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Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations

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Abstract

The high concentrations of cadmium recorded in the digestive gland of cephalopods from various temperate and subpolar waters suggest that these molluscs have developed efficient cadmium detoxification mechanisms. The subcellular distribution of cadmium in the digestive gland cells was investigated in seven cephalopod species from the Bay of Biscay (France) and the Faroe Islands. In most species, cadmium was mainly found in the cytosolic fraction of the digestive gland cells, reaching up to 86% of the total cadmium for the squid *Loligo vulgaris* from the Bay of Biscay. But species with the highest total level of cadmium showed a higher percentage of cadmium associated to insoluble compounds. The quantification of metallothioneins (MTs) by the polarographic method was performed in order to evaluate the involvement of these proteins in the detoxification of the high amounts of bioaccumulated cadmium. Metallothionein levels in cephalopods ranged form 742 ± 270 to 3478 ± 1572 μg/g wet weight. No relationship could be established between total cadmium, cytosolic cadmium and MT levels suggesting the occurrence of other Cd-binding ligands. Although these proteins

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have not been characterised, as cadmium in the digestive gland of cephalopods is mainly associated with soluble ligands, a high potential transfer to predators can be predicted.

Keywords: Cephalopods; Cadmium; Metallothionein, Detoxification

1. Introduction

Cephalopods are regarded as key species in many marine ecosystems (Amaratunga, 1983; Rodhouse, 1989). They represent an essential link in marine trophic chains and are eaten by many marine top predators, fish, birds and mammals (see the reviews of Clarke, 1996; Croxall & Prince, 1996; Klages, 1996; Smale, 1996). Owing to their ecological importance, the few high background levels of cadmium reported in these molluscs (Bustamante, 1998; Bustamante, Cherel, Caurant, & Miramand, 1998; Bustamante, Caurant, Fowler, & Miramand, 1998; Finger & Smith, 1987; Martin & Flegal, 1975; Miramand & Bentley, 1992; Miramand & Guary, 1980; Smith, Plues, Heyraud, & Cherry, 1984) are of particular interest. Moreover, species from subpolar areas showed higher levels of cadmium than those living in temperate ones (Bustamante, 1998; Bustamante, Cherel et al., 1998, Bustamante, Caurant et al., 1998) while exposure to soluble metals in natural seawater is extremely low in polar regions (Donat & Bruland, 1995; Mart et al., 1982). In both areas, cadmium is mainly accumulated in the digestive gland (Bustamante, 1998; Bustamante, Cherel et al., 1998; Finger & Smith, 1987; Miramand & Bentley, 1992; Miramand & Guary, 1980; Smith et al., 1984), reaching up to 98% of the total body cadmium in some species. Consequently, this organ appears to have a key function in the metabolism of cadmium in cephalopods. Very high levels of cadmium in the tissues of cephalopods would be expected to be toxic to the organisms unless efficient storage and detoxification mechanisms are available (Phillips & Rainbow, 1989; Simkiss & Taylor, 1982).

One well know detoxification strategy of marine invertebrates involves the binding of some trace metals to metallothioneins. These proteins which play a role in the homeostasis of the essential elements, copper and zinc (Cosson, Amiard-Triquet, & Amiard, 1991; Engle & Brouwer 1989; George & Olsson, 1994), are induced by various metals (i.e. Cd, Cu, Zn, Ag and Hg). As a consequence, metallothioneins are considered to be involved in cadmium detoxification (Dallinger, 1993, 1996; Roesijadi, 1992, 1996; Viarengo & Nott, 1993). The occurrence of metallothioneins in cephalopods has not been demonstrated although the occurrence of proteins with a molecular weight similar to metallothioneins has been shown. These are associated mainly with copper, and, to lesser extent, cadmium, but little zinc (Finger & Smith, 1987; Tanaka, Hayashi, & Ishizawa, 1983).

Taking into account the very high levels of cadmium observed in several cephalopod species, the aim of our study was to investigate how they manage to tolerate such amounts of toxic metal. Our analyses provided us with information on the subcellular distribution of cadmium and with the involvement of metallothioneins in

the detoxification processes. A comparison has been made between cephalopods from temperate and subpolar waters.

2. Materials and methods

The level of metallothionein (MTs) and the subcellular distribution of metals were determined in the digestive gland of several species of cephalopods collected by bottom trawl in waters of the Bay of Biscay and Faroe Islands during 1997.

2.1. Description of the samples

Cephalopods were sampled (1) during a cruise on the Magnus Heinason in August 1997 on the Faroe eastern continental shelf, at several stations located between 60°30′ and 62°50′ N, and 3°40′ and 7°60′ W; (2) during the RESSGASC cruise in February and May 1997 on the continental shelf of the Bay of Biscay (French Atlantic coast), at several stations located between 45° 14′ and 46°07′ N, and 01°24′ and 01°52′ W.

Only living animals were selected for on board-dissection (see later). We investigated both pelagic (i.e. squids) and benthic (i.e. cuttlefishes and octopuses) cephalopod species commonly encountered in French nearshore waters, i.e. Loliginidae and Ommastrephidae squids (*Loligo vulgaris*, *Illex coindetii*), cuttlefish (*Sepia officinalis*, *Sepia elegans*, *Sepia orbignyana*) and some other species from the Faroe Islands, i.e. the Ommastrephidae squid *Todarodes sagittatus* and the octopus *Eledone cirrhosa*. Table 1 gives the origin, number of individuals, mantle length and weight for each species of cephalopod.

2.2. Preparation of the samples

The digestive gland of each animal was isolated on board and deep-frozen in liquid nitrogen. In the laboratory, the samples were kept at −80 °C until used.

Table 1
Characteristics of the cephalopod samples

Family species	Number of individuals	Sex	Mantle length (mm)	Weight (g)	Origin
Sepiidae					
Sepia elegans	5	5♀	63±6	33±11	Bay of Biscay
Sepia officinalis	8	2 ♂, 6 ♀	153±63	507±522	Bay of Biscay
Sepia orbignyana	7	3 ♂, 4 ♀	84±20	153±53	Bay of Biscay
Loliginidae					
Loligo vulgaris	27	15 ♂, 12 ♀	233±88	434±302	Bay of Biscay
Ommastrephidae					
Illex coindetii	9	5 ♂, 4 ♀	206±43	368±109	Bay of Biscay
Todarodes sagittatus	21	11 ♂, 10 ♀	228±43	199±46	Faroe Islands
Octopodidae					
Eledone cirrhosa	4	4 ♂	101±8	448±85	Faroe Islands

Each sample was weighed (wet wt.) and homogenised with a mortar and pestle on ice with 10 volumes of a 0.02 M TRIS–HCl, 0.25 M sucrose buffer (Tanaka et al., 1983), containing 1 mM PMSF (phenylmethylsulfonylfluoride) as protease inhibitor and 5 mM DTT (dithiothreitol) as a reducing agent, at pH 8.6. The homogenates were centrifuged at 100 000 ***g*** for 1 h at 4 °C in a Berkman LE-70 ultracentrifuge. Particle-free supernatants (cytosols) were separated from the pellet containing cellular membranes, nucleus, lysosomal and mitochondrial material. Two aliquots (1 ml each) were removed from the supernatant for protein and metallothionein quantification. Aliquots of the homogenates, cytosols and pellets were analysed for cadmium.

2.3. Cadmium analyses

The content of cadmium in the different fractions was determined by atomic absorption spectrophotometry (AAS) after acid digestion with 4 ml of 65% HNO_3 and 1 ml of 70% $HClO_4$ for 24 h at 80 °C. The acid was removed from the samples by evaporation and the residues were diluted in 10 ml of 1 N nitric acid. Cadmium was determined by flame and flameless AAS using a Varian spectrophotometer Vectra 250 Plus with deuterium background correction. Blanks and reference materials (dogfish liver DOLT-2, NRCC) were taken through the procedure in the same way as the sample. Our results (21.2 ± 1.5 μg g^{-1} dry wt., $n = 3$) were in good agreement with certified values (20.8 ± 0.5 μg g^{-1} dry wt.). Levels of cadmium are given relative to the fresh weight of tissue.

2.4. Quantification of metallothioneins

The amounts of MTs were determined using the polarographic method (Olafson & Olsson, 1991; Olafson & Sim, 1979; Thompson & Cosson, 1984) in supernatants obtained after heat denaturation (70 °C, 15 min) and centrifugation (15 000 ***g***, 10 min). The levels of MTs were expressed relative to the fresh weight of tissues using rabbit liver metallothionein (Sigma) for the standard addition calibration. All the reagents used did not give a polarographic signal during the dosage of metallothioneins (Erk & Raspor, 2000).

2.5. Gel-filtration chromatography

Aliquots of heat-treated cytosol from the digestive gland of each species of cephalopods were chromatographed on a Waters Protein-Pack 125 (7.8×300 mm) column and eluted with 0.1 M TRIS–HCl, 0.25 M sucrose buffer, pH 8.6. The column was maintained at 4 °C and the samples were collected as 1.5 ml fractions. The ultraviolet absorbency of the eluate was measured at $\lambda = 254$ nm and $\lambda = 280$ nm. Differential pulse polarography was used to determine the thiolic content of each chromatographic fraction as explained earlier. The column was calibrated for molecular weight estimations with Bovine Serum Albumin (66 kDa), Rabbit Liver Metallothionein (12.5 kDa) and Aprotinine (6.5 kDa) as standard markers.

3. Results

3.1. Subcellular distribution

The partitioning of Cd between the pellet and the soluble fractions as a function of the total Cd content in the digestive gland is shown in Fig. 1. A significant negative correlation exists between these parameters (d.f. = 6, $r = 0.68$, $P < 0.05$). The distribution of cadmium between the pellet and the supernatant was fairly stable in each species. Two-way ANOVA did not show any significant differences among size and sex for the species with numerous individuals (i.e. *T. sagittatus* and *L. vulgaris*) (Fig. 2).

Partitioning of cadmium between the pellet and the supernatant was highly variable between species (Fig. 1). Nevertheless, more than 50% of cadmium was present in the soluble fraction in most of the species, reaching up to $86 \pm 12\%$ for the squid *L. vulgaris* from the Bay of Biscay; the only exception was the octopus *E. cirrhosa* from the Faroe Islands where only $42 \pm 17\%$ of the total cadmium was found associated to the soluble fraction. Species with the lowest cadmium levels displayed the highest percentage of soluble cadmium (e.g. *L. vulgaris*).

The subcellular distributions of cadmium in the digestive gland of the squids *L. vulgaris* from the Bay of Biscay and *T. sagittatus* from the Faroe Islands are shown in Fig. 3. The amount of cadmium in the cytosolic fraction increased significantly as a function of total cadmium level in the digestive gland of both species (d.f. = 16, $r = 0.991$, $P < 0.001$ for *L. vulgaris* and d.f. = 20, $r = 0.887$, $P < 0.001$ for *T. sagittatus*) whilst the amount of cadmium in the insoluble fraction increased significantly with the total cadmium level in the digestive gland only for *T. sagittatus* (d.f. = 20, $r = 0.906$, $P < 0.001$).

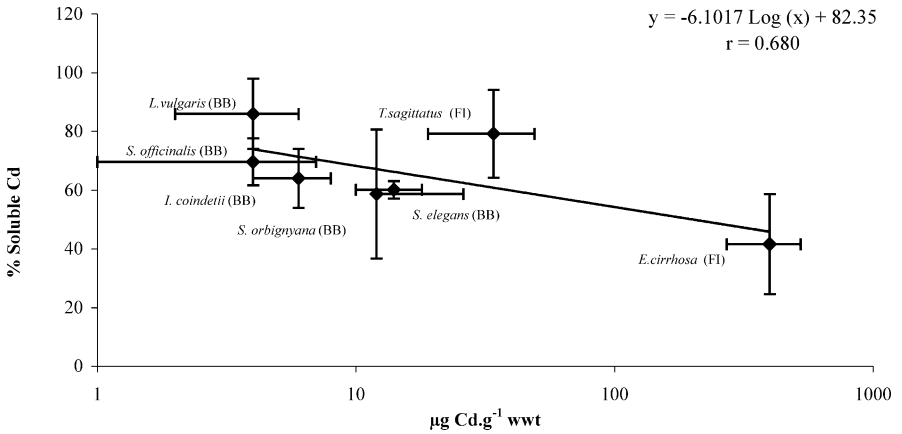

Fig. 1. Partitioning of cadmium (as % of soluble Cd) in the digestive gland of cephalopods as a function of total cadmium levels in this organ (expressed as µg Cd g^{-1} wet wt.). Scale bars represent 1 standard deviation. BB, Bay of Biscay; FI, Faroe Islands.

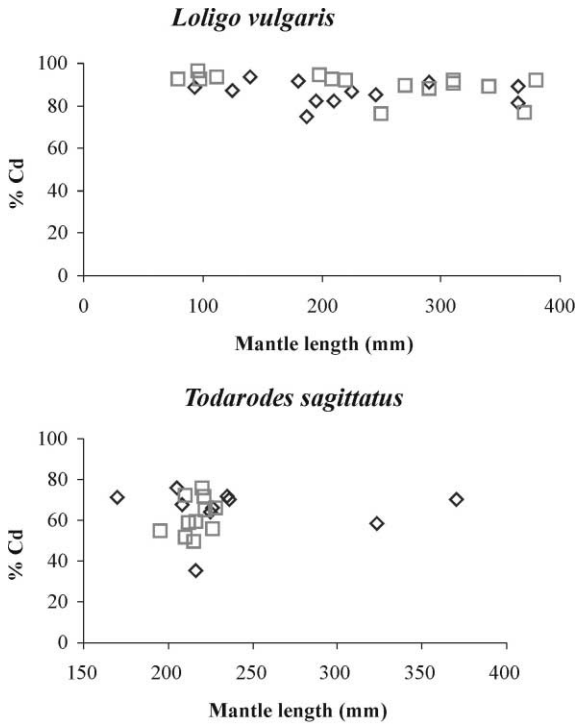

Fig. 2. Partitioning of cadmium in the cytosolic fraction (% of soluble Cd) of the digestive gland of two squid species, *Todarodes sagittatus* from Faroe Islands and *Loligo vulgaris* from the Bay of Biscay as a function of mantle length for males (□) and females (◇).

3.2. Metallothioneins

Typical chromatographic elution profiles of the absorbance ($\lambda = 254$, 280 nm) in heat treated cytosol from the digestive gland of cephalopods are shown in Fig. 4. Except for the squid *L. vulgaris*, the principal cadmium-binding component has an apparent molecular weight of approximately 6.5 kDa, eluting between fractions 6–7. However, all the cephalopods chromatographic profiles displayed absorbency peak around 12.5 kDa that corresponds to the metallothionein pool. Thus, 75% of the polarographic activity was detected in these fractions showing unequivocally that thiolic response in the heat-treated cytosol corresponds to metallothionein fractions. *S. officinalis* was the only exception with 44% of the polarographic activity at 12.5 kDa and 40% for molecular weight around 3 to 4 kDa. For *L. vulgaris*, another peak at 254 nm was detected in the fraction 2, corresponding to proteins having a molecular weight around 80 kDa.

The levels of metallothioneins in the heat-stable soluble fraction of the digestive gland of cephalopods and the levels of total cadmium in this tissue are shown in Fig. 5. Metallothionein levels were fairly stable within species but were highly

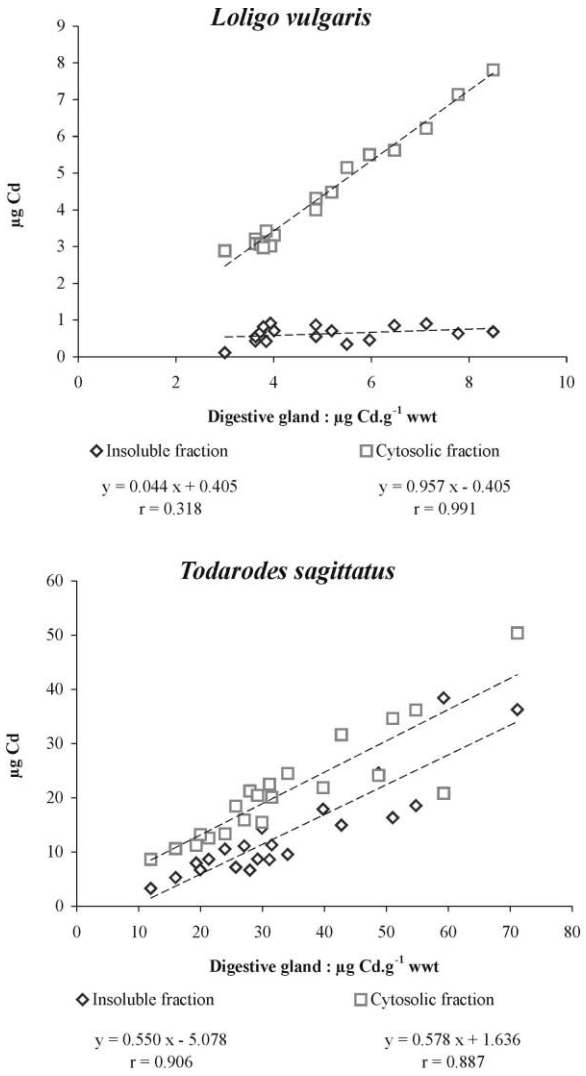

Fig. 3. Quantity of cadmium (as µg) in the soluble and insoluble fractions of the digestive gland of the squids *Loligo vulgaris* from the Bay of Biscay and *Todarodes sagittatus* from the Faroe Islands as a function of total cadmium level (µg Cd g^{-1} wet wt.) in this tissue.

variable between species exhibiting a significant decrease (d.f. = 6, $r = 0.81$, $P < 0.001$) when the Cd concentrations increased in the digestive gland. The concentrations ranged from 700 ± 300 µg g^{-1} wet weight in the octopus *E. cirrhosa* from the Faroe Islands up to 3500 ± 600 µg g^{-1} wet weight in the squid *L. vulgaris* from the Bay of Biscay and corresponded respectively to the highest and the lowest total cadmium levels in the digestive gland (i.e. 4 ± 2 µg g^{-1} wet wt. and 397 ± 126 µg g^{-1} wet wt.).

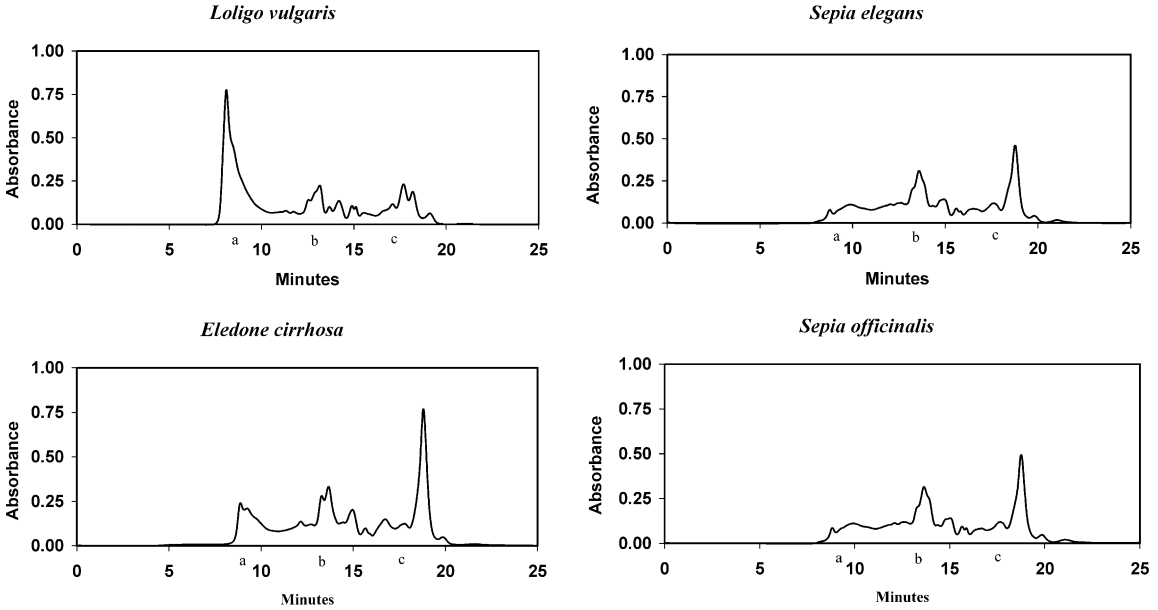

Fig. 4. Chromatographic profiles of heat-denatured homogenates from the digestive gland of cephalopods: Waters Protein-Pack 125 (7.8×300 mm) column; 100 mM Tris–HCl, pH 8.6; $\lambda = 254$ nm; a: Bovine Serum Albumine (66 kDa); b: Rabbit Liver Metallothionein (12.5 kDa); c: Aprotinine (6.5 kDa).

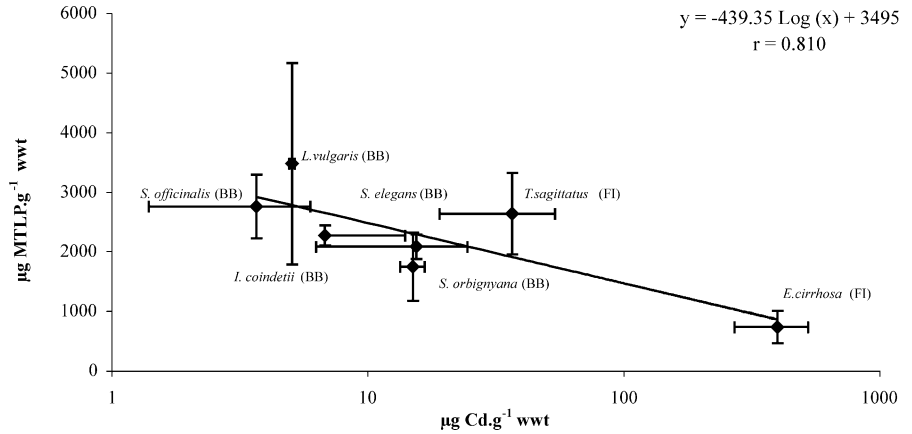

Fig. 5. Metallothionein-like protein levels in the heat-stable soluble fraction of the digestive gland of several cephalopod species as a function of the level of total cadmium in this tissue. Each point represents the mean ± 1 standard deviation for a single species. BB, Bay of Biscay; FI, Faroe Islands.

4. Discussion

In our study, except in *E. cirrhosa* from the Faroe Islands, cadmium was mainly located in the soluble fraction of the digestive gland cells, probably bound to cytosolic proteins. Very few studies have investigated the subcellular distribution of trace elements in the digestive gland of cephalopods and results are quite different between authors (Decleir Vlaeminck, Geladi, & Van Grieken, 1978; Finger & Smith, 1987; Rocca, 1969; Tanaka et al., 1983). Copper has been investigated in all these studies, but the subcellular distribution of cadmium has only been studied by Tanaka et al. (1983) and Finger and Smith (1987). Tanaka et al. (1983) found that most of the cadmium was bound to the organelles of the digestive gland cells of the squid *T. pacificus*, with only 26 ± 3% of the metal in the cytosolic fraction. On the contrary, most of this metal, i.e. 70 ± 10%, was bound to cytosolic proteins in the squid *Nototodarus gouldi* (Finger & Smith, 1987). Despite very different subcellular distributions between *T. pacificus* and *N. gouldi*, total cadmium levels in the digestive gland were in the same range within both species, ranging from 40 to 100 µg g^{-1} dry weight for *T. pacificus* and from 11 to 88 µg g^{-1} dry weight for *N. gouldi* (Finger & Smith, 1987; Tanaka et al., 1983). Our results (Table 2) concerning seven cephalopod species are in good agreement with those of Finger and Smith (1987) but not with the results of Tanaka et al. (1983; Fig. 1).

The subcellular distribution of cadmium seems to vary according to the levels of total cadmium in the tissue (Fig. 1). Thus, comparing species, as total cadmium levels increase in the tissue, the metal is proportionately more abundant in the insoluble fraction, bound to organelles and/or membranes. Trace element precipitation into metal-rich granules is an efficient metal detoxification mechanisms reported for a great number of invertebrate species (Brown, 1982; Coombs & George, 1978; Taylor & Simkiss, 1984). These granules function in the storage and

excretion of essential and non-essential metals, and their production is common in all major phyla (Brown 1982, George 1982). Thus, the observed increase of total cadmium levels in the digestive gland of cephalopods, resulting in the storage of cadmium in an insoluble form rather than via binding to cytosolic proteins, could be considered as more efficient to counteract the toxicity of the metal. Ultrastructural observations failed to show such insoluble compounds in several cephalopod species (Mangold & Bidder, 1989). Similar observations were made in the digestive gland cells of the squid *T. sagittatus* from the Faroe Islands, a species which exhibited relatively high cadmium levels (Bustamante, 1998): thus, microanalytical investigations of the digestive gland cells of this Faroese squid did not show any cadmium accumulations likely to explain the high cadmium levels of the digestive gland (Bustamante, 1998). The absence of granule-like structures has been noted for the digestive gland cells of the loliginid squids *L. forbesi* and *L. vulgaris*, and in the cuttlefish *S. officinalis* (Boucaud-Camou & Boucher-Rodoni, 1983; Boucher-Rodoni & Boucaud-Camou, 1987). Nevertheless, metal-rich spherules, mostly containing copper, have been described in *S. officinalis* (Martoja & Marcaillou, 1993). These authors suggested that the metals would be complexed by metallothioneins in these spherules. However, the presence of such structures remains controversial, as their existence has not been confirmed by other studies (Renata Boucher-Rodoni, personal communication).

Among organelles, lysosomes would be expected to play an important role in cadmium detoxification as has been reported for bivalves (Marigomez, Soto, & Cajaraville, 1995; Moore, 1990; Taylor, 1995). In invertebrate organisms, lysosomes are known to accumulate both essential and toxic metals from the cytosol of the digestive gland cells. Lysosomes contain several hydrolytic enzymes and function as a digestive system in the cells. Finally, at their last stage of maturity, they accumulate cellular waste products which cannot be degraded (Dallinger, 1993). It is therefore not surprising that metals are found in lysosomal residual bodies (Dallinger, 1995). In cephalopods, the digestive gland is involved in digestive and absorptive functions (Boucaud-Camou, 1974; Boucaud-Camou & Yim, 1980; Boucher-Rodoni & Boucaud-Camou, 1987). The lysosomal system is highly developed with several typical structures, i.e. heterolysosomes and heterophagosomes, "boules", residual bodies and brown bodies. Although microanalytical investigations have not shown any accumulation of cadmium (Bustamante, 1998), such lysosomal structures are probably involved in the compartmentalisation of metals in the digestive gland cells of cephalopods as has been reported for other mollusc species (Coombs & George, 1978; George & Viarengo, 1985; Viarengo & Nott, 1993; Marigomez et al., 1995). Moreover, lysosomes can contain degradation products of metallothionein indicating that they may serve as a final storage site of degraded metallothioneins and possibly, of other metal-binding proteins (Dallinger, 1995).

Cadmium sequestration seems to depend on levels of Cd contamination in the digestive gland. While the cytosolic content of cadmium increased significantly as a function of cadmium levels in the tissue in both *L. vulgaris* from the Bay of Biscay and *T. sagittatus* from the Faroe Islands (Fig. 3), the accumulation in the insoluble fraction was correlated with cadmium levels only for the Faroese squids. This

difference in cadmium partitioning could be explained by the following hypothesis. When a threshold level, located between the highest *L. vulgaris* and the lowest *T. sagittatus* total cadmium level in the digestive gland (i.e. 8 and 12 μg g^{-1} wet wt., respectively) is reached, the detoxification processes are modified and the storage of cadmium in organelles starts. Cd-binding proteins would be efficient enough to detoxify cadmium in loliginid squids from the Bay of Biscay while in ommastrephid ones, this system would be overloaded and lead to an alternative process assuring an efficient detoxification of cadmium. On an ultrastructural point of view, the digestive gland cells of loliginid squids are different from those of other cephalopods. They do not have the "boules" structures characteristic of most cephalopod species (Boucher-Rodoni & Boucaud-Camou, 1987). In the cuttlefish *S. officinalis*, some of these "boules" are considered as heterolysosomes and heterophagosomes involved in intracellular digestion (Boucaud-Camou, 1976; Boucaud-Camou & Yim, 1980). Although brown bodies which contain residues of the digestion, account for the digestion ability of endogenous or exogenous compounds by loliginid digestive cells, the lack of "boules" in these cells might mean that particle capture and intracellular digestion do not occur widely in their digestive glands (Boucher-Rodoni & Boucaud-Camou, 1987). Generally, in the Loliginidae family, the lysosomal system is less developed than in other cephalopod species. Consequently, loliginid squids could be physiologically limited to detoxify cadmium via binding to insoluble compounds. Alternatively, these squids could have developed mechanisms favouring the excretion of cadmium.

The occurrence of metallothioneins was suspected when considering the chromatograms of metalloproteins from the digestive gland of the squids *N. gouldi*, *T. pacificus* and *Ommastrephes bartrami*, eluting between 10 kDa and 18 kDa (Castillo & Maita, 1991; Finger & Smith, 1987; Tanaka et al., 1983). Since then, our investigations are among the first to attempt to determine the importance of these proteins in the detoxification processes of cadmium in cephalopods.

The quantification of metallothioneins showed up high specific differences, with the highest metallothionein levels corresponding to the lowest total cadmium levels in the digestive gland (i.e. *L. vulgaris* from the Bay of Biscay) and on the contrary, the lowest levels of metallothioneins corresponding to the highest total cadmium levels (i.e. *E. cirrhosa* from the Faroe Islands; Fig. 5). These observations are in accordance with the variation of the percentage of soluble cadmium (see earlier).

Metallothionein levels measured in cephalopods from this study (i.e. from 0.7 ± 0.3 to 3.5 ± 0.6 mg g^{-1} wet wt.) were of the same order of magnitude than those reported for other mollusc species. Thus, in the digestive gland of the mussel *Mytilus galloprovincialis* and the gastropod *Littorina littorea*, reported levels range from 0.6 to 2.1 mg g^{-1} wet weight (Bebianno, Langston, & Simkiss, 1992; Pavicic, Raspor, & Martincic, 1993). Cadmium levels in cephalopod digestive glands however were one or two orders of magnitude higher than those of these molluscs. Cadmium is largely considered as an inducer of the synthesis of metallothioneins in several phyla, and owing to the high cadmium levels in cephalopods, high metallothionein levels could be expected. It was not the case. On the contrary, considering the different cephalopod species in our study, metallothionein levels were negatively correlated with

total cadmium levels in the digestive gland (Fig. 5). In this mollusc class, metallothioneins do not represent the major response to the accumulation of cadmium in the tissue and cannot be considered as a biomarker of exposure to this metal. Cadmium detoxification could occur with other metalloproteins than metallothioneins. This hypothesis is strengthened by results found by other authors working on cephalopods. Tanaka et al. (1983) and Castillo, Kawaguchi, and Maiti (1990) have shown that most of the cytosolic cadmium in the digestive gland of the squids *T. pacificus* and *Onychoteuthis borealojaponica* was bound to proteins weighing more than 70 kDa. Only a small part of the soluble cadmium was bound to low molecular weight proteins (<3 kDa) or to proteins of size similar to metallothionein (10 kDa–16 kDa). Moreover, Finger and Smith (1987) have reported the occurrence of Cd-binding proteins with a high molecular weight ($>$ to 70 kDa) in the digestive gland of the squid *N. gouldi*.

5. Conclusions

The high levels of cadmium reported for cephalopod species from different areas of the world suggest that these organisms have developed efficient detoxification mechanisms. The subcellular distribution of cadmium in the digestive gland between cytosol and organelles suggests that the major part of this metal is associated with cytosolic proteins, except in *E. cirrhosa* from the Faroe Islands. The quantification of metallothioneins suggests a limited participation of these proteins in the detoxification of cadmium in cephalopods. An alternative mode of sequestration (lysosomal) could be activated when the level of cadmium in the digestive gland reaches a threshold value. Thus, further studies are needed to characterise the proteins involved in cadmium detoxification in cephalopods.

Table 2
Percentage of Cd, Cu and Zn in the soluble fraction of the cytosol from different cephalopod species

Family species	Number of individuals	Metal percentage in the soluble fraction			Origin
		Cd	Cu	Zn	
Sepiidae					
Sepia elegans	5	59±22	72±17	60±15	Bay of Biscay
Sepia officinalis	8	64±8	69±9	60±9	Bay of Biscay
Sepia orbignyana	7	70±8	69±6	72±10	Bay of Biscay
Loliginidae					
Loligo vulgaris	27	86±12	86±15	82±6	Bay of Biscay
Ommastrephidae					
Illex coindetii	9	79±15	84±12	79±17	Bay of Biscay
Todarodes sagittatus	21	64±10	70±8	61±9	Faroe Islands
Octopodidae					
Eledone cirrhosa	4	42±17	53±14	50±3	Faroe Islands

Cephalopods are considered to be a vector for the transfer of cadmium to top marine predators (Bustamante, Caurant et al., 1998; Honda & Tatsukawa, 1983; Muirhead & Furness, 1988). This is strengthened by our results which have demonstrated the subcellular localisation of cadmium. Recent investigations using radiollabelled food with ^{109}Cd have showed that ^{109}Cd bound to cytosolic proteins was transferred with a high efficiency and ^{109}Cd bound to insoluble compounds was relatively unavailable (Wallace & Lopez, 1997). Thus, the high bioavailability of cadmium in the digestive gland cells indicates a high potential for the trophic transfer of the metal to their predators such as marine mammals and seabirds.

Acknowledgements

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References

Amaratunga, T. (1983). The role of cephalopods in the marine ecosystem. In I. F. Caddy, *Advances in assessment of world cephalopod resources. FAO Fish Tech Pap, 231* (pp. 379–415).

Bebianno, M. J., Langston, W. J., & Simkiss, K. (1992). Metallothionein induction in *Littorina littorea* (Mollusca: Prosobranchia) on exposure to cadmium. *Journal of Marine Biology of Association of the United Kingdom*, *72*, 329–342.

Boucaud-Camou, E. (1974). Localisation d'activités enzymatiques impliquées dans la digestion chez *Sepia officinalis* L. *Archives de Zoologie expérimentale et générale*, *115*, 5–27.

Boucaud-Camou, E. (1976). Structure et fonction de la glande digestive ("foie") de la Seiche, *Sepia officinalis* L. (Mollusque, Céphalopode). *Bulletin de la Société Zoologique de France*, *101*, 887.

Boucaud-Camou, E., & Boucher-Rodoni, R. (1983). Feeding and digestion in Cephalopods. In A. S. M. Saleuddin, & K. M. Wilbur (Eds.), *The Mollusca (Vol. 5). Physiology, Part 2* (pp. 149–187). New York and London: Academic Press.

Boucaud-Camou, E., & Yim, M. (1980). Fine structure and function of the digestive cell of *Sepia officinalis* (Mollusca: Cephalopoda). *Journal of Zoology London*, *191*, 89–105.

Boucher-Rodoni, R., & Boucaud-Camou, E. (1987). Fine structure and absorption of ferritin in the digestive organs of *Loligo vulgaris* and *L. forbesi* (Cephalopoda, Teuthoidea). *Journal of Morphology*, *193*, 173–184.

Brown, B. E. (1982). The form and function of metal-containing 'granules' in invertebrate tissues. *Biol Rev Cam Philos Soc.*, *57*, 621–667.

Bustamante, P. (1998) Etude des processus de bioaccumulation et de détoxication d'éléments traces (métaux lourds et terres rares) chez les mollusques céphalopodes et bivalves pectinidés. Implication de leur biodisponibilité pour le transfert vers les prédateurs. Thesis, University of La Rochelle.

Bustamante, P., Caurant, F., Fowler, S. W., & Miramand, P. (1998). Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. *The Science of the Total Environment*, *220*, 71–80.

Bustamante, P., Cherel, Y., Caurant, F., & Miramand, P. (1998). Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. *Polar Biology*, *19*, 264–271.

Castillo, L. V., Kawaguchi, S., & Maita, Y. (1990). Evidence for the presence of heavy metal binding proteins in the squid, *Onychoteuthis borealijaponica*. In R. Hirano, & I. Hanyu (Eds.), *The second Asian fisheries forum* (pp. 991). Manila: Asian Fish. Soc.

Castillo, L. V., & Maita, Y. (1991). Isolation and partial characterisation of cadmium binding proteins from the oceanic squid, *Ommastrephes bartrami*. *Bull. Fac. Fish. Hokkaido Univ*, *42*(1), 26–34.

Clarke, M. R. (1996). Cephalopods as prey. III. Cetaceans. *Philosophical Transactions of the Royal Society London Series B*, *351*, 1053–1065.

Coombs, T. L., & George, S. G. (1978). Mechanisms of immobilisation and detoxification of metals in marine organisms. In D. S. McLusky, & A. J. Berry (Eds.), *Physiology and behaviour of marine organisms. Proceeding of the 12th European symposium on marine biology* (pp. 179–187). Oxford: Stirling, Pergammon Press.

Cosson, R. P., Amiard-Triquet, C., & Amiard, J. C. (1991). Metallothioneins and detoxification. Is the use of detoxification proteins for MTs a language abuse? *Water Air and Soil Pollution*, *57/58*, 555–567.

Croxall, J. P., & Prince, P. A. (1996). Cephalopods as prey. I. Seabirds. *Philosophical Transactions of the Royal Society London Series B*, *351*, 1023–1043.

Dallinger, R. (1993). Strategies of metal detoxification in terrestrial invertebrates: synopsis and perspectives. *Comparative Biochemistry and Physiology*, *113C*, 125–133.

Dallinger, R. (1995). Mechanisms of metal incorporation into cells. In M. P. Cajaraville (Ed.), *Cell biology in environmental toxicology* (pp. 135–154). Bilbo: University of the Basque Country Press Service.

Decleir, W., Vlaeminck, A., Geladi, P., & Van Grieken, R. (1978). Determination of protein-bound copper and zinc in some organs of the cuttlefish *Sepia officinalis* L. *Comparative Biochemistry and Physiology*, *60B*, 347–350.

Donat, J. R., & Bruland, K. W. (1995). Trace elements in the oceans. In B. Salbu, & E. Steinnes (Eds.), *Trace elements in natural waters* (pp. 247–281). Boca Raton: CRC Press.

Engle, D. W., & Brouwer, M. (1989). Metallothionein and metallothionein-like proteins: physiological importance. *Advances in Comparative Environmental Physiology*, *5*, 54–75.

Erk, M., & Raspor, B. (2000). Advantages and disadvantages of voltametric method in studying cadmium-metallothionein interactions. *Cellular and Molecular Biology*, *46*(2), 269–281.

Finger, J. M., & Smith, J. D. (1987). Molecular association of Cu, Zn, Cd and ^{210}Po in the digestive gland of the squid *Nototodarus gouldi*. *Marine Biology*, *95*, 87–91.

George, S. G. (1982). Subcellular accumulation and detoxification of metals in aquatic animals. In W. B. Vernberg, A. Calabresa, F. P. Thurnberg, & F. J. Vernberg (Eds.), *Physiological mechanisms of marine pollutant toxicity* (pp. 3–55). New York: Academic Press.

George, S. G., & Olsson, P. E. (1994). Metallothioneins as indicators of trace metal pollution. In K. J. M. Kramer (Ed.), *Biomonitoring of coastal waters estuaries* (pp. 151–171). Boca Raton: CRC Press.

George, S. G., & Viarengo, A. (1985). A model for heavy metal homeostasis and detoxification in mussels. In F. J. Thunrberg, E. Calabrese, & W. B. Vernberg (Eds.), *Marine pollution and physiology: recent advances* (pp. 125–143). Columbia: University South Carolina Press.

Honda, K., & Tatsukawa, R. (1983). Distribution of cadmium and zinc in tissues and organs, and their age-related changes in striped dolphin, *Stenella coeruleoalba*. *Archives of Environmental Contamination and Toxicology*, *12*, 543–550.

Klages, N. T. W. (1996). Cephalopods as prey. II. Seals. *Philosophical Transactions of the Royal Society London Series*, *351*, 1045–1052.

Mangold, K., & Bidder, A. M. (1989). L'appareil digestif et la digestion. In K. Mangold, *Céphalopodes*. In P. P. Grassé, *Traité de Zoologie Tome V* (pp. 321–373). Paris: Masson.

Marigomez, I., Soto, M., & Cajaraville, M. P. (1995). Morphological patterns of cell and tissue systems involved in metal handling and metabolism. In M. P. Cajaraville (Ed.), *Cell biology in environmental toxicology* (pp. 89–134). Bilbo: University of the Basque Country Press Service.

Mart, L., Rützel, H., Klahre, P., Sipos, L., Platzek, U., Valenta, P., & Nürnberg, H. W. (1982). Comparative studies on the distribution of heavy metals in the oceans and coastal waters. *The Science of the Total Environment*, *26*, 1–17.

Martin, J. H., & Flegal, A. R. (1975). High copper concentrations in squid livers in association with elevated levels of silver, cadmium, and zinc. *Marine Biology*, *30*, 51–55.

Martoja, M., & Marcaillou, C. (1993). Localisation cytologique du cuivre et de quelques autres métaux dans la glande digestive de la seiche, *Sepia officinalis* L. (Mollusque Céphalopode). *Canadian Journal of Fisheries and Aquatic Sciences*, *50*(3), 542–550.

Miramand, P., & 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. *Marine Biology*, *114*, 407–414.

Miramand, P., & Guary, J. C. (1980). High concentrations of some heavy metals in tissues of the Mediterranean octopus. *Bulletin of Environmental Contamination and Toxicology*, *24*, 783–788.

Moore, M. N. (1990). Lysosomal cytochemistry in marine environmental monitoring. *Histochemistry Journal*, *22*, 187–191.

Muirhead, S. J., & Furness, R. W. (1988). Heavy metal concentrations in the tissues of seabirds from Gough Island, South Atlantic Ocean. *Marine Pollution Bulletin*, *19*, 278–283.

Olafson, R. W., & Olsson, P. E. (1991). Electrochemical detection of metallothionein. *Methods in Enzymology*, *205*, 205–213.

Olafson, R. W., & Sim, R. G. (1979). An electrochemical approach to quantification and characterisation of metallothioneins. *Analytical Biochemistry*, *100*, 343–351.

Pavicic, J., Raspor, B., & Martincic, D. (1993). Quantitative determination of metallothionein-like proteins in mussels. Methodological approach and field evaluation. *Marine Biology*, *115*, 435–444.

Phillips, D. J. H., & Rainbow, P. S. (1989). Strategies of metal sequestration in aquatic organisms. *Marine Environmental Research*, *28*, 207–210.

Rocca, E. (1969). Copper distribution in *Octopus vulgaris* Lam. hepatopancreas. *Comparative Biochemistry and Physiology*, *28*, 67–82.

Rodhouse, P. G. (1989). Antarctic cephalopods—a living marine resource? *Ambio*, *18*(1), 56–59.

Roesijadi, G. (1992). Metallothionein in metal regulation and toxicity in aquatic animals. *Aquatic Toxicology*, *22*, 81–114.

Roesijadi, G. (1996). Metallothionein and its role in toxic metal regulation. *Comparative Biochemistry and Physiology*, *113C*, 117–123.

Simkiss, K., & Taylor, M. G. (1982). Metal detoxification and bioaccumulation in molluscs. *Marine Biological Letters*, *3*, 187–201.

Smale, M. J. (1996). Cephalopods as prey. IV. Fishes. *Philosophical Transactions of the Royal Society London Series B*, *351*, 1067–1081.

Smith, J. D., Plues, L., Heyraud, M., & Cherry, R. D. (1984). Concentrations of the elements Ag, Al, Ca, Cd, Cu, Fe, Mg, Pb and Zn, and the radionuclides ^{210}Pb and ^{210}Po in the digestive gland of the squid *Nototodarus gouldi*. *Marine Environmental Research*, *13*, 55–68.

Tanaka, T., Hayashi, Y., & Ishizawa, M. (1983). Subcellular distribution and binding of heavy metals in the untreated liver of the squid; comparison with data from the livers of cadmium and silver exposed rats. *Experientia*, *39*, 746–748.

Taylor, M. G. (1995). Mechanisms of metal immobilisation and transport in cells. In M. P. Cajaraville (Ed.), *Cell biology in environmental toxicology* (pp. 155–169). Bilbo: University of the Basque Country Press Service.

Taylor, M. G., & Simkiss, K. (1984). Inorganic deposits in invertebrates tissues. *Environmental Chemistry*, *3*, 102–138.

Thompson, J. A. J., & Cosson, R. P. (1984). An improved electrochemical method for the quantification of metallothioneins in marine organisms. *Marine Environmental Research*, *11*, 137–152.

Viarengo, A., & Nott, J. A. (1993). Mini-review. Mechanisms of heavy metal cation homeostasis in marine invertebrates. *Comparative Biochemistry and Physiology*, *104C*, 355–372.

Wallace, W. G., & Lopez, G. R. (1997). Bioavailability of biologically sequestered cadmium and the implications of metal detoxification. *Marine Ecology Progress Series*, *147*, 149–157.