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Total lead and its stable isotopes in the digestive gland of *Octopus vulgaris* as a fingerprint

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ABSTRACT: We hypothesised that the isotopic signature of Pb in the digestive gland of the common octopus reflects the organisms' sources of Pb, and investigated whether isotopic Pb ratios are useful in characterising octopus populations. A total of 47 *Octopus vulgaris* individuals were captured between November 2005 and September 2006 in 2 areas of the Portuguese coast, near Matosinhos (Area A; NW coast) and Olhão (Area B; south coast), and digestive glands were analysed for total Pb and its stable isotopes. The same determinations were performed in 22 samples of surface sediments from the 2 areas. Pb concentrations in the digestive gland of specimens from Area B (2.8 to 13.0 µg g^{-1}) exceeded the values found in Area A (1.3 to 8.3 µg g^{-1}). A similar pattern was found for the isotopic Pb ratios: $^{206}Pb/^{207}Pb$ was 1.173 to 1.185 for Area A and 1.165 to 1.172 for B; $^{206}Pb/^{208}Pb$ was 0.476 to 0.487 for Area A and 0.318 to 0.483 for B. The different signatures of the digestive glands are in line with those observed in the surface sediments of the 2 coastal areas (e.g. $^{206}Pb/^{207}Pb$ was 1.179 to 1.207 for Area A and 1.171 to 1.181 for B). However, the isotopic Pb signature of octopus was less radiogenic than that of sediments. Because octopus has a short life span (up to 24 mo) the signature reflects recent sources of Pb that have a less radiogenic signature. The Pb signature of surface sediments tends to integrate the record of the previous few years or decades, due to the frequent resuspension of the upper layer of coastal sediments. The mixing of sediments deposited during those periods results in higher isotopic Pb ratios (more radiogenic). The consistent differences between the 2 areas, in sediments and octopus, points towards the isotopic Pb signature as a possible useful tool to distinguish octopus populations.

KEY WORDS: Cephalopods · Lead · Stable lead isotopes · Bioaccumulation · Portugal

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INTRODUCTION

The marine biogeochemical cycle of Pb has been greatly affected by human activity in the last century (Komárek et al. 2008). Industrial emissions and gasoline exhaust fumes have led to an increase in Pb deposition into the marine environment (Alleman et al. 2000). In the past, understanding Pb bioaccumulation relied mainly on concentration measurements. Because Pb isotope ratios vary according to the origin of this element, the inclusion of these values in environmental studies allowed researchers to distinguish the pathways of Pb from distinct sources (Komárek et al. 2008). There are 4 stable isotopes with mass numbers: ^{204}Pb (primordial), ^{206}Pb, ^{207}Pb and ^{208}Pb (radiogenic). The last 3 isotopes are products of the radioactive decay of ^{238}U, ^{235}U and ^{232}Th, respectively (Scheuhammer & Templeton 1998). The isotopic composition of anthropogenic and natural Pb generally differs and is seldom affected by kinetic processes (Gobeil et al. 2001).

The high persistence of Pb in abiotic compartments and its accumulation in living organisms has allowed researchers to determine baseline values and spatial distribution of Pb in sediments and biota, and to study its behaviour in the coastal zone (e.g. Prego & Cobelo-Garcia 2004). In the last decade, determination of Pb

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concentration in sediments has been coupled with measurement of Pb isotope ratios to better understand the fate of this element within coastal ecosystems. However, the isotopic Pb signature in marine organisms has been poorly documented. Only a few studies have indicated that biological samples can provide a fingerprint for sources of Pb (Spencer et al. 2000, Ip et al. 2005).

Octopus vulgaris, common octopus, is a benthic species and exclusively neritic, with the exception of the larval phase, which is planktonic. *O. vulgaris* has a short lifespan, fast growth and high reproductive potential (Rocha et al. 2001). This species is normally distributed on rocky, sandy and muddy bottoms (Mangold 1983). Octopus has been long considered to have a cosmopolitan occurrence in temperate and tropical seas (Roper et al. 1984), although a possible occurrence of cryptic species among *O. vulgaris*-like octopods is also reported (Guerra et al. 1999). Thus, the distribution of *O. vulgaris* in a strict sense may be restricted to the Mediterranean Sea and eastern Atlantic Ocean (Mangold 1983). Octopus undergoes vertical seasonal migrations, moving close to the shore for reproduction (Mangold & von Boletzky 1973).

Bioaccumulation studies have reported that storage of Pb in cephalopods occurs mainly in the digestive gland (e.g. Miramand & Bentley 1992, Nessim & Riad 2003, Seixas et al. 2005, Bustamante et al. 2008, Raimundo & Vale 2008). Furthermore, accumulated Pb levels appear to depend on the availability of Pb in water and food (Bustamante et al. 1998, Raimundo et al. 2004, 2005, Napoleão et al. 2005). However, to our knowledge no attempt has been made to clarify whether accumulated Pb values in the digestive gland reflect an anthropogenic versus natural origin.

Contrasting geomorphological features and oceanographic conditions were reported for the Portuguese coast (Fiuza 1983): the NW region is an exposed coast characterised by several estuarine systems crossing the shore, while the southern region is a sheltered coast with extensive inner coastal lagoons. The Iberian Peninsula is crossed by a giant massive sulphide deposit in the southern region (Iberian Pyrite Belt), mined since the Roman Age (Palanques et al. 1995), and has a relatively homogeneous isotopic Pb signature (Marcoux 1997). Water surveys have shown different availability of trace elements in the NW and southern coastal waters (Caetano & Vale 2003). River flow regime and pyrite belt location were invoked as major factors influencing these differences. A similar geographic contrast was found for Zn, Pb, Cd and Hg concentrations in the digestive gland of *Octopus vulgaris* (Raimundo et al. 2004, Napoleão et al. 2005, Seixas et al. 2005): enhanced levels of Pb, Hg, and Zn were found in individuals from the south coast and higher accumulation of Cd was found in specimens captured in NW stations.

The 2 areas are, therefore, suitable for testing the hypothesis that the isotopic Pb signature in the digestive gland of octopus reflects the animals' sources of Pb, and for investigating whether Pb isotope ratios are useful for characterising octopus populations. We measured total and stable Pb isotopes in the digestive gland of *Octopus vulgaris* from the NW and southern areas of Portugal, as well as Al and Pb concentrations and stable Pb isotopes in sediments, which we used to distinguish Pb signatures between the 2 areas.

MATERIALS AND METHODS

Samples. A total of 47 *Octopus vulgaris* individuals were collected between November 2005 and September 2006 from commercial catches landed in Matosinhos (NW coast; hereinafter Area A) and Olhão (southern coast; Area B). Specimens were captured within 6 n miles (9.7 km) radius centred from each of Areas A and B (Fig. 1). Total body weight, mantle length and sex were determined for each individual. Specimens were stored in individual plastic bags and frozen (–80°C) in order to minimise mobilisation of metals among organs and tissues (Martin & Flegal 1975). In the laboratory, the digestive gland was totally removed under partially defrosted conditions without rupture of the tissue, then freeze-dried, grounded and homogenised.

Surface sediments were collected in February 2006 in Areas A (12 samples) and B (10 samples), using a Van Veen grab from RV 'Noruega'. The top 5 cm sediment layer was sampled. Each sediment sample was oven-dried to constant weight at 40°C, sieved through a 2 mm mesh and ground with an agate mortar.

Analytical methodology. ***Sample pre-treatment:*** Samples of digestive gland (~200 mg) were digested with a mixture of HNO_3 (supra pure, 65% v/v) and H_2O_2 (supra pure, 30% v/v) at 60°C for 12 h, 100°C for 1 h and 1 h at 80°C according to Ferreira et al. (1990). Two mineralisation procedures were used for sediment samples: (1) digestion for Al quantification using HF (supra pure, 40% v/v), aqua regia (HCl 36%:HNO_3 65%; 3:1) and H_3BO_4 following Rantala & Loring (1975); and (2) mineralisation for analysis of Pb concentration and stable Pb isotopes by using the first step of Procedure (1), and evaporating to near-dryness and eluting with HNO_3 (double-distilled) and Milli-Q water (18.2 MΩcm) (Caetano et al. 2007). Procedural blanks were prepared using the same analytical procedure and reagents, and included within each batch of 10 samples.

Methods: Al was analysed by flame atomic absorption spectrometry (Perkin Elmer AA100) with a nitrous oxide-acetylene flame and concentrations determined

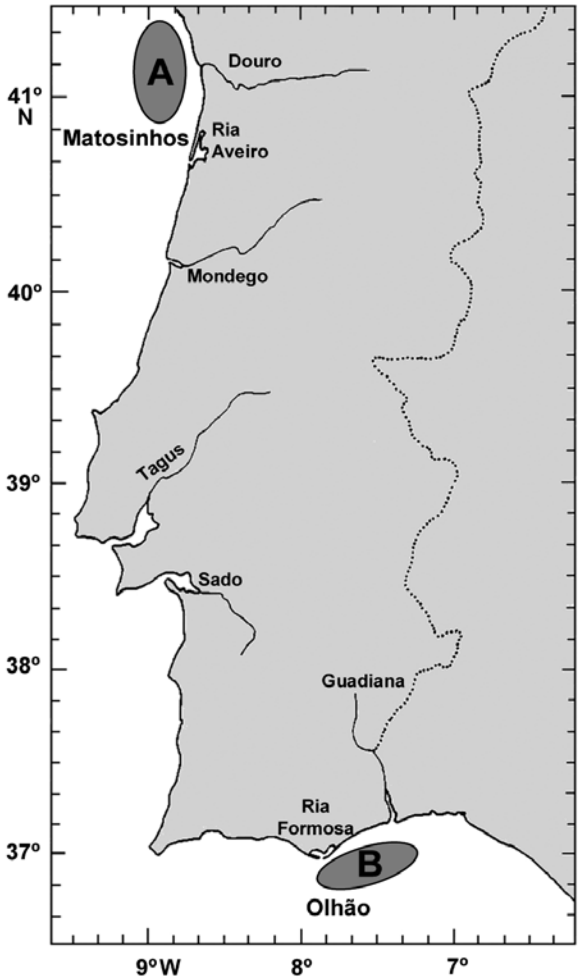

Fig. 1. Location of the 2 sampling sites on the Portuguese coast: A (Matosinhos) and B (Olhão)

with the standard addition method. Total Pb concentration and stable Pb isotopes (^{206}Pb, ^{207}Pb and ^{208}Pb) were determined in the same samples but in separate runs using a quadropole inductively coupled plasma mass spectrometer (ICP-MS; Thermo Elemental, X-Series) equipped with a Peltier Impact bead spray chamber and a concentric Meinhard nebuliser. A 7-point calibration within a range of 1 to 100 µg l^{-1} was used to quantify total Pb. The precision and accuracy of the Pb concentration measurements, determined through repeated analysis of reference materials (BCSS-1 and MESS-3 for sediment, and TORT-1 and TORT-2 lobster hepatopancreas for organisms), using ^{115}In as the internal standard, was better than 2% (Table 1). Procedural blanks always accounted for <1% of the total Pb in the samples. For Pb isotope determinations, between every 2 samples, corrections for mass fractionation were applied using NIST-SRM981 reference material. The isotopic Pb composition of procedural blanks did not significantly influence the ^{206}Pb/^{207}Pb and ^{206}Pb/^{208}Pb ratios. The coefficients of variation of the NIST-SRM981 reference material obtained in between-batch external quality control were 0.37% for ^{206}Pb/^{207}Pb and 0.22% for ^{206}Pb/^{208}Pb ratios.

Statistical analysis. Prior to statistical analysis, metal concentrations and biological parameters were tested for normality and equality of variances. The non-parametric Kruskal-Wallis test (KW *H*) was applied to all data in order to detect differences between metal concentrations and biological parameters and in the 2 study areas. The statistical analyses were performed using the STATISTICA 6.0 Statistical Software System.

RESULTS

Biological parameters in octopus

The octopus sampled in Area A included 13 males and 11 females, and the specimens in Area B were 12 males and 11 females. Size and weight of the sampled individuals varied over broad ranges: Area A, 125 to 170 mm and 578 to 1433 g; Area B, 113 to 165 mm and 698 to 1520 g, respectively. Size, weight and sex were not significantly ($p > 0.05$) different between areas. Differences between sampling periods were also not significant ($p > 0.05$).

Pb concentration and isotopic ratios in digestive gland

Levels of total Pb were significantly lower in Area A (Fig. 2a). Pb isotope ratios in specimens from Area A were significantly higher than those from Area B (Fig. 2b,c). No statistical differences ($p > 0.05$) were found between Pb concentration or isotopic Pb ratios and the measured biological parameters. Differences between sampling periods were also not significant.

Table 1. Pb and Al concentrations (mean ± SE) of lobster hepatopancreas (TORT-1 and TORT-2) and marine sediments (BCSS-1 and MESS-3) (National Research Council of Canada) in the present study and certified values; nd: not determined

Standard		Pb (µg g^{-1}, dry wt)	Al (%, dry wt)
TORT-1	Obtained	9.4 ± 1.9	nd
	Certified	10.4 ± 2	nd
TORT-2	Obtained	0.43 ± 0.27	nd
	Certified	0.35 ± 0.13	nd
BCSS-1	Obtained	23.0 ± 3.7	6.56 ± 0.17
	Certified	22.7 ± 3.4	6.26 ± 0.22
MESS-3	Obtained	22.1 ± 3.1	9.17 ± 0.23
	Certified	21.9 ± 1.2	8.59 ± 0.23

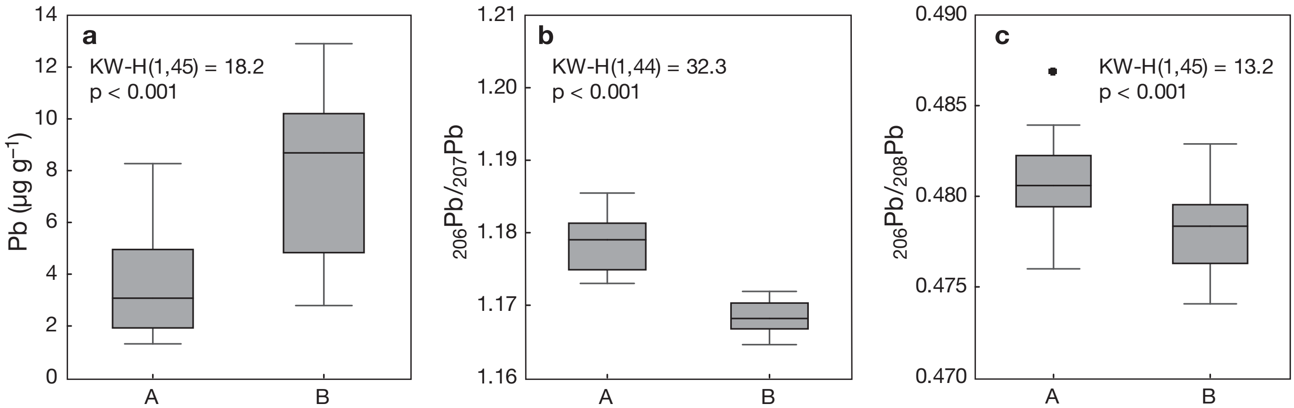

Fig. 2. *Octopus vulgaris.* (a) Pb concentrations (µg g^{-1} dry wt); (b) $^{206}Pb/^{207}Pb$ and (c) $^{206}Pb/^{208}Pb$ ratios in the digestive gland at Matosinhos (A) and Olhão (B). Data are median, 25th and 75th percentiles, minimum and maximum, outliers (•), Kruskal-Wallis test (KW *H*) and p

Al and Pb concentrations in sediment

Al content in surface sediments from Areas A and B ranged within broad intervals: 1.9 to 6.8% and 0.58 to 8.7%, respectively. These results indicate that sediments sampled in the 2 areas present a wide mixture of coarse (low Al content) and fine-grained particles (high Al content). The sediment samples of the 2 areas showed no significant ($p > 0.05$) differences in Al content. When metal concentrations are compared in a sediment set containing different grain sizes it is recommended to normalise levels to Al in order to minimise differences associated with sediment nature (Windom et al. 1989). For this reason Pb concentrations in the present study were normalised to Al content. Pb concentrations varied in a wide range, with levels in Area B significantly higher than in Area A (Fig. 3). Normalising Pb to Al separated the 2 areas pronouncedly; elevated Pb concentrations in Area B were not due to a more abundant fine fraction. The values of Pb/Al in Area B were significantly higher than those from Area A (Fig. 3b.

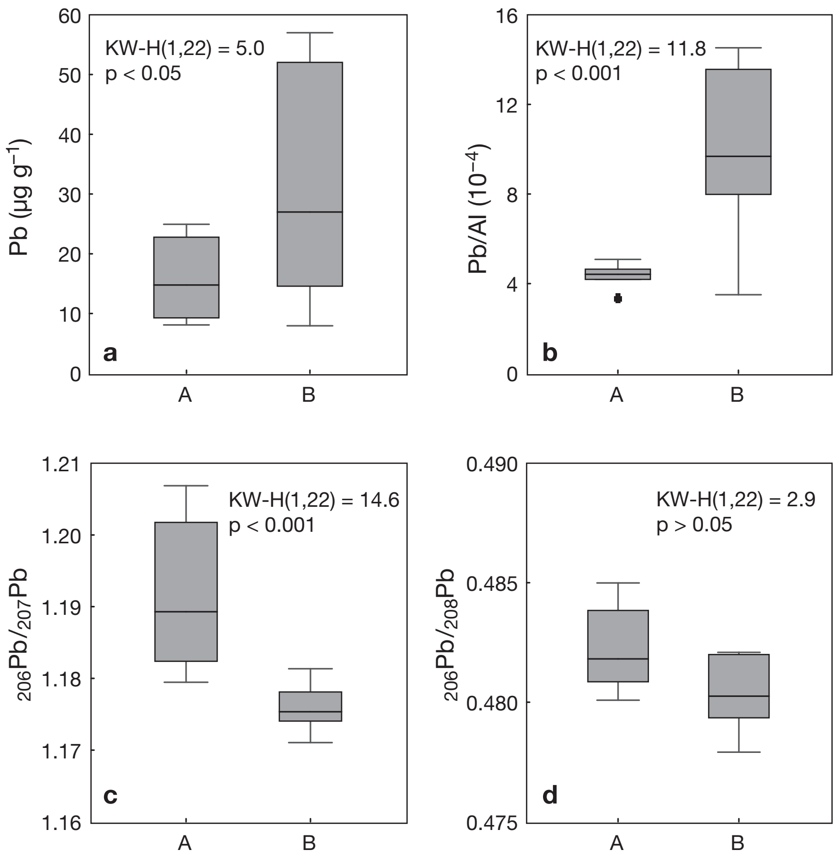

Fig. 3. (a) Pb concentrations (µg g^{-1} dry wt) and (b) Pb/Al (10^{-4}), (c) $^{206}Pb/^{207}Pb$ and (d) $^{206}Pb/^{208}Pb$ ratios in surface sediments at Matosinhos (A) and Olhão (B). Data are median, 25th and 75th percentiles, minimum and maximum, outliers (•), Kruskal-Wallis test (KW *H*) and p

Isotopic Pb ratios in sediment

Surface sediments from Area A showed a more radiogenic signature of $^{206}Pb/^{207}Pb$ than those from Area B (Fig. 3c). Moreover, a broader range of this ratio was found in sediments from Area A. However, no significant differences of $^{206}Pb/^{208}Pb$ ratios were found between the 2 areas (Fig. 3d).

DISCUSSION

The broad range of Al content in surface sediments indicates the existence of a wide combination of coarse and fine-grained materials in the 2 study areas. Despite that variability, Pb/Al ratios were consistently higher in Area B. The elevated ratios appear to result mainly from the geological feature of the Iberian Pyrite Belt, since anthropogenic Pb sources were minor. Indeed, the narrow range of isotopic Pb signature of sediments ($^{206}Pb/^{207}Pb$ was 1.171 to 1.181) matches data of Caetano et al. (2007) on sediments from Guadiana River, the main river that crosses the sulphide deposit area ($^{206}Pb/^{207}Pb = 1.172 \pm 0.003$). The obtained isotopic Pb signature in coastal sediments from Area B suggests a mixing of particles derived from the Pyrite Belt and pre-industrial sediments, with minor inputs of anthropogenic Pb (Caetano et al. 2007). In contrast, sediments from Area A exhibited a broader range of $^{206}Pb/^{207}Pb$ ratios, suggesting that Pb concentration in sediments was influenced by Pb from various origins. The observed $^{206}Pb/^{207}Pb$ ratios (1.179 to 1.207) were comprised of values reported for sediments contaminated by industrial effluents (1.166 to 1.170; Sundby et al. 2005) and pre-pollution Pb signature as recorded in NW Spain (1.235; Kylander et al. 2005) or in pre-industrial sediments from the North Atlantic (1.197 to 1.220; Sun 1980). Area A receives the discharges of the Douro River, which crosses an extensive rural area, and of the urban effluents of Porto (Caetano & Vale 2003). The isotopic Pb signature of coarse and fine sediments from Area A may thus mirror the mixture of high-radiogenic background Pb and low-radiogenic contaminant Pb emissions of alkyl-lead gasoline (1.06 to 1.09; Gobeil et al. 2001).

Pb concentrations in the digestive gland of *Octopus vulgaris* captured in the 2 areas ranged within the intervals observed in previous studies (Raimundo et al. 2004, 2008, Napoleão et al. 2005). The elevated concentration of Pb in sediments (Area B), as well as the Pb/Al ratios, match the increased values in the digestive gland of specimens from the same area. This response to the environmental availability is consistent with findings of other cephalopod studies (Bustamante et al. 1998, Koyama et al. 2000, Raimundo et al. 2004, Napoleão et al. 2005). Our results do not allow for the evaluation of the preferential pathway of accumulation; however, given the sedentary habits of octopus, both water and food should be considered vehicles for Pb uptake. It is expected that Pb in sediments influences the levels existing in benthic organisms that constitute the octopus diet, including crabs and bivalves (Mangold 1983). The uptake of Pb from different pathways presupposes the accumulation of Pb with distinct signatures. Thus the observed signature is an integration of all local sources. Specimens from Area B exhibited lower isotopic ratios ($^{206}Pb/^{207}Pb$ ratios were 1.165 to 1.172) than from Area A (1.173 to 1.185), mirroring the Pb signature of each area. This parallelism has been rarely reported for marine organisms.

However, isotopic signatures in octopus and sediments did not show the same range of values. These differences are consistent with findings from Ip et al. (2005) that showed lower isotopic Pb ratios in molluscs, crustaceans and fish than in sediments. Because octopus has a short lifespan (up to 24 mo; Mangold 1997), isotopic Pb ratios in the digestive gland should reflect recent sources of Pb that, in comparison to previous sources, have a less radiogenic signature. The 2 coastal areas are frequently subjected to suspension of surface sediments and settling events due to wave or wind storms. As a result, the isotopic Pb signature of the collected sediments tends to integrate the record of the previous few years or decades. This is why the Pb signature in sediments showed higher isotopic ratios than octopus tissues. The hypothesis of segregated accumulation of Pb isotopes by *Octopus vulgaris* or differential isotope detoxification is beyond the scope of this work. These results indicate that isotopic Pb signature in the digestive gland of octopus reflects the Pb sources of the animal and offers a useful tool for distinguishing octopus populations.

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