyzed, can be explained by the low concentration of the elements in the sediment, since they were generally at lower concentrations than those found in sites considered pristine, such as Antarctic sediments (Ahn et al. 1996). Furthermore, except for As, the water column and interstitial water also showed very low element concentrations, as they were below the detection limits of the equipment used. The higher concentration in As in the interstitial water compared with other elements may explain the relatively high concentration of this element in both clam species. The two species showed similar absorption patterns of elements: The concentrations of Zn, Hg, As, and Cd were higher in the organisms (BAF>1) while, for the remaining elements, the concentrations were higher in the sediments (BAF<1). Nevertheless, *R. decussatus* showed higher ability to accumulate most of the elements analyzed, except for Ni and Pb, compared with *R. philippinarum*. This trend was confirmed by laboratory experiments conducted by the present authors, where both species were submitted to identical concentration ranges of Cd (Figueira et al. 2012), As, Hg, and Pb (data not published). The same authors showed that *R. decussatus* accumulated higher concentrations of As, Hg, and Cd, whereas *R. philippinarum* accumulated higher concentrations of Pb. Usero et al. (1997) compared the accumulation of different elements in *R. decussatus* and *R. philippinarum* from sites with different contamination levels and as in the present study; *R. decussatus* accumulated higher concentrations of most elements when both species were collected from the same site. Baudrimont et al. (2005) compared the bioaccumulation of three bivalve species from the Girond estuary: *Cerastoderma edule* and *R. philippinarum* accumulated similar concentrations of Cu, Cd, and Zn but much less than *Crassostrea gigas* and *R. philippinarum*, and *C. gigas* accumulated similar concentrations of Hg, but much

Table 3 Concentrations of different elements in *R. decussatus* and *R. philippinarum* from Ria de Aveiro and the amount of clams that a 70-kg adult needs to consume to exceed PTWI

	Cr	Ni	Cu	Zn	As	Cd	Pb	Hg	Reference
ML (mg kg^{-1})									
EFSA						1.0	1.5	0.5	EC 2006
USFDA	13	80			86	4	1.7	1	USFDA 2001
FSANZ					1	2	2	0.5	Vannoort and Thomson 2005
Total element concentration in clams after depuration (mg kg^{-1})									
R. philippinarum	0.065a	0.500 a	0.562 a	6.67 a	1.19 a	0.016 a	0.211 a	0.008 a	
R. decussatus	0.107 a	0.479 a	0.690 a	5.48 a	1.98 b	0.055 b	0.176 a	0.009 a	
PTWI (mg kg^{-1} $week^{-1}$)									
JECFA			3.5	7.0	0.015	0.007	0.025	0.005	JECFA 2004
FSANZ					0.015	0.007	0.025	0.005	Vannoort and Thomson 2005
Amount of clams consumed to exceed PTWI after depuration (kg)									
R. philippinarum			435.6 a	73.5 a	0.9 a	31.3 a	8.3 a	43.0 a	
R. decussatus			354.8 a	89.4 a	0.5 b	8.9 b	9.9 a	37.1 a	

For each element, different letters (a–b) represent significant differences ($p<0.05$) between species

Current consumption guidelines for elements set by different organizations are also presented: *JECFA* Joint FAO/WHO Expert Committee on Food Additives, *USFDA* United States Food and Drug Administration, *EFSA* European Food Safe Authorities, *FSANZ* Food Standards Australia and New Zealand, *ML* maximum levels in shellfish (milligrams per kilogram fresh weight), *PTWI* provisional tolerable week intake (milligrams per kilogram per week)

less than *C. edule*. These absorption features can be important in systems where the concentration of inorganic elements is high, since the two clam species under analysis can easily accumulate high concentrations of Cd, As, Hg, and Zn, and at the same conditions, *R. decussatus* will accumulate higher amounts of these elements than *R. philippinarum*. However, in systems heavily polluted by Ni or Pb. the ability of *R. philippinarum* to avoid biomagnification of these two elements (BAF<1) may not be enough to prevent their accumulation at concentrations that can cause toxicity.

The toxicity of an element is not only dependent on the total amount accumulated but on its partition as well. Elements in solution interfere with macromolecules with metabolically important functions, such as enzymes, transporters, or DNA and therefore are more toxic than insoluble elements (Valko et al. 2005; Pytharopoulou et al. 2008; Zhang et al. 2010). In the two clams, the majority of the elements are in the insoluble fraction, and both species showed similar partition features for the different elements, except for Pb. The concentration of Pb in the soluble fraction is almost three times higher in *R. decussatus* than in *R. philippinarum*. Elements such as Cd and As, that are accumulated in higher proportions in the soluble fraction, are potentially more toxic than the others. Freitas et al. (2012c) determined the percentage of several inorganic elements in the soluble fraction of *C. edule*, demonstrating that, in this species, Cd and As were also the elements with higher percentages in the soluble fraction.

Except for Ni, depuration lowered the element burden between 17 % (As for *R. decussatus*) and 78 % (Cr for *R. philippinarum*). Other works also reported reductions in the element loads of bivalves submitted to depuration (Freitas et al. 2012c; Chong and Wang 2000; El-Shenawy 2004; Han et al. 1993). Reductions are higher in the first 24 h (Chong and Wang 2000), being species-dependent. In the present work, the decrease in element concentrations caused by depuration was similar in both species. However, the concentration of Zn reduced more in *R. decussatus* and As in *R. philippinarum*. While *R. decussatus* was not able to reduce the Cd concentration after depuration, *R. philippinarum* reduced by 70 % the Cd load. These results are important since they evidence changes in the element burden of organisms subjected to depuration, but also emphasize differences between the two species. As and Cd are 1.7× and 3.5× higher, respectively, in *R. decussatus* than in *R. philippinarum* after depuration. Zn concentration, that was initially lower, became higher in *R. philippinarum* than in *R. decussatus* after depuration. Depuration also interfered with the partition of elements. In *R. philippinarum*, element concentrations in the soluble fraction was maintained or decreased, whereas in *R. decussatus* increased. In fact, *R. decussatus* accumulated 1.4 to 3.4× higher concentrations of elements in the soluble fraction than *R. philippinarum*. These results suggest a higher availability of elements in *R. decussatus*, even when the total concentration is equal in the two species.

Clams are one of the main shellfish resources in the world, with 3.5 million tonnes produced in 2010 (FAO 2011). In European countries, the transaction of *R. philippinarum* has been gradually replacing *R. decussatus*, and implications of this substitution for public health was never evaluated. The results presented in this work show that the risk of dietary exposure to inorganic elements from clam consumption is predominantly from As. Only the concentrations of As were above the reference values for FSANZ, and the PTWI is exceeded for As when more than 0.5 kg for *R. decussatus* and 0.9 kg for *R. philippinarum* of clam flesh is consumed, in 1 week, by an adult (70 kg). However, the concentration of Cd and Pb should be of general concern, since Ria de Aveiro presents extremely low Cd and Pb concentrations compared with the mean of the worldwide coasts and estuaries—1.1 and 34 $mg kg^{-1}$ dry weight for Cd and Pb, respectively (Ahn et al. 1996; Cheggour et al. 2005; Ramos-Gómez et al. 2011), but the PTWI is exceeded for Cd and Pb when more than 8.9 kg for *R. decussatus* and 8.3 kg for *R. philippinarum* of clam flesh are consumed by an adult (70 kg) in 1 week. When comparing total element concentration of clams with cockles (*C. edule*) also from an area in Ria de Aveiro with similar element concentrations (Figueira et al. 2011), we can conclude that cockles accumulated higher levels of most elements, except for Hg and As which were similar. Thus, the health risk of consuming clams from this coastal system is lower than consuming cockles. When comparing with other systems worldwide, consumers of depurated clams from this coastal system have a similar or lower risk of exceeding the PTWI for Cd, As, Pb, and Hg than clam (Smaoui-Damak et al. 2004; Hamza-Chaffai et al. 2000; Bebiano et al. 1993; Kucuksezgin et al. 2010; Usero et al. 1997; Wang et al. 2012) and shellfish (Baraj et al. 2003; Bargagli et al. 1985; Bebianno and Machado 1997; Beliaeff et al. 1998; Fung et al. 2004; Liang et al. 2004; O'Connor 2002; Otchere et al. 2003; Whyte et al. 2009; Yap et al. 2004) consumers from other countries.

This and other work (Figueira et al. 2011; Usero et al. 1997) evidence that, even at low-contamination areas, the MLs for some elements can easily be achieved, prohibiting marketing and inviabilizing clam culture for commercial purposes in many areas. Differences in element accumulation between species exist. In this work, it was observed that *R. philippinarum* generally accumulates lower concentrations of elements than *R. decussatus*, except for Zn and Pb. Stakeholders and ecosystem managers should take into consideration the features of element accumulation of each shellfish species and the level of contamination of different elements in the sediment, when evaluating an area for bivalve production, in order to select the most appropriate species for a particular area. This procedure will guarantee that the use of available areas for shellfish culture can produce bivalves not only below standard maximum levels but also with the lowest element load possible and thus be more safe to consumers. Element partitioning in organisms is not currently considered in shellfish marketing, but it affects their bioavailability and thus is also a feature important to consumers. While the soluble fraction is readily available, the absorption of the insoluble fraction is dependent on the digestive capacity of consumers (Rainbow and Smith 2010; Metian et al. 2009). Bivalve species that have a higher proportion of elements in solution potentially constitute a higher risk to consumers than species accumulating most of the element burden in insoluble forms. By accumulating a larger fraction of elements in the soluble fraction, *R. decussatus* constitutes a higher health concern than *R. philippinarum*, since a higher proportion of the elements consumed will be absorbed when equal amounts of both clam species are consumed.

Conclusion

In summary, this study provides evidence that the recently introduced Indo-Pacific clam, *R. philippinarum*, accumulates less 20 to 70 % of the most health-threatening elements (Cd, Hg, and As) than the native *R. decussatus*, except for Pb. *R. philippinarum* also reduces more the element burden when subjected to depuration than *R. decussatus*. Moreover, *R. philippinarum* allocates a lower proportion of the accumulated elements in the soluble fraction. Thus, it is safer to consume *R. philippinarum* instead of *R. decussatus*, except when clams come from areas heavily polluted by Pb.

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