BIOACCUMULATION OF LEAD, CALCIUM AND STRONTIUM AND THEIR RELATIONSHIPS IN THE OCTOPUS *OCTOPUS VULGARIS*

SÓNIA SEIXAS[1,*] and GRAHAM PIERCE[2]
[1]*Universidade Aberta, Rua Escola Politecnica, 147, 1269-001 Lisboa, Portugal;* [2]*Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, U.K.*
*(*author for correspondence, e-mail: sonia@univ-ab.pt, fax: +351-213969293)*

(Received 23 July 2004; accepted 19 December 2004)

Abstract. Octopuses (*Octopus vulgaris*) landed from commercial fishing were sampled and the concentrations of lead, calcium and strontium, in digestive gland, branchial heart, gills, mantle and arms, were determined using PIXE (particle-induced X-ray emission). Lead was detected in all tissues, although not in samples from all animals analysed. Female octopuses accumulated more lead in digestive gland than did males. The digestive gland index, an indicator of nutritional status, showed a negative correlation with lead concentration in both sexes. There were positive correlations between the concentration of lead and those of calcium and strontium in digestive gland and a negative correlation between lead and calcium in branchial heart tissue. Concentrations of calcium in arms were lower in autumn and spring than in winter and summer. Lead content increased with increasing body weight and mantle length so we can conclude that lead continues to accumulate during the animal's life. Concentrations of lead in two samples were higher than the maximum legally permitted concentration of lead in food.

Keywords: calcium, cephalopods, contamination, lead, *Octopus vulgaris*, rubidium

1. Introduction

Lead is a non-essential metal and is poisonous at very low concentrations. Amounts in the environment have increased substantially during the past 200 years (NAS, 1991). Many countries now restrict the use of lead; one example is the adoption of unleaded petrol. Studies in 1983 in the north Atlantic revealed that the concentration in seawater was between 1 and 15 ng/L (Stum, 1983 *in* Buffle, 1994).

Aquatic animals take up and accumulate lead from water, sediment and food. In bivalve molluscs, lead in seawater is fixed by lysosomes when the water crosses the branchial epithelium. The uptake of lead from water and sediment is influenced by various environmental factors such as temperature, salinity and pH (WHO, 2000). When lead in food arrives in the digestive tract there is immobilization by interstitial cellules and capture by amoebocites (Amiard, 1988; Galdies and Axiak, 1992). Animals that consume food contaminated with lead often have high concentrations of lead in their tissues, although there is no biomagnification (WHO, 1995). Sydeman and Jarman (1998) noted that concentration of lead have been reported to decrease with increasing trophic level.

In animals, lead affects numerous enzymes and physiological systems, provoking deleterious effects at the cellular level (WHO, 1995). In the nucleus, it forms a lead–protein complex that affects the promoter systems which regulate DNA (Duc *et al.*, 1994).

Calcium is essential to many bodily functions including nerve transmission, muscle contraction, renal function and respiration. Calcium ions have a ubiquitous role as intracellular messengers, transducing electrical and hormonal signals (NAS, 1993).

Lead perturbs calcium-regulated and calcium-mediated bodily functions (Fullmer, 1992; Pounds, 1984) and interferes with calcium metabolism, due to the physico-chemical similarity between Pb^{2+} and Ca^{2+}. Pb^{2+} substitutes for Ca^{2+} in many cellular processes and interferes with reactions that require Ca^{2+} (Barton *et al.*, 1978; Kerper and Hinkle, 1997). Lead interferes with Ca^{2+} transport, synthesis and with the release of neurotransmitters. Lead toxicity in the nervous system seems to be related to specific effects on calcium metabolism and, in particular, calcium transport (Rius *et al.*, 1986; Duc *et al.*, 1994).

Strontium is an essential element for the cephalopods, it was demonstrated that statoliths did not develop when strontium was absent (Hanlon *et al.*, 1989).

Strontium mimics calcium in the body. Studies on squid axons showed that strontium seems to be handled on an essentially similar manner to calcium (Baker and Singh, 1982). Physiological and nutritional variables that affect strontium metabolism are similar to those that affect calcium metabolism and usually operate in the same direction. One of the differences that is observed between calcium and strontium is that the rates of most calcium-mediated processes, including muscular contraction, are slowed when strontium is substituted for calcium (NAS, 2000), this was observed in squid giant fibre lobe neurons (Amstrong and Palti, 1991). In cephalopod statoliths (functionally equivalent to fish otoliths), strontium/calcium ratios showed tare affected by environmental conditions (Biemann and Piatkowski, 2000) such as water temperature (Yatsu *et al.*, 1998) and salinity (Zumholz *et al.*, 2004).

Cephalopods are known to accumulate high levels of heavy metals and it has been argued that the digestive gland of cephalopods constitutes a good potential indicator of heavy metal concentrations in the marine environment (Miramand and Bentley, 1992). At least in the case of cadmium, cephalopods have been shown to represent an important route for the transfer of heavy metals to higher predators (Bustamante *et al.*, 1998). Seixas *et al.* (in press a) analysed levels of mercury in octopus (*Octopus vulgaris*) tissues. However, very few studies have looked at lead levels in cephalopods. In a previous preliminary study, Seixas *et al.* (2002) summarised lead levels in octopus from two locations on the Portuguese coast, while Seixas *et al.* (in press b) surveyed levels of a range of trace elements in the edible parts (arms) of octopus sampled from the Portuguese coast in spring 2002 and 2003.

The octopus *Octopus vulgaris* is an important fishery resource throughout southern Europe. It is a target species for some fishing fleets in Portuguese waters. As such, it is important to monitor the levels of potentially harmful metals in octopus tissues. Since lead interferes with biological systems that use calcium we also measured calcium concentration, as well as the concentration of strontium, another element that interacts with calcium metabolism. The present study is based on samples collected over four seasons in 1999–2000. In addition to quantifying concentrations of these elements in various body tissues, we examine whether there is seasonal, maturity, sex or size-related variation in concentrations.

2. Material and Methods

2.1. Sampling and sample preparation

Animals landed by commercial fisheries were sampled in Cascais, which is situated mid-way down the coast of Portugal, with a strong influence of the Tagus River (the largest river in Portugal).

Animals were sampled in the four seasons of the year, autumn (November, 1999), winter (January, 2000), spring (May, 2000) and summer (July, 2000). In each season, 10 animals (5 males and 5 females) were collected.

Total length, mantle length, total weight, sex and maturation state were determined for each animal. The maturation state was determined by direct observation of colours of reproductive structures (Gonçalves, 1993). The maturity index used was adapted for use on octopus by Guerra (1975).

To provide an indicator of condition, we determined the digestive gland index (DGI), which is defined as:

$$\mathrm{DGI} = \frac{\text{Weight of digestive gland}}{\text{Body weight}} \times 100$$

This index has previously been used in studies of biology of *Octopus vulgaris* (Silva *et al.*, 2002) and in many other studies on cephalopods. Regoli (1998) called this index the condition index.

The tissues collected for analysis were digestive gland, branchial heart, gills, mantle and arms. Tissue samples were stored frozen between −20 and −40 °C prior to analysis. Samples of branchial heart and mantle from winter and summer were lost due to problems that occurred during the storage of the samples.

2.2. Analytical procedure

The concentration of lead was determined using PIXE (particle induced X-ray emission). Samples were freeze-dried, followed by microwave acid digestion with

9:1 v/v HNO_3 and H_2O_2, 4 min at 300 W. We used yttrium (Y) as the internal standard. Three aliquots of 10 μL of sample from each animal and tissue were then analysed. The technique of PIXE was available in a Van der Graff accelerator at the Technological and Nuclear Institute of Portugal (ITN). The sample was irradiated by a sheaf of protons (2.2 MeV, 5 mm diameter, 6.5 nA mm^{-2}) in a vacuum. A Mylar^{TM} of 350 μm thickness was used to eliminate the contribution of X-rays of low energy (lower than potassium). The crystal where the emitted X-rays were detected was made of Si(Li). For each X-ray detected one electrical signal is produced, which is processed in a multi-channel system (MCA). The spectra were analysed with AXIL software (Van Espen *et al.*, 1986) and calculations of thick target concentration were performed using DATTPIXE software (Reis and Alves, 1992). The methodology was validated using certified reference materials (Barreiros *et al.*, 2001). The results for each tissue are given relative to the dry weight (mg/kg dry weight). Total lead content in each tissue was estimated as the product of concentration (mg/kg wet weight) and organ weight.

2.3. Statistical procedures

Statistical analysis of the data was carried out using STATISTICA (StatSoft, Inc., 2001). Gender and seasonal differences in concentrations were analysed using ANOVA (factors: sex and season). Student's *t*-tests were used to identify which pairs of seasons differed from each other. For analysis of data from branchial heart and gills samples, when the level was below the detection limit it was assumed to be zero.

To analyse the correlations between state of maturation (an ordinal variable) and concentration of elements we used Spearman rank correlations. For quantifying relationships between other parameters, such as total length, mantle length and total weight and concentrations of elements we used the Pearson coefficient of correlation.

3. Results

The weight, total length and mantle length of the females and males studied in the four seasons are shown in Table I. There were significant differences between females and males in spring in weight ($t = 4.79$, $N = 40$, $P = 0.00$), total length ($t = 3.42$, $N = 40$, $P = 0.01$) and mantle length ($t = 4.39$, $N = 40$, $P = 0.00$).

3.1. Lead

Lead was detected in all digestive gland samples analysed; concentrations are shown in Figure 1 and the estimated total amounts are shown in Figure 2. Lead was detected in branchial hearts of 12 animals (8 out of 10 females analysed and 4 out of 10 males

TABLE I
Weight, total and mantle length of the animals studied in different seasons

Season	Sex	*N*	Weight (g)	Total length (mm)	Mantle length (mm)
Autumn	Females	5	1266 ± 660	872 ± 123	154 ± 26
	Males	5	1524 ± 628	892 ± 120	166 ± 29
Winter	Females	5	1393 ± 259	796 ± 75	149 ± 12
	Males	5	1374 ± 95	794 ± 15	154 ± 7
Spring	Females	5	2183 ± 424	914 ± 61	192 ± 16
	Males	5	1052 ± 414	746 ± 91	146 ± 17
Summer	Females	5	1760 ± 719	950 ± 111	188 ± 35
	Males	5	1663 ± 772	933 ± 93	165 ± 23

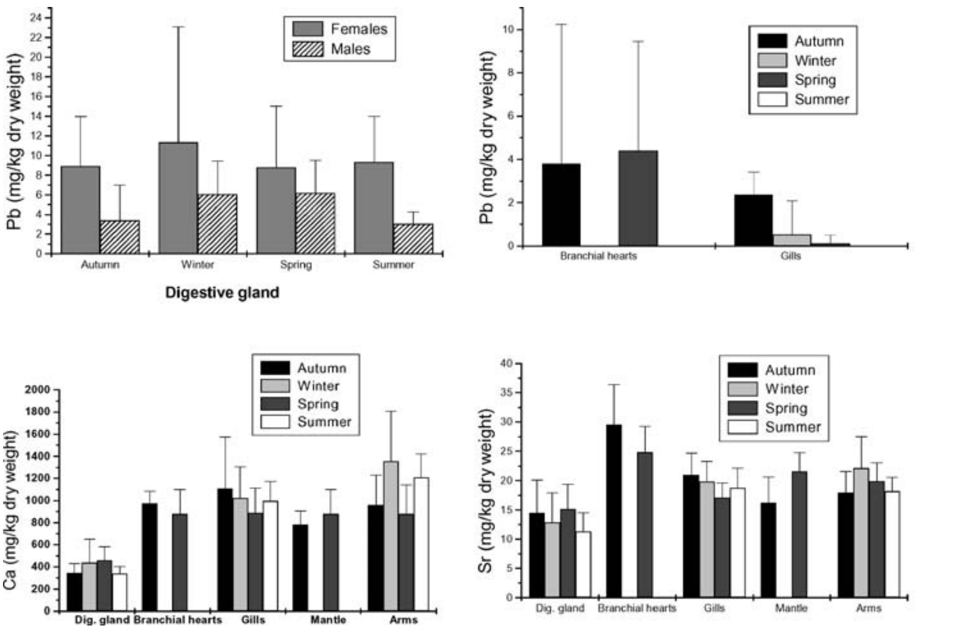

Figure 1. Concentration of lead in digestive gland, branchial hearts and gills, calcium and strontium in octopus tissues analysed in the four seasons.

analysed) (Figure 2). Lead was detected in six gills samples (3 out of 20 females and 3 out of 20 males analysed), while lead was only detected in one mantle of 20 analysed, and one arm of 40 analysed.

The highest concentrations and quantities of lead were mainly found in digestive gland. Maximum values for concentration and quantity of lead in the digestive gland were recorded in females, in winter and summer respectively. There were no significant correlations between concentrations of lead in different tissues.

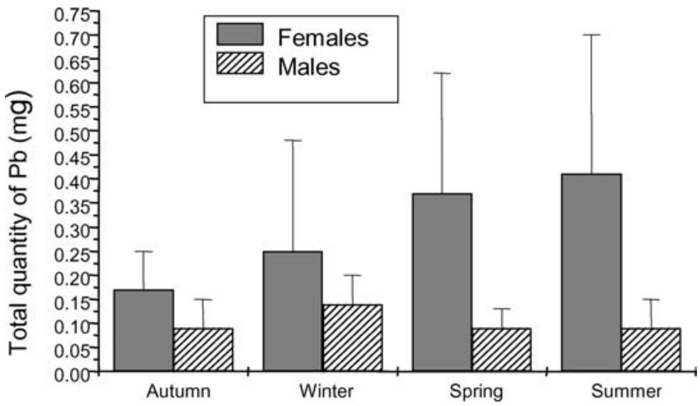

Figure 2. Total quantity of lead in digestive gland in females and males in the four seasons of the year.

Correlations of concentrations of lead in digestive gland and branchial hearts with weight, total and mantle length were mainly non-significant, the exception being the correlation between concentration in branchial hearts and total length ($r = -0.59$, $N = 12$, $P = 0.04$).

Correlations of total amounts of lead with weight, total and mantle length were significant only in the case of the amount in digestive gland, in relation to both weight ($t = 0.40$, $N = 40$, $P = 0.02$), and mantle length ($t = 0.42$, $N = 20$, $P = 0.01$). There were no correlations between maturation state and the concentration of lead or total amount of lead, in either females or males.

There were negative correlations between DGI and lead concentrations in digestive gland, for both females and males (Figure 3). Two possible explanations can

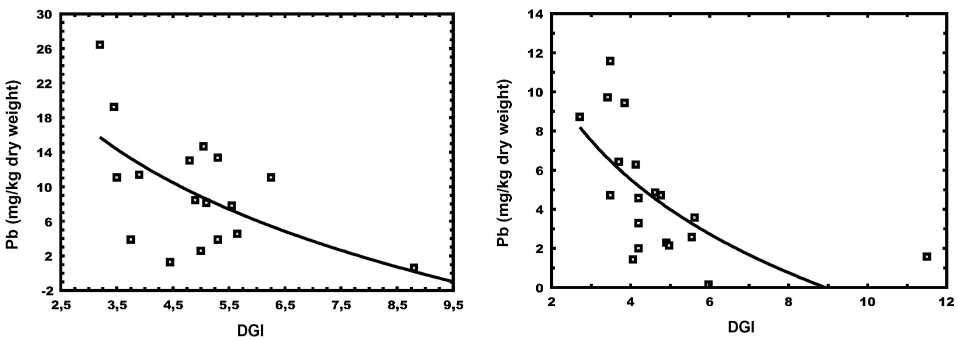

Figure 3. Correlation between DGI and concentration of lead in digestive gland, in female (left): Pb = (22.913−2.713)DGI ($r = -0.53$, $P = 0.03$); and male (right): Pb = (9.108−0.926)DGI ($r = -0.53$, $P = 0.02$).

TABLE II
Results for two-way ANOVA for effects of sex and season on concentrations of Pb, Ca and Sr

Tissue	Factor	Pb	Ca	Sr
Digestive gland	Sex	**6.84 (40, 0.01)**	3.22 (40, 0.08)	2.32 (40, 0.14)
	Season	0.42 (40, 0.74)	1.87 (40, 0.16)	0.80 (40, 0.51)
Branchial heart	Sex	0.48 (20, 0.81)	0.33 (20, 0.57)	2.56 (20, 0.13)
	Season	1.80 (20, 0.34)	1.35 (20, 0.26)	3.50 (20, 0.08)
Gills	Sex	0.43 (20, 0.73)	1.36 (40, 0.25)	1.12 (40, 0.30)
	Season	2.31 (20, 0.10)	0.90 (40, 0.45)	2.47 (40, 0.08)
Mantle	Sex	–	0.84 (20, 0.37)	1.63 (20, 0.22)
	Season	–	1.34 (20, 0.27)	**9.82 (20, 0.01)**
Arm	Sex	–	0.39 (40, 0.54)	1.57 (40, 0.22)
	Season	–	**5.06 (40, 0.01)**	2.30 (40, 0.10)

The table shows the values of F, followed by the sample size (N) and associated probability (p) in parentheses. Significant correlations are shown in boldface. Empty cells represent analyses for which insufficient data were available.

be suggested: lead is simply more diluted in the animals with high DGI or animals with a good reserves concentrated lower quantities of lead. To analyse this we determined the concentration of lead in relation to wet weight and then calculated the quantity of lead in each digestive gland. There was no significant correlation between the total quantity of lead in digestive gland and DGI ($r = -0.17$, $P = 0.31$), so the hypothesis of reduced accumulation can be ruled out, and the dilution effect remains plausible.

The concentration of lead in digestive gland samples differed significantly between sexes but not between seasons (ANOVA, Table II). Females accumulated more lead in digestive gland than males (females: 9.51 ± 6.78 mg/kg, males: 4.34 ± 3.22 mg/kg).

3.2. Calcium and strontium

Calcium and strontium were detected in all samples analysed in all tissues, although concentrations of calcium were almost two orders of magnitude higher than those of strontium (Figure 1).

Most correlations between body size and concentrations of calcium and strontium were non-significant, the exception being with the concentration of calcium in digestive gland and total length ($r = -0.34$, $N = 40$, $P = 0.04$; Figure 4). In relation to maturation, only the concentration of strontium in mantle tissues of females was correlated with maturity stage ($R = 0.72$, $N = 10$, $P = 0.02$).

Concentrations of calcium in arm samples in winter and summer were higher than in autumn and spring (Tables II and III; Figure 2). There was

TABLE III

Results of t-tests for between-season comparison of calcium concentrations in octopus arms (values of t and P in parenthesis)

Seasons	Winter	Spring	Summer
Autumn	**−2.37 (0.03) Aut < Win**	0.64 (0.53) Aut = Sp	**−2.22 (0.04) Aut < Sum**
Winter		**2.76 (0.01) Win > Sp**	0.87 (0.39) Win = Sum
Spring			**−2.95 (0.01) Sp < Sum**

Significant correlations are shown in boldface.

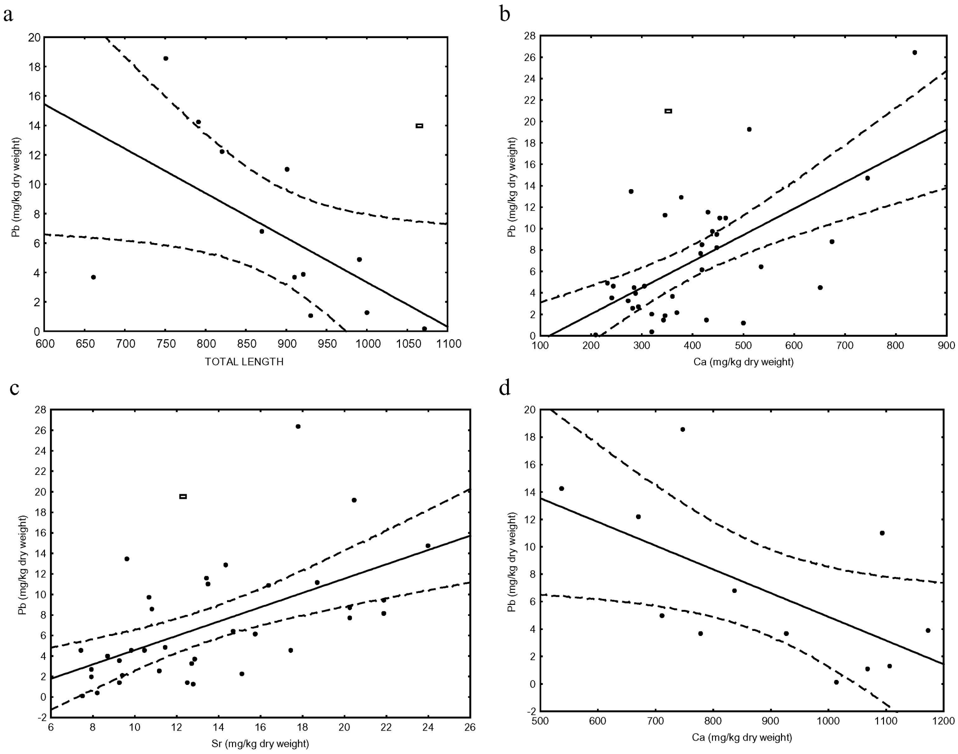

Figure 4. Correlations between (a) concentration of lead in branchial hearts and total length (TL) Pb = (17.997−0.0158)TL, (b) concentrations of lead and calcium in digestive gland Pb = (−2.903 + 0.0246)Ca, (c) concentrations of lead and strontium in digestive gland Pb = (−2.405 + 0.697)Sr, and (d) concentrations of calcium and lead in branchial hearts (Pb = (22.19−0.018)Ca. The graphs show the fitted lines with 95% confidence limits.

no significant seasonal variation in calcium concentration in the other tissues (Table II). The concentration of strontium in mantle tissue differed significantly between seasons (Table II), levels being higher in spring than in autumn.

TABLE IV

Results of Pearson correlation between the elements analysed in the different tissues

Tissues	Pb/Ca	Pb/Sr	Ca/Sr
Digestive Gland	**0.63 (0.00)**	**0.58 (0.00)**	**0.72 (0.00)**
Branchial heart	**−0.60 (0.04)**	−0.38 (0.22)	**0.57 (0.04)**
Gills	0.07 (0.90)	0.32 (0.53)	**0.84 (0.00)**
Mantle	–	–	**0.60 (0.01)**
Arms	–	–	**0.74 (0.00)**

Values of the correlation coefficient (r) and associated probability (P) are shown in parentheses. Significant correlations are shown in boldface.

3.3. Relationships between different elements

Concentrations of calcium and strontium were significantly positively correlated in all tissues (Table IV, Figure 4). In digestive gland tissue, there were positive correlations between concentrations of lead and those of calcium and strontium, and there was a negative correlation between concentrations of lead and calcium in branchial heart samples (Table IV, Figure 4).

4. Discussion

4.1. Lead

Lead was detected in many of the samples analysed, most consistently in the digestive gland, perhaps because this tissue is a short-term storage location for digested food. Several authors note that, in molluscs, toxic metals are stored in the digestive gland, in lysosomes (Marigomez *et al.*, 1986; Reico *et al.*, 1988; Domouhtsidou and Dimitriades, 2000; Iriato *et al.*, 2003).

Seixas *et al.* (in press b) recorded lead concentrations of 3–4 mg kg^{-1} for arm samples collected from the Portuguese coast in spring of 2002 and 2003 (measured using inductively coupled plasma – atomic emission spectrometry). This is somewhat lower than most values recorded for octopus tissues in the present study.

The highest single value for lead concentration recorded in the present study was in a mantle tissue sample but generally the highest concentrations were recorded in digestive gland and lowest concentrations in arms, where the values were usually below the detection limit for the technique. This result is consistent with previous studies, although Seixas *et al.* (2002) also recorded high lead levels in branchial heart samples of *Octopus vulgaris*.

Values from the present study were generally high when compared with published values for lead concentrations in cephalopods (Table V), with the exception

TABLE V

Values for concentration of lead from this study and from the literature on cephalopods

Species	Locality	Digestive gland	Branchial hearts	Gills	Mantle	Arms	Authors
Octopus vulgaris	Cascais, Portugal	F: 9.51 ± 6.78; M: 4.75 ± 3.22	4.11 ± 5.64	0.36 ± 0.96	48.49[d]	8.74[d]	Present study
	Viana, Portugal	4.9 ± 1.94[a]	8.4 ± 6.22[a]	2.4[d]	3.0 ± 2.5[a]		Seixas *et al.* (2002)
	Viana + Cascais, Portugal					3–4	Seixas *et al.* (in press b)
Eledone cirrhosa	English Channel, France	F: 1.10 ± 0.1; M: 1.23 ± 0.06	0.39 ± 0.04	0.62 ± 0.11	0.11 ± 0.05		Miramand and Bentley (1992)
Vulcanoctopus hydrothermalis	Hydrothermal vent				10		González *et al.* (1998)
Todarodes pacificus	Pacific coast, Japan	0.24[c]			0.040[b,c]		Ichihashi *et al.* (2001b)
	Sea of Japan	0.64[c]			0.042[b,c]		Ichihashi *et al.* (2001b)
	Nemuro Strait, Japan	0.14[c]			0.044[b,c]		Ichihashi *et al.* (2001b)
Loligo forbesi	West coast of Scotland, UK	64.3[c]			0.6[c]		Craig and Overnell (2003)
Loligo opalescens	Monterey, USA	1.4 ± 0.6[c]			1.1 ± 0.5[c]	0.86 ± 0.44[c]	Falandysz (1991)
Squid	Fraserburgh, UK				0.02[b,c]		MAFF (1998)
Squid	Newlyn, UK				0.01[b,c]		MAFF (1998)
Ilex argentinus	Argentina	0.24–0.60[c]					Falandysz (1988)
Stenoteuthis oualaniensis	Iriomote Island, Japan	0.44[c]					Ichihashi *et al.* (2001a)
Sepia officinallis	English Channel, France	1.10 ± 0.06	0.99 ± 0.06	0.39 ± 0.06	0.17 ± 0.07		Miramand and Bentley (1992)

Note: Values are in mg/kg dry weight.
[a]Not detected in all analysed samples.
[b]Muscle.
[c]Wet weight.
[d]Only detected in one animal.

of lead in the mantle of the octopus *Vulcanoctopus hydrothermalis* (González *et al.*, 1998). This species inhabits hydrothermal vents, which are characterised by high concentrations of heavy metals (e.g. Ruelas-Inzunza *et al.*, 2003).

The high lead levels in animals in Cascais may be related to the proximity of the estuary of Tagus River, as well as other smaller river basins, to the fishing grounds. The level of lead in seawater in Cascais region ranged from 0.7 to 1.7 μg/L (Costa *et al.*, 1999). These high values are probably the consequence of high lead concentrations in the estuaries of the rivers Tagus (7 μg/L) and Sado (up to 19 mg/L); data from INAG (National Institute of Water). Of these two estuaries, the Tagus is probably the more influential as it is both nearer to and north of the sampling site and the prevailing ocean currents in this area are from north to south.

Octopus vulgaris feeds mainly on crustaceans and fishes, although it occasionally includes gastropods and other cephalopods in its diet (Quetglas *et al.*, 1998). In the Tagus estuary, levels of lead in these organisms are likely to be elevated because of the high quantity of lead in the water.

The legal limit (for human consumption) set by the European Commission for the lead content of cephalopods (without viscera) is 1.0 mg/kg fresh weight (EC rule no. 466/2001). These values correspond, in terms of dry weight, to approximately 5 mg/kg. We found, in one arm, a value of 8.7 mg/kg dry weight (out of 40 arms tested), and in one mantle sample a value of 44.8 mg/kg dry weight (out of 20 mantles tested), concentrations that are higher than the levels allowed by EC law. The number of arms and mantle that contained detectable amounts of lead was low when compared to the number of samples examined but, if our samples are representative, lead concentrations could be dangerously high in a significant proportion of octopus. At very least, more extensive data are needed. It is possible that, in future, lead pollution must be controlled or consumption of octopus from certain localities restricted. Furthermore, in Portugal, families of fishermen and sellers of fresh cephalopods in the market habitually eat the gills of octopus. This is a particular cause for concern because levels found in gills were generally higher than the legal limits.

Another problem related to the high values of lead found in some tissues of cephalopods arises when marine mammals consume octopus. In marine mammals, lead tends to accumulate in bone tissue, following a similar metabolic path to calcium (Becker, 2000). Lead was found in livers of seals, porpoises and dolphins from waters around British Isles in concentrations up to 4.3 mg/kg wet weight (Law *et al.*, 1991). Blubber and skin samples of monk seals from Greece exhibited concentrations of lead that reached 65.2 mg/kg dry weight (Dosi, 2000). Cephalopods (although usually not coastal octopods) are also important in the diets of some of the larger Odontocete cetaceans, such as beaked whales and the sperm whale (Santos *et al.*, 1999, 2001a,b,c).

The present study found that lead levels in different tissues of octopus were uncorrelated. Furthermore, there was no consistent relationship between lead concentrations and size or maturity, although lead concentrations in the branchial heart samples decreased at larger size. However, total lead content increased with increasing body size so we can conclude that lead continues to accumulate during the animal's life, for example due to dietary intake.

Female octopus accumulated more lead than did males in digestive gland. This may be because digestive gland is an organ where lipids are accumulated (Mangold and Bidder, 1989). Females need higher levels of lipids to incorporate in ovocytes during the maturation process. A study in *Sepia officinalis* found that, in digestive gland, females (13%) have higher quantities of lipids in relation to males (9%) (Blanchier and Boucaud-Camou, 1980). This accumulation of lipids could also indicate higher metabolic rates in females, hence a higher dietary intake of lead.

The negative correlation between the DGI, which may be thought of as a feeding or condition index, and lead concentration, in both sexes, was also found by Regoli and Orlando (1993) for mussels *Mytilus galloprovinciallis* in the North Tyrrhenian Sea. These authors suggested that this was an effect of maturation: as the gonads grew, DGI diminished. If lead remains in the digestive gland as reserves are mobilised, its concentration would increase as DGI decreased. Certainly, in the present study the total lead content of the digestive gland was not related to the DGI.

In the clam *Ruditapes decussates*, the presence of lead reduced the growth rate of the post-larvae significantly (Seixas, 1995). In relation to octopus we have no information about effects of lead on growth, and future studies are required. However, some insights concerning the action of lead may arise from studying the relationships between lead levels and those of calcium and strontium.

4.2. Calcium and strontium

In relation to calcium, in the present study the highest levels were found in arms, probably because each arm has a longitudinal nerve, which contains nerve fibres and ganglions (Mangold *et al.*, 1989). The next highest values were recorded in gills, which may be related to the presence of muscular fibres that contract during ventilation (Wells and Smith, 1987).

Calcium concentration in *Octopus cyanea* is 0.9% of dry weight, of the whole animal, and in the squid *Loligo* sp. it is 0.1% (Goodman-Lowe *et al.*, 1999). A report from MAFF (1998) indicated that concentrations of elements in relation to fresh weight of the edible portion of squid were 220 mg/kg for calcium and 2.2 mg/kg for strontium. Given that figures for octopus in the present study are given in terms of dry weight, and assuming a 5:1 ratio of wet to dry weight, it appears that concentrations in octopus and squid tissues were rather similar.

In the present study, calcium concentrations in arms were lower in autumn and spring than in winter and summer. There was no significant correlation between maturation and concentration of calcium in arms but the seasons during which the levels were lower are those when the octopus reproduces (Silva *et al.*, 2002).

4.3. Relationships between different elements

In the digestive gland there was a positive correlation between levels of lead and calcium. At present, we can only speculate as to the possible significance of this relationship. In humans, an increase in the concentration of lead appears to result in an increase in intracellular calcium stores, in every tissue studied by NAS (1993). In branchial hearts of octopus there was a negative correlation between concentrations of lead and calcium.

In digestive gland, branchial heart, gills, mantle and arms there were positive correlations between concentrations of calcium and strontium. This can be explained because the action of strontium is closely related to that of calcium and mimics its effects (Sukhanov *et al.*, 1990). In statoliths of neon flying squid, Sr/Ca ratios gradually increased with age from juveniles to adults (Yatsu *et al.*, 1998).

Acknowledgments

Staff at the Instituto Tecnólogico e Nuclear provided assistance and facilities. The work was funded under grant number PRAXIS 2/2.1/MAR/1707/95.

References

Amiard, J.: 1988, 'Les mecanimes de transfer des elements metaliques dans la chaine alimentaires aboutissant à lhuitre et à la moule, mollusques filtreurs, formes chimiques de stokage, consequences ecotoxicologiques', *Oceanus* **14**, 283–287.

Armstrong, C. and Palti, Y.: 1991, 'Potassium channel block by internal calcium and strontium', *J. Gen. Physiol.* **97**, 627–638.

Baker, P. and Singh, R.: 1982, 'Metabolism and transport of strontium in giant axons of Loligo', *J. Physiol. London* **330**, 373–392.

Barreiros, M., Pinheiro, T., Araújo, M., Costa, M., Palha, M. and Silva, R.: 2001, 'Quality assurance of X-ray spectrometry for chemical analysis', *Spectrochim. Acta B* **56**, 2095–2106.

Barton, J., Conrad, M., Harrison, L. and Nuby, S.: 1978, 'Effects of calcium on the absorption and retention of lead', *J. Lab. Clin. Med.* **91**, 366–376.

Becker, P.: 2000, 'Concentration of chlorinated hydrocarbons and heavy metals in Alaska Arctic marine mammals', *Mar. Pollut. Bull.* **40**, 819–829.

Biemann, M. and Piatkowski, U.: 2001, 'Amounts and composition of trace elements in the statoliths of loliginid squids: Reflection of environmental conditions?', International Council for the Exploration of the Sea CM 2001/K:05.

Blanchier, B. and Boucaud-Camou, E.: 1980, 'Contenu lipidique de la glande digestive de *Sepia officinalis* à maturité sexuelle', *Haliotis* **10**, 1–20.

Buffle, J.: 1994, 'The ecological role of aquatic organic and inorganic components, deduced from their nature, circulation and interactions', in *Environmental Chemistry Inorganic Pollutants – a Course on Risk Assessment of Metals Compounds and Other Inorganics*, European Environmental Research Organization, pp. 23–26.

Bustamante, P., Caurant, F., Fowler, S. W. and Miramand, P.: 1998, 'Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean', *Sci. Total Environ.* **220**, 71–80.

Costa, M., Barreiros, M., Carvalho, M. and Queralt, I.: 1999, 'Multi-element characterization of estuarine sediments and waters', *X-Ray Spectrom.* **28**, 410–413.

Domouhtsidou, G. and Dimitriadis, V.: 2000, 'Ultrastrutural localization of heavy metals (Hg, Ag, Pb, and Cu) in gills and digestive gland of mussels, *Mytilus galloprovinciallis* (L.)', *Arch. Environ. Contam. Toxicol.* **38**, 472–478.

Dosi, A.: 2000, 'Heavy metals in blubber and skin of Mediterranean monk seal, *Monachus monachus* from Greek waters', *PhD Thesis*, University of Bangor, 85 pp.

Duc, M., Kaminsky, P. and Klein, M.: 1994, 'Intoxication par plomb et ses sels', *Encycl. Méd. Chir.* Paris. Toxicologie-Pathologie professionelle, 16-007, 10 pp.

Falandysz, J.: 1988, 'Trace metals in squid *Illex argentinus*', *Z. Lebensm. Unters. For.* **187**, 359–361.

Fullmer, C.: 1992, 'Intestinal interactions of lead and calcium', *Neurotoxicology* **13**, 799–807.

Galdies, Ch. and Axiak, V.: 1992, 'The fate of lead in a benthic bivalve (*Venus verrucosa*)', *MAP Tech. Rep. Ser.* **69**, 135–141.

Goodman-Lowe, G., Carpenter, J., Atkinson, S. and Ako, H.: 1999, 'Nutrient, fatty acid, amino acid and mineral analysis of natural prey of the Hawaiian monk seal, *Monachus schauinslandi*', *Comput. Biochem. Phys. A* **123**, 137–146.

Gonçalves, J.: 1993, '*Octopus vulgaris Cuvier, 1797 (polvo comum): Sinopse da biologia e exploração*', *Provas de Aptidão Pedagógica e Científica*, Universidade dos Açores, 447 pp.

González, A., Guerra, A., Pascual, S. and Briand, P.: 1998, '*Vulcanoptus hydrothermalis* gen. Et sp. Nov. (Mollusca, Cephalopoda): An octopod from a deep–sea hydrothermal vent site', *Cah. Biol. Mar.* **39**, 169–184.

Guerra, A.: 1975, 'Determinación de las diferentes fases del desarrollo sexual de *Octopus vulgaris* Lamarck, mediante un índice de madurez', *Invest. Pesq.* **39**, 397–416.

Hanlon, R., Bidwell, J. and Tait, R.: 1989, 'Strontium is required for statolith development and thus normal swimming behaviour of hatchling cephalopods', *J. Exp. Biol.* **141**, 187–195.

Ichihashi, H., Kohno, H., Kannan, K., Tsumura, A. and Yamasaki, S.: 2001a, 'Multielemental analysis of purpleback flying squid using high resolution inductively coupled plasma-mass spectrometry (HR ICP-MS)', *Environ. Sci. Technol.* **35**, 3103–3108.

Ichihashi, H., Nakamura, Y., Kannan, K., Tsumura, A. and Yamasaki, S.: 2001b, 'Multi-elemental concentration in tissues of Japanese common squid (*Todarodes pacificus*)', *Arch. Environ. Contam. Toxicol.* **41**, 483–490.

Iriato, P., Santovito, G., Cassini, A., Piccinni, E. and Albergoni, V.: 2003, 'Metal accumulation and binding protein induction in *Mytilus galloprovincialis, Scapharca inaequivalvis*, and *Tapes philippinarum* from the Lagoon of Venice', *Arch. Environ. Contam. Toxicol.* **44**, 476–484.

Kerper, L. and Hinkle, P.: 1997, 'Cellular uptake of lead is activated by depletion of intracellular calcium stores', *J. Biol. Chem.* **272**, 8346–8352.

Law, R., Fileman, C., Hopkins, A., Baker, R., Harwood, J., Jackson, D., Kennedy, S., Martin, A. and Morris, R.: 1991, 'Concentrations of trace metals in the livers of marine mammals (seals, porpoises and dolphins) from waters around the British Isles', *Mar. Pollut. Bull.* **22**, 183–191.

MAFF 1998, 'Concentrations of metals and other elements in marine fish and shellfish', *Food Surveill. Informat. Sheet* **151**, 1–13.

Mangold, K. and Bidder, A.: 1989, 'Láppareil digestif et la digestion', in K. Mangold (ed.), P.P. Grassé (Series ed.), *Céphalopodes, Tome V, Traité de Zoologie Anatomie, Systématique, Biologie*, Masson, Paris, pp. 321–373.

Mangold, K., Bidder, A. and Portmann, A.: 1989, 'Structures cutanées', in K. Mangold (ed.), P.P. Grassé (Series ed.), *Céphalopodes. Tome V. Traité de Zoologie Anatomie, Systématique, Biologie*, Masson, Paris, pp. 121–162.

Marigomez, I., Angulo, E. and Moya, J.: 1986, 'Copper treatment of the digestive gland of the slug, Arion ater L. 1. Bioassay conduction and histochemical analysis', *Biol. Environ. Contam. Toxicol.* **36**, 600–607.

Miramand, P. and Bentley, D.: 1992, 'Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledone cirrhosa* and *Sepia officinalis*, from the French coast of the English Channel', *Mar. Biol.* **114**, 407–414.

NAS: 1991, *Seafood Safety*, National Academy of Science, Washington, 452 pp.

NAS: 1993, *Measuring Lead Exposure in Infants, Children, and Other Sensitive Populations*, National Academy of Science, Washington, 356 pp.

NAS: 2000, *Mineral Tolerance of Domestic Animals*, National Academy Press, 3rd edn., Washington, 577 pp.

Pounds, J.: 1984, 'Effect of lead intoxication on calcium homeostasis and calcium-mediated cell function: A review', *Neurotoxicology* **5**, 295–331.

Quetglas, A., Alemany, F., Carbonell, A., Merella, P. and Sánchez, P.: 1998, 'Biology and fishery of *Octopus vulgaris* Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, Western Mediterranean)', *Fish. Res.* **36**, 237–249.

Regoli, F.: 1998, 'Trace metals and antioxidant enzymes in gills and digestive gland of the Mediterranean mussel *Mytilus gallopronvincialis*', *Arch. Environ. Contam. Toxicol.* **34**, 48–63.

Regoli, F. and Orlando, E.: 1993, '*Mytilus gallopronvicialis* as a bioindicator of lead pollution: Biological variables and cellular responses', *Sci. Total Environ.*, Supplement 1993, 1283–1293.

Reico, A., Marigomez, J., Angula, E. and Moya, J.: 1988, 'Zinc treatment of the digestive gland of the slug Arion ater (L.) 1. Cellular distribuition of zinc and calcium', *Biol. Environ. Contam. Toxicol.* **41**, 858–864.

Reis, M. and Alves, L.: 1992, 'DATTPIXE, a computer package for TTPIXE data analysis', *Nucl. Instrum. Meth. B* **68**, 300–304.

Rius, R., Govoni, S. and Trabucchi, M.: 1986, 'Regional modification of brain calcium antagonist binding after in vivo chronic lead exposure', *Toxicology* **40**, 191–197.

Ruelas-Inzunza, J., Soto, L. A. and Paez-Osuna, F.: 2003. 'Heavy-metal accumulation in the hydrothermal vent clam *Vesicomya gigas* from Guaymas basin, Gulf of California', *Deep-Sea Res. Pt 1* **50**, 757–761.

Santos, M., Pierce, G., Boyle, P., Reid, R., Ross, H., Patterson, I., Kinze, C., Tougaard, S., Lick, R., Piatkowski, U. and Hernández-García, V.: 1999, 'Stomach contents of sperm whales *Physeter macrocephalus* stranded in the North Sea 1990–1996', *Mar. Ecol. Prog. Ser.* **183**, 281–294.

Santos, M., Clarke, M. and Pierce, G.: 2001a, 'Assessing the importance of cephalopods in diets of marine mammals and other top predators: Problems and solutions', *Fish. Res.* **52**, 121–139.

Santos, M., Pierce, G., Reid, R., Patterson, I., Ross, H. and Mente, E.: 2001b, 'Stomach contents of bottlenose dolphins (*Tursiops truncatus*) in Scottish waters', *J. Mar. Biol. Assoc. U.K.* **81**, 873–878.

Santos, M., Pierce, G., Smeenk, C., Addink, M., Kinze, C., Tougaard, S. and Herman, J.: 2001c, 'Stomach contents of northern bottlenose whales *Hyperoodon ampullatus* stranded in the North Sea', *J. Mar. Biol. Assoc. U.K.* **81**, 143–150.

Seixas, S.: 1995, 'Efeitos da Acumulação de chumbo em pós-larvas de amêijoa boa (Ruditapes decussatus L.)' *Tese de Mestrado em Estudos Marinhos e Costeiros*, Universidade do Algarve, 88 pp.

Seixas, S., Bustamante, P. and Pierce, G. J.: in press a, 'Accumulation of mercury in the tissues of the common octopus *Octopus vulgaris* (L.) in two localities on the Portuguese coast', *Sci. Total Environ*. (in press).

Seixas, S., Bustamante, P. and Pierce, G.J.: in press b, 'Interannual patterns of variation in concentrations of trace elements in arms of *Octopus vulgaris*' *Chemosphere* (in press).

Seixas, S., Pinheiro, T. and Sousa Reis, C.: 2002, 'Lead in octopus (*Octopus vulgaris*) in Portugal: A preliminary study', *Biol. Mar. Sci.* **71**, 1091–1093.

Silva, L., Sobrino, I. and Ramos, F.: 2002, 'Reproductive biology of the common octopus, *Octopus vulgaris* Cuvier, 1797 (Cephalopoda: Octopodidae) in the Gulf of Cádiz (SW Spain)', *Biol. Mar. Sci.* **71**, 837–850.

Sukhanov, B., Korolev, A., Marninchuk, A. and Merzliakova, N.: 1990, 'Experimental study of the protective role of calcium in lead poisoning', *Gigiena i Sanitariya* **12**, 47–49.

Sydeman, W. and Jarman, W.: 1998, 'Trace metals in seabirds, Steller sea lion, and forage fish and zooplankton from Central California', *Mar. Pollut. Bull.* **36**, 828–832.

Van Espen, P., Janssens, K. and Swentens, I.: 1986, *AXIL X-ray Analysis Software-Users Manual*, Camberra Packard, Benelux.

Wells, M. and Smith, P.: 1987, 'The performance of the octopus circulatory system: A triumph of engineering over design', *Experientia* **43**, 487–499.

WHO: 1995, 'Inorganic Lead' *Enviromental Health Criteria* **165**. World Health Organisation, Geneva, 206 pp.

WHO: 2000, 'Lead' *WHO Food Additives Series* **44**. World Health Organization, Geneva, 39 pp.

Yatsu, A., Mochioka, N., Morishita, K. and Toh, H.: 1998, 'Strontium/calcium ratios in statoliths of the neon flying squid, *Ommastrephes bartrami* (Cephalopoda), in North Pacific Ocean', *Mar. Biol.* **131**, 275–282.

Zumholz, K., Piatkowski, U. and Hansteen, T.: 2004, 'Trace element distributions in cuttlefish (*Sepia officinalis*) statoliths in response to a manipulated environment', International Council for the Exploration of the Sea CM 2004 CC:38.