

Note

Lead concentrations and size dependence of lead accumulation in the clam *Dosinia exoleta* from shellfish extraction areas in the Galician Rías (NW Spain)

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Abstract – To protect public health, the European Commission established maximum levels of certain contaminants permitted in foodstuffs. The maximum amount of lead allowed in bivalve mollusc is $1.5 \mu\text{g g}^{-1}$ wet weight. In the Galician Rías, which are important areas of shellfish production in Spain, high levels of lead have been detected in the commercial bivalve *Dosinia exoleta* (Veneridae). Given the environmental and socio-economical problems this could represent, Pb concentration was tested in *D. exoleta* from two Rías, and the relation of lead accumulation with body size studied in detail. Implications for fisheries management are also discussed in this paper. Results showed a strong dependence of Pb accumulation on body size, with lead concentrations increasing exponentially with shell length. Larger animals presented a 5-fold increase in lead concentrations above the maximum permitted level. The size limit (length beyond which *D. exoleta* should not be extracted for commercial purposes) was initially established at 40 mm; but a more comprehensive geographical study of Pb concentrations in individuals from 35 to 40 mm long revealed that this size limit was not protective enough, and 35 mm is proposed as a safer limit.

Key words: Metal accumulation / Pollution / Size effect / Lead / Clam / *Dosinia exoleta*

1 Introduction

Lead is one of the metals most frequently enriched by man because of its use in piping, building materials, storage batteries, paints and other chemicals (Sadiq 1992). Large amounts of Pb and its compounds have been emitted into the atmosphere in past decades as a result of leaded gasoline combustion. Consequently, Pb has become ubiquitous in our environment, presenting a potential hazard for both animals and humans. One of the well known toxic effects of inorganic lead is its inhibition of hemoglobin synthesis, leading to the development of anemia. Since Pb ions have an ionic structure similar to calcium ions, they may be taken into nervous system cells and mitochondria in a similar manner to Ca (Chang and Cockerham 1994), and may hence reduce cognitive development and intellectual performance in children, and cause blood pressure and cardiovascular diseases in adults. In order to protect public health, and with the objective of further lowering the mean levels of lead in foodstuffs, the European Commission established

limits on the maximum levels of Pb permitted in different food sources for humans. In the case of bivalve molluscs, the maximum established level was $1.5 \mu\text{g g}^{-1}$ wet weight (Anonymous 2002).

Shellfish production in Galicia (NW Spain) represents more than 90% of total shellfish production in Spain, in which the clam *Dosinia exoleta* represents an important fraction. In 2005, *D. exoleta* production reached 1452 tons. This is equivalent to 16% of the total extracted bivalves, which include 17 different species (Anonymous 2005). According to work by Beiras et al. (2003a,b) and Prego and Cobelo-García (2003), the Galician Rías present a low degree of pollution, mainly restricted to localised areas. However, Pb is the metal with the narrowest safety margin in the Galician Rías, and concentrations exceeding legal standards have been reported in non-commercial wild mussels from the inner parts of the Rías (Beiras et al. 2003c).

The present study was incited by the fishers' associations of the Rías of Pontevedra and Arousa, important shellfish production areas in Galicia, after local authorities found high

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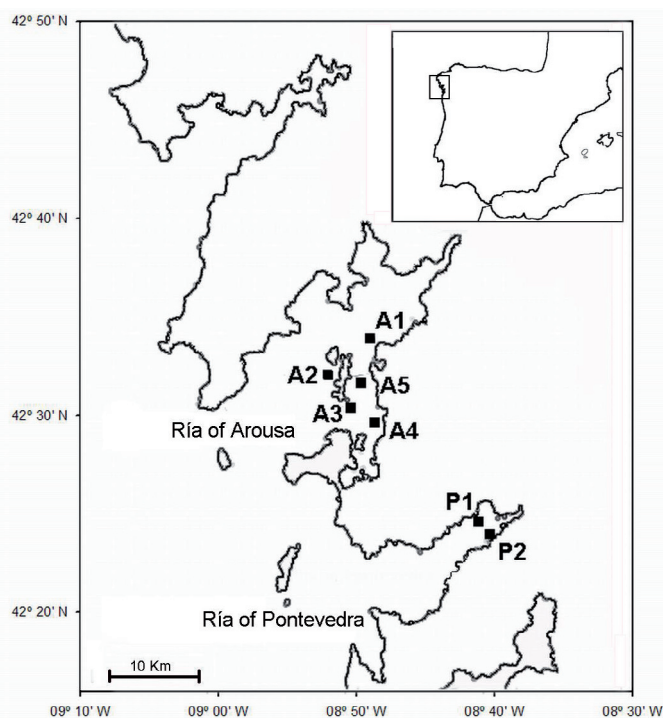


Fig. 1. Position of the Rías of Arousa and Pontevedra on the Atlantic coast of the Iberian Peninsula and localisation of sampling sites.

levels of Pb in this clam. Three different studies were conducted. The first tested whether the average Pb concentration in *D. exoleta* from representative banks in the Galician Rías conformed to European legislation. The second study investigated the quantitative relationship between body size and Pb concentration in a commercial bank, in order to identify the size class whose Pb concentration corresponded to the maximum permitted by EC standards. The third study focused on the geographical variation of the Pb concentration in that size class, in order to establish a maximum length whose Pb concentration would not exceed EC standards.

2 Material and methods

2.1 Collection and pre-treatment of the samples

Samples of *D. exoleta* were collected on three sampling dates, in May, October and November 2006 in different shellfish extraction areas of the Rías of Pontevedra and Arousa. Each sampling date corresponded to one of the three studies performed. Samples were immediately transported to the laboratory, and conserved frozen until their treatment and analysis.

The preliminary study (May 2006) covered 6 locations: A1, A2, A3 and A4 in Arousa and P1 and P2 in Pontevedra (Fig. 1). Seven individuals of a similar size (from 35 to 45 mm) were chosen from each location.

For the second study (on Pb concentration in clams of different sizes), a total of 50 individuals with the largest range of sizes possible were sampled at one location (A5, Fig. 1) in October 2006.

The clams were opened with a scalpel, and rinsed with artificial seawater (free of metals) and ultrapure water. Excess water was drained from the clams and they were allowed to air-dry for 5 min on drying paper. The tissue was separated from the valves using plastic tools, and deposited in pre-weighed polypropylene vials. Both wet and dry weight were determined, the latter after drying for 72 h in an oven at 90 °C.

In the third study (November 2006), instead of analyzing each individual separately, a homogenized pool was obtained in order to conform with the methodology described in the EC directive (Anonymous 2001). Therefore, 20 individuals between 35 and 40 mm were used for each of three homogenized pools, corresponding to locations A1, A2 and A4. Clam tissues were deposited on nylon meshes, lyophilized for 72 h and ground using a tungsten-blade grinder. Three replicates of 0.5 g were deposited in polypropylene vials for digestion and analysis.

2.2 Sample digestion and metal analysis by ICP-Mass spectrometry

Samples were digested with 1 ml of HNO₃ for trace analysis (Scharlau Chemie, S.A, Barcelona, Spain) and 200 µl of H₂O₂ (Tracepur, Merck, Darmstadt, Germany) per 100 mg of dried sample, using a microwave-assisted technique, following a method modified from De Wit and Blust (1998). After addition of HNO₃, samples were left to react for 48 h at room temperature. After this period, H₂O₂ was added and samples were left at room temperature for 24 h. Occasional agitation was applied using a vortex. Then samples were put in a microwave oven inside an airtight polycarbonate Bio-Safe carrier box (Nalgene, Nalge, Rochester, NY, USA). The microwave oven was operated according to the following procedure: 1 min (×2) at 90 W, 2 min (×2) at 90 W. The same was then repeated at 160 and 350 W. The final step, of 2 min at 350 W, was repeated several times until the samples were completely digested (start of ebullition, translucent colour and non-formation of foam).

After digestion, samples were diluted to a 3% acid concentration with a mixture of ultrapure water and internal standards, to obtain a final concentration of 5 µg L⁻¹ of the internal standards. Pb was measured in all analyses from each sampling. In the final study, Cu, Zn and Cd were also measured. Internal standards used were: Tl (for Pb correction), Rh (for Cd) and Ge (for Cu and Zn). Samples were analyzed by inductively coupled plasma mass spectrometry using an X Series ICP-MS, (Thermo Elemental, Cheshire, UK). Procedure blanks and certified reference material ERM-CE278 (mussel tissue) were included in the sample treatment and analysis.

All the material in contact with the samples had been previously washed with 10% nitric acid and rinsed with ultrapure water.

2.3 Data analysis and statistics

Normality and homocedasticity of the data were first verified using Kolmogorov-Smirnov and Levene tests respectively. Statistical differences between lead concentrations in different

Table 1. Comparison of measured and certified values of ERM-CE278 (mussel tissue).

	Pb ($\mu\text{g g}^{-1}$)	Cu ($\mu\text{g g}^{-1}$)	Zn ($\mu\text{g g}^{-1}$)	Cd ($\mu\text{g g}^{-1}$)
<i>n</i>	11	3	3	3
Measured	1.88 ± 0.07	9.35 ± 0.09	82.73 ± 0.01	0.35 ± 0.001
Certified	2.00 ± 0.04	9.45 ± 0.13	83.1 ± 1.7	0.35 ± 0.001
%Recovery	94%	99%	100%	100%

Values expressed as arithmetic mean ± standard deviation; “*n*”: number of samples measured.

locations were analysed by one-way ANOVA, applying post hoc HSD Tukey tests with the software SPSS version 15.0.1 for Windows (2006, SPSS Inc). To compare two means, or a mean to a known value, a *t*-test was used. The null hypothesis was accepted at a level of significance of 0.05. Curve fitting was done by least squares regression analysis using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, California, USA).

3 Results

Measured values of Pb, Cu, Zn and Cd in the ERM-CE278 reference (mussel tissue) are shown in Table 1. Recovery was 94% for Pb, 99% for Cu and 100% for Zn and Cd. Metal concentration in the blanks was below 1% of metal concentration in the samples. Certified reference material was measured at the beginning and at the end of each run to detect the effectiveness of internal standards for drift correction. The correction was satisfactory in all cases.

3.1 Preliminary study

Mean Pb concentrations in *D. exoleta* from the six sampled locations are shown in Table 2. Pb concentrations in clams showed no significant differences between sampling points within the same Ría. However, clams from the Ría of Pontevedra showed on average a higher ($p < 0.001$, *t*-test) Pb concentration ($1.29 \pm 0.63 \mu\text{g g}^{-1}$ ww, $n = 14$) than clams from Arousa ($0.63 \pm 0.39 \mu\text{g g}^{-1}$ ww, $n = 28$). Even though the mean concentration did not exceed the maximum permitted level in any of the studied Rías, results showed high inter-individual variability. In the Pontevedra Ría, 3 of the 14 clams analyzed presented Pb concentrations higher than $1.5 \mu\text{g g}^{-1}$ ww; and in the Arousa Ría, 2 of the 28 analyzed individuals presented concentrations above $1.5 \mu\text{g g}^{-1}$ ww.

3.2 Study of Pb concentration in clams of different size

The exponential increase in Pb concentration with size in *D. exoleta* individuals from a commercial bank in the Ría of Arousa is shown in Figure 2. For small individuals, from 20 to 40 mm long, Pb concentration increased slowly with length: the mean Pb concentration in small (<40 mm) clams was $0.7 \pm 0.9 \mu\text{g g}^{-1}$ ww ($n = 24$). For individuals larger than 40 mm though, Pb concentration increased very sharply with

Table 2. Lead concentrations in *Dosinia exoleta* in different locations in the Rías of Arousa (A) and Pontevedra (P).

Location	Pb ($\mu\text{g g}^{-1}$ ww)
A1	0.82 ± 0.15
A2	0.30 ± 0.07
A3	0.60 ± 0.45
A4	0.80 ± 0.51
P1	1.33 ± 0.87
P2	1.24 ± 0.33

Values given as mean ± sd of 7 individuals between 35 and 45 mm length.

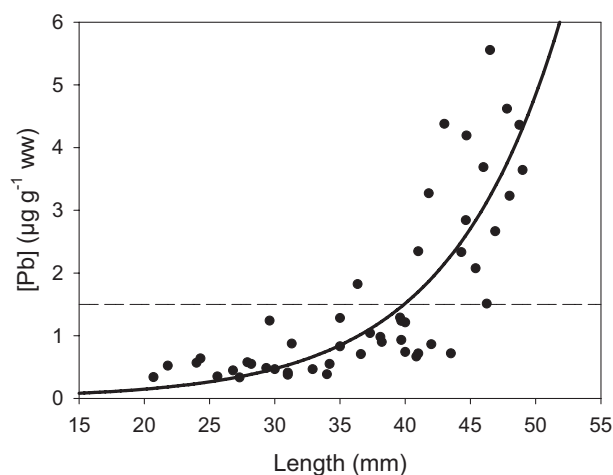


Fig. 2. Pb concentrations in *Dosinia exoleta* ($\mu\text{g g}^{-1}$ ww) in relation to individual length (mm). Each point represents a single individual. The solid line represents the fitted curve following the equation: $[\text{Pb}] = 0.015 \cdot \exp(0.116 \cdot D)$, D : shell length, $R^2 = 0.67$. The horizontal dashed line marks the $1.5 \mu\text{g g}^{-1}$ ww limit for Pb in shellfish for human consumption, established by the European Commission.

size. Values in large clams reached $5 \mu\text{g g}^{-1}$ ww, more than five times higher than younger clams living in the same site.

The fitted curve followed the equation: $[\text{Pb}] = a \exp(b \cdot D)$; where D is shell length. Adjusted parameters were: $a = 0.015 \pm 0.010 \mu\text{g g}^{-1}$ ww and $b = 0.116 \pm 0.010$ ($R^2 = 0.67$). The size that would have a Pb concentration of $1.5 \mu\text{g g}^{-1}$ ww was calculated to be 39.9 mm, thus agreeing with the initial observation of 40 mm as the limit size. The mean Pb concentration in individuals just below this limit (35–40 mm) was 1.1 ± 0.3 ($n = 10$), which is significantly lower

Table 3. Metal concentrations ($\mu\text{g g}^{-1}$ dw) in *Dosinia exoleta* (35–40 mm long) from different locations in the Ría de Arousa.

Location	Cu	Zn	Cd	Pb	
				dw	ww
A1	8.67 \pm 0.54	203 \pm 14	0.29 \pm 0.03	4.65 \pm 0.34	0.93 \pm 0.07
A2	9.13 \pm 0.72	176 \pm 14	0.25 \pm 0.01	2.45 \pm 0.21	0.49 \pm 0.04
A4	16.10 \pm 1.58	301 \pm 9	0.75 \pm 0.06	7.89 \pm 0.28	1.58 \pm 0.06

Values given as mean \pm sd ($n = 3$) of a homogenized pool of 20 *D. exoleta* individuals. Pb values are expressed in $\mu\text{g g}^{-1}$ dw (dry weight) and also in $\mu\text{g g}^{-1}$ ww (wet weight) to allow comparison with the EC norms.

than the 1.5 limit ($p = 0.01$, t -test). However, the security margin is very narrow, and variations in the Pb pollution level between different sites could influence clam flesh Pb concentration. For this reason, a spatial study of clams between 35 and 40 mm long was performed.

3.3 Geographical verification of Pb concentration within the selected size class

Results of the analysis of Pb, Cu, Zn and Cd in homogenized pools of *D. exoleta* from 35 to 40 mm long in three commercial banks of the Arousa Ría (A1, A2 and A4) are shown in Table 3. Results are expressed on the basis of dry weight (dw) since the digestion procedure is optimal using dried material. In the case of Pb results were transformed to $\mu\text{g g}^{-1}$ wet weight (ww), to allow comparison with the EC standards, by multiplying by a factor of 0.19, ($dw = 0.19 ww$, $R^2 = 0.98$; $n = 50$).

In clams from the A1 and A2 locations, Pb concentrations were below the EC standard. However, in location A4, mean Pb concentration in individuals between 35 and 40 mm long was above the critical threshold, though this was not significant (t -test; $p = 0.05$). These results show that Pb concentration is dependent not only on size but also on location (t -test; $p < 0.001$). The location presenting the lowest Pb concentration, A2, is the most external of the ría,; while A4, the most polluted site, is closer to the river Umia. This river is known to carry a charge of urban and industrial effluents, in which local Pb inputs have been detected (Prego and Cobelo-García, 2003). The other metals measured showed the same pattern, with maximum levels in A4, and minimum in A2 (Table 3).

4 Discussion

Lead concentrations in *D. exoleta* from the two studied Galician Rías ranged between 1.5 to 8.5 $\mu\text{g g}^{-1}$ dw for individuals under 40 mm in length, but reached concentrations up to 20 $\mu\text{g g}^{-1}$ dw in individuals around 45 mm. Pb concentrations in the Ría of Pontevedra were significantly higher than those in the Ría of Arousa, which is consistent with previous findings (Besada et al. 2002; Beiras et al. 2003a, 2003b; Prego and Cobelo-García 2003).

Pb concentrations reported in different species of infaunal bivalve (*Macoma balthica*, *Macoma nasuta*, *Chione subrugosa*, *Cerastoderma edule*) around the world are normally

between 1 and 4 $\mu\text{g g}^{-1}$ dw, reaching values of 8 $\mu\text{g g}^{-1}$ in some moderately polluted sites (Szefer et al. 1998; Sokolowski et al. 2002, 2007; Lu et al. 2005; Jung et al. 2006). Very high Pb concentrations, up to values of few hundreds of $\mu\text{g g}^{-1}$ dw, have been reported for clams inhabiting extremely polluted estuaries (Bryan et al. 1985; Southgate et al. 1983). In the Galician Rías, Pb concentrations in *Venerupis pullastra* gills ranged from 0.2 to 1.9 $\mu\text{g g}^{-1}$ dw (Sánchez-Marín et al., unpublished data) similar to those found for small *D. exoleta* in the present study. Similar concentrations were also reported by Saavedra et al. (2004) in *Venerupis pullastra* and *Cerastoderma edule*. The high Pb accumulation by large individuals of *D. exoleta* observed in our present study is unusual given the low degree of Pb pollution in the Ría of Arousa, and suggests that this may be a specific feature of this particular species.

Metal accumulation by bivalves and other biota is influenced by several biological and environmental factors, such as season, metal compartmentalization in the sediment, size or age of the individual and reproductive status. Several studies have related size with metal accumulation in bivalves, and three different patterns (negative relationship, positive relationship and lack of relationship) have been found depending on the species and the metal considered (reviewed by Wallace et al. 2003). The positive relationship, as found in the present work, has often been observed in bivalves inhabiting contaminated sites, and the storage of metals in metal concretions has been proposed as a possible mechanism to explain these observations (Johnels et al. 1967; Strong and Luoma 1981). The retention of metal in granular deposits has been observed to result in extremely high metal body burdens (Brown 1982). Given the long life span of *D. exoleta*, it is possible that this detoxification mechanism could be responsible for the high Pb concentration found in larger (older) clams.

From the present study, it could be inferred that clams less than 40 mm long in the Ría of Arousa are suitable for human consumption with regards to Pb levels, given that none of the studied locations presented Pb concentrations significantly higher than 1.5 $\mu\text{g g}^{-1}$ ww in clams from 35 to 40 mm long. However, this limit cannot be considered as safe enough because Pb concentration in one location was just above the limit (though not significantly), and other factors (such as seasonal variations) could increase Pb concentrations in any of the studied areas. Furthermore, extension of the study to more contaminated Rías (e.g. Ría of Pontevedra) would most probably result in an increase in Pb values for the same size class. For these reasons, we propose 35 mm as a safer limit until more detailed studies can be made.

5 Conclusion

Pb accumulation in *D. exoleta* has been shown to be highly size dependant, with larger individuals reaching Pb concentrations of $25 \mu\text{g g}^{-1} dw$ ($5 \mu\text{g g}^{-1} ww$). This high Pb concentration threatens the commercialization of this species for human consumption, and makes it essential to fix a maximum size that can be extracted and commercialized without health risks for consumers.

This size limit was observed to be 40 mm for one studied location. However, when the study was extended to other more polluted sites, it was seen that the 40 mm limit would not offer sufficient protection: 35 mm is therefore advised as a safer limit.

This work highlights the necessity for detailed study on Pb concentrations in *D. exoleta* in relation with size for all areas where this species is extracted, in order to establish a specific size limit for each area or to define a safe limit size for all areas based on a significant amount of data. Other factors that can influence metal accumulation, such as the effect of season or physiological status, should also be studied.

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