Science of The Total Environment
Vol. 361, Issues 1-3 , 15 May 2006, P.132-143
http://dx.doi.org/10.1016/j.scitotenv.2005.10.018

Variation of heavy metal concentrations (Ag, Cd, Co, Cu, Fe, Pb, V, and Zn) during the life cycle of the common cuttlefish *Sepia officinalis*

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Abstract:

The developmental changes in the concentration of 8 essential and non-essential heavy metals (Ag, Cd, Cu, Co, Fe, Pb, V, Zn) in the tissues (digestive gland, cuttlebone and whole animal) of the common cuttlefish Sepia officinalis collected in the bay of the river Seine were monitored from the end of the embryogenesis until the adult reproductive stage. Compared to embryos, juveniles after hatching displayed much higher concentrations of Ag, Cu, Fe and Zn, suggesting an efficient incorporation from seawater. Conversely, the amounts of Cd, Pb and V in hatchlings remained constant suggesting that these metals are barely bioavailable for juveniles. Once the juveniles start to feed, the digestive gland appears to play a major role in the storage of all metals. After only one month of benthic life, the digestive gland already contains up to 90% of the total metal body burden, indicating that it plays a major role in the storage and presumed detoxification of the selected metals. Metal concentrations in the digestive gland increase in a logarithmic fashion with age during the entire life of cuttlefish, except for Ag, which decreases as soon as cuttlefish migrate to open sea. This strongly suggests that (1) Ag is excreted from the digestive gland in relation to presumably lower exposure in less contaminated environments compared to coastal waters and (2) the digestive gland of cephalopods could be a very good indicator of Ag contamination in the marine environment.

Keywords: Trace element; Bioaccumulation; Detoxification; Cephalopods; Sexual maturity; Embryogenesis

ABSTRACT

The evolution of the concentration of 8 essential and non essential heavy metals (Ag, Cd, Cu, Co, Fe, Pb, V, Zn) in the tissues (digestive gland, cuttlebone and whole animal) of the common cuttlefish *Sepia officinalis* collected in the Bay of Seine has been tracked since the end of the embryogenesis until the reproduction period. Compared to the embryos, the juveniles after hatchling display much higher concentrations of Ag, Cu, Fe and Zn suggesting an efficient incorporation from seawater. Conversely, the amounts of Cd, Pb and V in hatchlings remain suggesting that these metals are barely bioavailable for the juveniles. Once the juveniles start to feed, the digestive gland appears to play a major role in the storage of all metals. After only one month of benthic life, the digestive gland already contains up to 90% of the total metal body burden, indicating that digestive gland plays a major role in the storage and presumed detoxification of the selected metals. Metal concentrations in the digestive gland increase in a logarithmic fashion with age during the entire life of cuttlefish, except for Ag which decreases as soon as cuttlefish migrate to open sea. This strongly suggests that (1) Ag is depurated from the digestive gland in relation to supposedly lower exposure in less contaminated environments compare to coastal waters and (2) the digestive gland of cephalopod could be a very good indicator of Ag contamination in the marine environment.

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Keywords: Trace element; bioaccumulation; detoxification; cephalopods; sexual maturity; embryogenesis

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INTRODUCTION

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As other cephalopods, the cuttlefish *Sepia officinalis* has a very short life cycle during which migrations related to growth and reproduction take place. In the English Channel and the Atlantic areas, the reproduction period after which these organisms die, occurs between April and August of their second year of life, when they are aged between 14 and 18 months (Boucaud-Camou et al., 1991; Legoff and Daguzan, 1991). Only some male individuals not attaining their sexual maturity during this period can survive for another year. Therefore, the life of a cuttlefish never exceeds 2 years in those areas (Richard, 1971). During this period, the growth rate of cuttlefish is very high. Their weight can even be multiplied by a factor of 2,000, cuttlefish weighing 0.25 g after hatching and more than 600 g at the sexual maturity

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(Richard, 1971; Pascual, 1978; Forsythe et al., 1994). This exceptional growth rate can be explained in terms of their active metabolism owing to their carnivore diet (Mangold, 1989). Regardless of such a short life cycle, the strong capability of cuttlefish to concentrate a large number of metals in their tissues has been previously shown, as well as the major role of the digestive gland in the bioaccumulation processes (e.g. Decleir et al., 1978; Miramand and Bentley, 1992; Bustamante et al., 1998; 2002a). This capability seems to be shared by many other species of cephalopods, octopodidae, teuthoidae or nautilidae (Rocca, 1969; Nardi et al., 1971; Schipp and Hevert, 1978; Miramand and Guary, 1980; Smith et al., 1984; Finger and Smith, 1987; Bustamante, 1998; Bustamante et al., 1998; 2000). More recently, radiotracer experimental studies have shown the relative importance of the transfer pathways (water, food, sediments) in the bioaccumulation of 4 heavy metals (Ag, Cd, Co, Zn) in juvenile and adult cuttlefish (Bustamante et al., 2002b; 2004), confirming the storing nature of the digestive gland regardless of the uptake pathway.

However, little is known about the evolution of metal concentrations and burdens during the embryogenesis and growing of *Sepia officinalis*. The aim of this study was therefore to investigate the variation of the concentrations of 8 essential and non essential (Ag, Cd, Cu, Co, Fe, V, Pb, Zn) heavy metals during the growth of the cuttlefish from the embryogenesis to the reproduction period. Eggs, hatched juveniles, juveniles during their 2 first months of benthic life, immature individuals and mature adults returning to the coast to mate were analysed. Thus, both the physiological changes and the migrations have been considered. The former occur during the life cycle of cuttlefish and may modify the bioaccumulation processes of metals, whereas during the migrations the animals move from coastal areas (with metallic inputs of anthropogenic origin) to the open sea (supposed to be less polluted than the coast) hence potentially modifying the metal concentrations in their tissues. In this work, special attention has been paid to the digestive gland owing to its major role in the metabolism of metals. To this end, the zone of study chosen is particularly interesting since direct arrivals of metallic contaminants, especially Ag (RNO, 2001) and Cd (Chiffoleau et al., 1994; 1996; Miramand et al., 2001) are poured by the Seine River. Furthermore, the bay of Seine constitutes a laying and nursery area for the common cuttlefish that is subjected to fishery activities of important economical impact in the region (Boucaud-Camou and Boismery, 1991).

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MATERIALS AND METHODS

Biological material

1) Animals from the field

Between July 1989 and July 1991, 153 cuttlefish were collected in 6 times in the bay of Seine off the harbour of Ouistreham (Normandy, France). The age of animals was estimated according to the previous works of Medhioub (1986) and Boucaud-Camou et al. (1991) using the model of seasonal growth established by Pauly and Gaschütz (1979). According to their mantle length (ML), the animals were pooled in estimated age classes for heavy metal analysis as follows:

- about 1-week old: 3 pools of 25 individuals (mean ML =10 ± 1 mm);
- about 2-weeks old: 4 pools of 8 individuals (mean ML =18 ± 3 mm);
- about 1-month old: 3 pools of 4 individuals (mean ML = 33 ± 4 mm);
- about 2-months old: 4 pools of 4 individuals (mean ML =59 ± 6 mm);
- about 12-months old (immature cuttlefish): 3 pools of 5 individuals (mean ML = 133 ± 19 mm);
- about 18-months old (mature cuttlefish): 3 pools of 1 individual (mean ML = 215 ± 5 mm).

The cuttlefish specimens were immediately frozen on board and stored at –20° C. After a short frozen period (< 1 month), each individual was dissected in the laboratory: digestive gland and cuttlebone were removed from the remaining tissues and pooled to allow metal analyses in minute organs and tissues. Our sampling of cuttlefish is supposed to represent a single troop, at least for the individuals of the first year.

2) Eggs reared in the laboratory

Eggs collected in the same area and the same period as the cuttlefish were incubated at the laboratory in aquaria with an open circuit system. 4 pools of 25 eggs were dissected and embryos, yolk and eggshells were separated for analysis. The embryos collected from the eggs were about to hatching and were completely formed but still attached to the yolk sack, which was not completely resorbed. The rest of the eggs were maintained in aquaria until hatchling, and the former 25 juvenile hatched (ML = 10 ± 1 mm) were dissected as previously described.

Sample preparation and analytical procedure

All the samples were dried at 60°C for several days to constant weight and then reduced to powder using porcelain mortar and pestle. Aliquots ranging from 10 to 300 mg of the homogenised samples were digested with 4 ml of 14N ultrapur HNO_3 and 1 ml of 22N ultrapur $HClO_4$ at 100°C on a hot plate during 3 days. After evaporation of the acids, the

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residues were taken up in 5 ml 0.3 N HNO_3. Ag, Cd, Co, Cu, Pb and V were analyzed by Zeeman graphite furnace atomic absorption spectrophotometry and Fe and Zn by flame atomic absorption spectrophotometry. Heavy metal concentrations in whole individuals were calculated for reconstructed individuals from analysis of remaining tissues, cuttlebone and digestive gland. However, the high Ca content in the cuttlebone hinder Co measures in this organ, not allowing calculations for the whole juveniles and adults.

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Quality control was assay by heavy metal analyses in blanks and reference materials. Thus, Orchard–Leaves (National Bureau of Standards) and MA-A-1, MA-A-2, respectively copepods and fish flesh standard (IAEA) were treated and analysed in the same way as the samples. Our results for the standard reference materials were in good agreement with the certified values (Table 1). The detection limits were (µg.g^{-1} dry weight): 0.004 (Cd), 0.02 (Ag), 0.1 (Co, Pb), 0.5 (Cu, V, and Zn) and 2.5 (Fe). Results are also expressed in micrograms per gram of the dry tissue weight (µg.g^{-1} dwt).

Statistical analysis

Comparison of the metal concentrations between eggshell and embryos/yolk were assessed using *t*-test comparison of means. Differences between metal concentrations at the different ages were tested using the non-parametric Kruskall-Wallis test. Regressions between the concentrations measured in the cuttlefish digestive gland and mantle length were tested using regression procedures for linear and non linear fitted models. Accumulation of metals in the cuttlefish was described by linear and logarithmic fitted models, with the exception of Ag in the digestive gland which is described by a two-order linear component model. Statistical analyses were performed using XLStat Pro 7.0. The level of significance was always set at a=0.05.

RESULTS

Levels of concentration and distribution among compartments

The heavy metal concentrations in eggs, embryos, juveniles of different sizes, immature and mature *Sepia officinalis* are given in Table 2.

At the end of the embryo development, the eggs were dissected between eggshell, yolk and embryo. Heavy metal concentrations were similar between yolk and embryo, except for Cd and Cu. However, when compared metal concentrations of those internal compartments with the external one (i.e. the eggshell), results appear contrasted. For Co, Fe, Pb, and V, the

Mis en forme: Retrait : Première ligne : 0 cm concentrations in the eggshell were significantly (P_{t-test}<0.0001 for the 4 metals) higher than in yolk and embryo whereas they were significantly lower for Ag (P_{t-test} =0.015) and Cu (P_{t-test} <0.0001).

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Overall, metal concentrations in the embryos extracted from the eggs were generally lower (Ag, Cu, Fe, and Zn) or in the same range (Cd, Pb and V) than in hatchlings. These concentrations were between 2 and 4 times lower than those measured in mature individuals. During the whole-life cycle of cuttlefish, metal concentrations showed significant variations (H-value ranges from 15.32 to 17.34, and $P_{Krsukall-Wallis}$-value ranges from 0.004 to 0.009). Overall, the highest metal concentrations correspond to those of the essential metals Cu, Fe and Zn (between 30 and 200 µg.g^{-1} dwt) and the lowest to non essential metals (between 0.13 and 2.4 µg.g^{-1} dwt).

The dissection of cuttlefish from 7 days old to 2 years old showed that the digestive gland exhibited about one order of magnitude higher metal concentrations compared to the whole animal (Table 3). Consequently, this organ generally contained a great proportion of the whole-body burden of the metals (Table 4). In contrast, the cuttlebone displayed very low non-essential metal concentrations (Table 5). In this organ, Pb and V concentrations remained close to the detection limit during the whole-life cycle and Ag and Cd concentrations were one order of magnitude lower than those measured in the whole animal. Therefore, the cuttlebone only contained a small fraction of the total non essential metal load (Table 6). Finally, the concentrations of the essential metals measured in the cuttlebone are similar (Fe and Zn) or lower (Cu) than those found in the whole organisms (Table 5).

Variation of metal concentrations and burdens with age

Immediately after hatching, the concentrations of Cu, Fe, and Zn have strongly increased in cuttlefish (Table 2). Indeed, the very young cuttlefish displayed concentrations values of 1.5 to 2 (Cu and Zn) and 6 (Fe) times higher than those found in the embryos taken from the eggs immediately before hatching. However, considering the whole-body concentrations, Cu and Zn increased significantly with age following a logarithmic fashion (R=0.408, ddl=18, P<0.05 and R=0.865, ddl=18, P<0.0001, respectively) whereas Fe tended to decrease significantly (R=0.657, ddl=18, P<0.001). Among toxic elements, Ag was the only metal showing a significant tendency to decrease with age (R=0.558 ddl=18, P<0.01) whereas Cd, Pb and V globally increase (Table 2). However, such variations in the whole-body animals are likely to be due to the variations in the storage organs such as the digestive gland. These tissue-specific variations are actually faked by natural dilution by non-storage organs. Therefore, the tissues presently dissected at all stages of the life cycle, i.e. the digestive gland and the cuttlebone, were considered to investigate metal variations with age (Figure 1 & 2, respectively).

Regardless of the essential or non essential role of the metals, their concentrations in the digestive gland showed significant variations during the whole-life of cuttlefish (Table 3). The amounts of metals contained in the digestive gland relatively to the whole-body burden also show remarkable changes during the life cycle (Table 4). With the exception of Ag, all metal concentrations and burdens in the digestive gland increase significantly with age following a logarithmic fashion (Figures 1 & 3). Ag concentrations increase linearly (R=0.812, ddl=13, P<0.001) until the age of 2 months (ML = 59 mm), then decrease linearly (R=0.855, ddl=9, P<0.001) when cuttlefish migrate to open ocean waters.

In the cuttlebone, the concentrations of Ag, Cu and Fe decrease with age following a logarithmic fashion (R=0.861, ddl=18, P<0.001, R=0.910, ddl=18, P<0.001, R=0.660, ddl=18, P<0.005, respectively) whereas Zn display a linear accumulation (R=0.989, ddl=18, P<0.001) (Figure 2). The other metals either varied randomly (i.e. Cd) or were below the detection limit (i.e. Pb and V).

Figure 3 shows the variation of metal burdens in relation to the mantle length (age) of the cuttlefish. Both essential and non essential element burdens clearly increase during the whole life cycle of the cuttlefish, with the exception of Ag whose burdens have decreased in mature individuals.

DISCUSSION

Metal bioaccumulation in eggs and hatchlings

At the end of the embryonic development, the embryos showed very low metal concentrations compared to older cuttlefish (Table 2). Such concentrations are close or even identical to those measured in the vitellus of the early spawned eggs, suggesting that the vitellus contains a sufficient amount of essential metals (Cu, Fe, and Zn) necessary for the development of the embryo. Previous studies with radio-labelled metals have demonstrated that ^{65}Zn, ^{109}Cd and ^{57}Co were mainly retained on the eggshell (96% after 11d of exposure), acting as a protective barrier limiting/hindering the incorporation of waterborne metals. However, the eggshell the thickness of the eggshell changes during embryonic development. When spawned, its thickness is in the mm-region, i.e. ± 1.5 mm (Lemaire, 1971) and is composed of albumin and other proteins, hardening when put into contact with seawater. Then, it becomes thinner during embryonic development, being almost transparent at the moment of eclosion (Wolf et

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Various studies have shown the ability of cephalopods to concentrate large amounts of trace elements within their tissues. Among cephalopod tissues, the digestive gland has been previously shown to have a key function in metal storage and detoxification. Up to date these processes have been mainly studied for Cd, which has been shown to be mainly associated to cytolosic compounds in the digestive gland of these cephalopods (Bustamante et al. 2002a). Therefore, a significant fraction of Cd could be likely detoxificated by association with cytolosic proteins of the same type of metallothioneins as reported for different squid species (Tanaka et al. 1983; Finger & Smith, 1987; Castillo & Maita, 1991). Nevertheless, there might be other detoxification processes of this metal in cephalopods as the ratio of Cd associated to cytolosic compounds decreases when the concentration of total Cd increases in the digestive gland (Bustamante et al. 2002a). Despite an important number of studies (Rocca, 1969; Martin & Flegal, 1975; Tanaka et al. 1983, Smith et al. 1984; Finger & Smith, 1987; Bustamante et al. 1998; 2002ab, 2004), such processes are still not well understood for Cd whereas some other elements as Pb and V have not yet been studied.¶
Furthermore, the dynamic of the bioaccumulation processes of metals during the whole-life cycle of these molluscs have never been investigated. In that aim, cuttlefish was chosen as a cephalopod model and accumulation of metals has been studied at different stage of its life cycle, from the embryonic development in the eggs to the mature pre-spawning adults ... [1]

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al., 1985). Very weak concentrations of Pb and V in the embryos also suggest a specific limitation of their incorporation into the eggs, but this issue should be addressed specifically in the future.

Immediately after hatching, the rapid increase of Cu, Fe and Zn concentrations in cuttlefish tissues (Table 2) suggests that the hatchlings are highly dependent on the essential metals to fulfil their metabolic demands. It therefore follows that the metals are rapidly uptaked once they are in contact with seawater. At this stage, the accumulation only occurs through the dissolved pathways by branchial absorption and tegumental adsorption, the hatchlings still living only on their vitellian reserves for a week (Mangold and Bidder, 1989). High ^{65}Zn accumulation rate has been experimentally shown for juveniles exposed to radiolabelled seawater (Bustamante et al., 2002b). Similarly to essential elements, Ag concentrations are 10 times higher in hatchlings than in the embryos (Table 2), showing a very rapid bioaccumulation of the metal. Similarly to ^{65}Zn, exposure of hatchlings to ^{110m}Ag via seawater leads to elevated concentration factors (i.e. 320) after a period of only 36h (Bustamante et al., 2004). Therefore, the dissolved pathway appears as a very important route for essential elements and Ag, whereas its role appears rather limited for Cd (Bustamante et al., 2002b), or Pb and V (Miramand et al., 1980, 1981) whose concentrations remained very low. Further investigations on the uptake of V and Pb are specifically needed to asses this hypothesis.

Metal bioaccumulation during growth

After a week of benthic life, the hatchlings use up their vitellian reserves and therefore need to eat. Once they start to feed, cuttlefish rapidly grown (data not shown) but at the same time, are exposed to a new source of metals. Despite dietary intakes, the concentrations of most of the metals in the whole organisms did not vary significantly and only a few metals show a significant increase of the concentrations in the whole cuttlefish (i.e. Cd, Fe, Table 2). This could be due either to 1) control of essential element concentrations through homeostatic regulation, or to 2) dilution of non essential accumulated metals related to very fast somatic growth. With the exception of Fe, the essential metals seem to be well regulated and Cu and Zn showed remarkably homogenous concentrations during the whole life (Table 2). Among non essential elements, Ag concentration is also slightly influenced by dietary supplies. Concentrations of Pb and V in the whole cuttlefish remain very low and will only be over the detection limit only after 1 and 2 months of benthic life, respectively. Therefore, in what concerns the whole animal, cuttlefish bioaccumulate poorly these 2 metals.

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Therefore, eggshell seems to constitute a protective barrier limiting or hindering the incorporation of waterborne metals into the eggs during the development of the embryo. This barrier seems to be particularly effective in the case of Pb and V since the concentrations measured in the embryos were very weak, respectively under 0.1 and 0.5 µg g^{-1} dry wt, which corresponds to the detection limit of the analytical technique. The high concentrations of Pb and V but also of Fe and Co in the eggshell suggest that these metals are adsorbed at the surface but do not penetrate into the egg. This observation is in good agreement with the results obtained with radio-labelled metals such as ^{65}Zn, ^{109}Cd and ^{57}Co (Bustamante et al. 2002b; 2004), which were associated in more than 96% to the eggshell af ... [2]

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Because of the metal dilution with the somatic growth, it was necessary to consider the tissues separately and particularly the digestive gland as a storage organ of metals. With the first feedings, the immature digestive gland of the juveniles starts to evolve to be fully functional at the age of one month (Yim and Boucaud-Camou, 1980). The food intake provokes the beginning of the digestive processes, i.e. secretion of enzymes, absorption of nutrients, and excretion of digestive residues. In a few weeks, the digestive gland takes a dark colour due to the development of digestive cells called "Boules" cells (e.g. Boucaud-Camou, 1973; Boucaud-Camou et al., 1983; Boucher-Rodoni et al., 1987). The development of the digestive gland in the retention process of metals is very important and is reflected by the increased fraction of the whole body burdens of all metals in this organ (Table 4). This process is particularly significant in the case of Fe, for which the metal content stored in the digestive gland raises respectively, from 6 to about 50% before and after the digestive gland development. After only two months of benthic life, the digestive gland contains between 30 (Pb) and 90 % (Ag) of the total amount of metals (Table 4). These contents are identical to those previously measured in adult cuttlefish collected in the same area (Miramand and Bentley, 1992).

Thus, the digestive gland plays a major role in the metabolism of all metals in cuttlefish, and especially Cd (Figure 1). Indeed, during the period of development of the digestive gland (i.e. the first month of benthic life), Cd concentrations are increased 9 times compared to a factor 2 for the remaining metals. During this period, the digestive gland weight increased 30 times and the Cd amounts in this organ were multiplied by 230, i.e. 3 to 5 times higher than for the other metals (Figure 3). The digestive gland has an primarily role in the accumulation of Cd in cuttlefish, as also reported after both field and experimental studies (Miramand and Bentley, 1992; Bustamante, 1998; Bustamante et al., 2002b). Such a role of the digestive gland can be related both to the very efficient detoxification processes of the metal occurring in this organ (Bustamante et al., 2002a) and to the very high Cd assimilation efficiency (*viz*. 62 % for juvenile cuttlefish). Both processes lead to a strong retention of the metal in the digestive gland, which biological half-life ($T_{b\frac{1}{2}}$) exceeds 8 months in juveniles (Bustamante et al., 2002b).

Influence of age

The process of bioaccumulation of all metals except Ag appears as age dependent in the digestive gland (Figure 1). Indeed, the digestive gland weight is increased by 3,500 between hatching and sexual maturity whereas its metal amounts is increased between 6,000 and 9,000

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for the essential metals (Cu, Fe, Zn), more than 20,000 (Cd and V) and about 70,000 (Co) (Figure 3). Thus, the digestive gland contains after 2 years of life between 15 and 90 µg of Ag, Co, Pb and V, 165 µg of Cd and between 3 and 7 mg of Cu, Fe and Zn (Figure 3). Therefore, the digestive gland bioaccumulate large concentrations of the metals even those not mostly incorporated through the food pathway, such as for Ag. It is therefore noteworthy that different uptake pathway for metals, i.e. via seawater for Ag and via food for Cd, lead to large concentrations of both toxic metals in the digestive gland. Since the beginning of cuttlefish benthic life, the digestive gland shows 5 to 6 times higher Ag and Cd concentrations compared to those measured in the whole organisms (Tables 2 & 3) and already accounts for 50 to 90% of the total amount of these two metals (Table 4).

Consequently to its storage capacities, all the metals concentrations increased following a logarithmic shape in the digestive gland. Ag is the only exception to this pattern (Figure 1) and its concentrations seem strongly influenced by the migration behaviour of the cephalopods. Indeed, after a few months in coastal areas, juveniles migrate to deeper ocean waters and return to coastal ground to mate only the following year (Richard, 1971). The migration to less polluted area will provoke a decrease in metal concentrations when they have a relatively rapid turn over. In cuttlefish, Ag has short biological half-life either following exposure from seawater or from food (less than 2 wks), as a consequence of its high depuration rates (Bustamante et al., 2004). Our results are likely due to the equilibrium established between contamination (when the animals live by the coast) and decontamination (when the animals live in the open sea) episodes. Therefore, both the elevated bioaccumulation of Ag and its weak retention in the digestive gland of cephalopods will allow the use of these molluscs to monitor Ag concentrations in the marine environment.

The decontamination is actually much slower in the case of other metals as Cd or Co for which the biological ½ life measured in adults after incorporation of Cd or Co by the food pathway is largely higher than the lifetime of cuttlefish (Bustamante et al., 2002b; 2004). Owing to this, it is very likely that since the beginning of the migration to the open sea, the digestive gland of adults is strongly decontaminated in Ag and very little in Co and Cd. Since the main incorporation mechanism of V to the marine organisms seems to be the food pathway (Miramand et al., 1981, Miramand and Fowler, 1998), the behaviour of V is similar to Cd and Co and is strongly retained by cuttlefish. Thus, it would be very interesting to confirm experimentally such hypothesis.

In contrast to the digestive gland, the concentrations measured in the cuttlebone of juveniles are very low, with most of the metals showing concentrations close to those observed in

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juvenile just after hatching (Table 2 & 5). The shell in cuttlefish is internal, therefore not exposed to metal adsorption phenomena by direct contact with seawater, unlike other molluscs, especially mussels (Van Weers, 1973; Fowler & Benayoun, 1976; Ünlü & Fowler, 1976; Miramand et al. 1980). As discussed above, albeit the significant penetration of the metals into the tissues, internal transfer of metals to the cuttlebone appear to be very slow and somatic growth of this organ lead to overall dilution of most metals. The only exception was Zn which concentrations significantly increase with age whereas the other analysed elements tended to decrease (Figure 2). Consequently, the metal amounts contained in the cuttlebone correspond in fact either to their weight importance (Cu, Pb, Fe) or are clearly lower (Ag, Cd, V and Zn) (Table 6). This observation is in good agreement with the experimental radiotracer results obtained for Ag, Cd and Zn (Bustamante et al. 2002b, 2004).

Influence of sexual maturation

In two years old individuals (sexually mature), Ag, Cd, Cu and Pb concentrations are lower than those in one-year old animals (immature) against those of Fe, V, and Zn (Table 2). This would be due to physiological changes related to the sexual maturation as well as to the growth of the gonads. These organs exhibit globally low metal levels in cephalopods (Miramand and Bentley, 1992) and contribute to dilute the non-essential metal (Ag, Cd and Pb) concentrations in the whole animals. This is clearly exemplified by the fact that, with the exception of Zn, metal concentrations in the digestive gland do not significantly differ between mature and immature individuals (Table 3). High levels of Zn in the genital tract of the males and females of *Sepia officinalis* (190 ± 22 and 123 ± 3 $\mu g.g^{-1}$ dwt, respectively) have been reported (Miramand and Bentley, 1992), which would explain that Zn concentrations still increased after sexual maturation. This 1.5-fold increase observed in the whole animals is in all likelihood due to a Zn accumulation in the digestive gland. This value represents a very significant concentration in the mature individuals and is 3 times and even 2 times higher than that measured in the juveniles and in the immature individuals, respectively (Table 3). During this period, the cuttlebone also showed a concentration of Zn higher than that measured in the juveniles and in the one-year old immature individuals. This observation could be translated by an increased flux of Zn in the haemolymphe of cuttlefish during this period of sexual maturity. This metal could therefore be partially accumulated by the cuttlebone. In this way, in the animals collected at the end of their sexual maturity the amounts of Zn associated to the cuttlebone (expressed as total Zn % contained in the organisms) are almost 2 times higher compared to the contents calculated in the juveniles and

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in the one-year old immatures. Interestingly enough, this phenomenon seems to be limited to Zn. The concentrations of the remaining 7 metals measured in the cuttlebone and the percentages they represent do not vary during this period (Tables 5 & 6).

Acknowledgements: We are grateful to Professor E. Boucaud-Camou for its fruitful advises on this work. Statistical analysis and writing up of this work was supported by the EC under the CEPHSTOCK project (QLRT-2001-00962).

Supprimé : *Conclusion*

This study on metal bioaccumulation at various stages of the life cycle of *Sepia officinalis* highlights: 1) The selectivity of the cuttlefish eggshell against metal penetration; further studies are needed in order to better understand the processes involved in the specific shield behaviour of the cuttlefish eggshel l. 2) The preferential pathway of uptake of the metals, non essential elements being mainly accumulated through the food pathway and strongly retained in the digestive gland. Ag has a completely different behaviour, with a major incorporation through the dissolved pathway and short time retention in the tissues. Because of that, cuttlefish depurate Ag during the open sea phase of their life cycle. The elevated bioaccumulation of Ag and its weak retention in the digestive gland of cephalopods will allow the use of these molluscs to monitor Ag concentrations in the marine environment.

REFERENCES

Boucaud-Camou E. Etude de l'appareil digestif de *Sepia officinalis* L. (Mollusque Céphalopode). Essai d'analyse expérimentale des phénomènes digestifs. PhD Thesis, University of Caen, France. 1973.

Boucaud-Camou E. Exploitation de la seiche (*Sepia officinalis* L.) en Basse-Normandie. Bulletin de la Société Zoologique de France 1988;113(3):305-310.

Boucaud-Camou E, Boucher-Rodoni R. Feeding and digestion in cephalopods. In: The Mollusca, Vol 5, Part 2. Saleudin ASM, Wilbur KM editors. New York, London: Academic Press, 1983. p.149-87.

Boucaud-Camou E, Boismery J. The migration of the cuttlefish *Sepia officinalis* L. in the English Channel. In: "The Cuttlefish" Act. Proceedings of the first international Symposium on the cuttlefish *Sepia*. Boucaud-Camou E editor. Caen: Centre de Publications de l'Université de Caen; 1991. p. 179-89.

Boucaud-Camou E, Kouéta N, Boismery J, Medhioub A. The sexual cycle of *Sepia officinalis* L. from the bay of Seine. In: "The Cuttlefish" Act. Proceedings of the first international Symposium on the cuttlefish *Sepia*. Boucaud-Camou E editor. Caen: Centre de Publications de l'Université de Caen; 1991. p. 141-51.

Boucher-Rodoni R, Boucaud-Camou E, Mangold K. Feeding and digestion. In: Cephalopod Life Cycles vol II. Comparative reviews. Boyle PR editor. New York, London: Academic press; 1987. p. 85-108.

Bustamante P. 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. PhD thesis, University of La Rochelle, France; 1998.

Bustamante P, Chérel Y, Caurant F, Miramand P. Cadmium, copper and zinc in octopuses from Kerguelen Islands, Southern Indian Ocean. Polar Biology 1998;19:264-271.

Bustamante P, Grigioni S, Boucher-Rodoni R, Caurant F, Miramand P. Bioaccumulation of 12 trace elements in the tissues of the nautilus *Nautilus macromphalus* from New Caledonia. Marine Pollution Bulletin 2000;40:688-696.

Bustamante P, Cosson RP, Gallien I, Caurant F, Miramand P. Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. Marine Environmental Research 2002a;53:227-241.

Bustamante P, Teyssié JL, Fowler SW, Cotret O, Danis B, Miramand P, Warnau M. Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish Sepia officinalis. Marine Ecology Progress Series, 2002b;231:167-177.

Bustamante P, Teyssié JL, Danis B, Fowler SW, Miramand P, Cotret O, Warnau M. Uptake, transfer and distribution of silver and cobalt in tissues of the common cuttlefish *Sepia* officinalis at different stages of its life cycle. Marine Ecology Progress Series 2004;269:185-195.

Calabrese A, Collier RS, Nelson DA, MacInnes JR. The toxicity of heavy metals to embryos of the American oyster *Crassostrea virginica*. Marine Biology 1973;18:162-166.

Castillo LV, Maita Y. Isolation and partial characterisation of cadmium binding proteins from the oceanic squid, *Ommastrephes bartrami.* Bulletin Faculty Fisheries Hokkaido University 1991;42:26-34.

Chiffoleau JF, Cossa D, Auger D, Truquet I. Trace metal distribution, partition and fluxes in the Seine estuary (France) in low discharge regime. Marine Chemistry 1994;47:145-158.

Chiffoleau JF, Michel P, Cossa D, Auger D, Averty B, Chartier E, SanJuan J, Truquet I. Distribution des contaminants métalliques dans l'estuaire de Seine en période de crue (Février 1995). *Rapport 1995/fin-3. Programme Scientifique Seine Aval Thème Chimie des Contaminants,* Rouen; 1996.

Decleir W, Vlaeminck A, Geladi P, Van Grieken R. Determination of protein-bound copper and zinc in some organs of the cuttlefish *Sepia officinalis* L.. Comparative Biochemistry and Physiology 1978.60B:347-350.

Denis V, Robin JP. Present status of the French Atlantic fishery for cuttlefish (*Sepia officinalis*). Fisheries Research 2001;52:11-22.

Finger JM, Smith JD. Molecular association of Cu, Zn, Cd, and ^{210}Po in the digestive gland of the squid *Nototodarus gouldi*. Marine Biology 1987;95:87-91.

Fowler SW, Benayoun G. Accumulation and distribution of selenium in mussel and shrimp tissues. Bulletin of Environmental Contamination and Toxicology 1976;16:339-346.

Forsythe JW, DeRusha RH, Hanlon RT. Growth, reproduction and life of *sepia officinalis* (Cephalopoda: Mollusca) cultured through seven consecutive generations. Journal of Zoology, London 1994;223:175-192.

Legoff R, Daguzan J. Etude des déplacements de la seiche commune *Sepia officinalis* L. dans le Golfe du Morbihan au cours de la période de reproduction : premiers résultats. In: "The Cuttlefish" Act. Proceedings of the first international Symposium on the cuttlefish *Sepia*. Boucaud-Camou E editor. Caen: Centre de Publications de l'Université de Caen; 1991. p. 167-77.

Lemaire J. Etude du développement embryonnaire de *Sepia officinalis* L. PhD Thesis, University of Lille, France; 1971.

Mangold K. Reproduction, croissance et durée de vie. In: Traité de Zoologie, Tome V. Céphalopodes. Grassé PP editor. Paris:Masson; 1989.

Mangold K, Bidder AM. L'appareil digestif et la digestion. In: Traité de Zoologie, Tome V. Céphalopodes. Grassé PP editor. Paris:Masson; 1989.

Martin JH, Flegal AR. High copper concentrations in squid livers in association with elevated levels of silver, cadmium and zinc. Marine Biology 1975;30: 51-55.

Martin M, Osborn KE, Billig P, Glickstein N. Toxicities of ten metals to *Crassostrea gigas* and *Mytilus edulis* embryos and *Cancer magister* larvae. Marine Pollution Bulletin 1981;12:183-188.

Medhioub A. Etude de la croissance et du cycle sexuel de la seiche (*Sepia officinalis*) des côtes normandes. PhD Thesis, University of Caen, France; 1986.

Miramand P, Guary JC. High concentrations of some heavy metals in tissues of the Mediterranean *Octopus*. Bulletin of Environmental Contamination and Toxicology 1980;24:738-788.

Miramand P, Guary JC, Fowler SW. Vanadium transfer in the mussel *Mytilus galloprovincialis*. Marine Biology 1980;56:281-293.

Miramand P, Guary JC, Fowler SW. Uptake, assimilation and excretion of vanadium in the shrimp *Lysmata seticaudata* (Risso) and the crab *Carcinus maenas* (L.). Journal of Experimental Marine Biology and Ecology 1981;49:267-287.

Miramand P, Bentley D. 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 1992;114:407-414.

Miramand P, Guyot T, Rybarczyk H, Elkaïm B, Mouny P, Dauvin JC, Bessineton C. Contamination of the biological compartment in the Seine estuary by Cd, Cu, Pb and Zn. Estuaries 2001;24,6B:1056-1065.

Miramand P, Fowler SW. Bioaccumulation and transfer of vanadium in marine organisms. In: Vanadium in the Environment Part 1: Chemistry and Biochemistry. Nriagu JO editor. John Wiley & Sons New York, Vol. 30 in the Wiley Series in Advances in Environmental Sciences and Technology; 1998. p. 167-97.

Nardi G, Muzii E, Puca M. Ferritin in the hepatopancreas of *Octopus vulgaris* Lam. Comparative Biochemistry and Physiology 1971;48B:199-205.

Pascual E. Crecimiento y alimentación de tres generaciones de *Sepia officinalis* in cultivo. Investigaciones Pesqueras 1978;42:412- 442.

Pauly D, Gaschütz G. A simple method for fitting oscillating growth data, with a program for pocket calculators. Conseil International pour l'Exploration de la Mer 1979;CM/G:24-25.

Richard A. Contribution à l'étude expérimentale de la croissance et de la maturation sexuelle de *Sepia officinalis* L. (Mollusque céphalopode). PhD Thesis, University of Lille, France; 1971.

RNO. Surveillance du milieu marin, travaux du RNO-Edition 2001. L'argent, le cobalt, le nickel et le vanadium dans les mollusques du littoral français. Paris, Nantes: IFREMER et Ministère de l'Environnement; 2001.

Rocca E. Copper distribution in *Octopus vulgaris* Lam. Hepatopancreas. Comparative Biochemistry and Physiology 1969;28:67-82.

Schipp R, Hevert F. Distribution of copper and iron in some central organs of *Sepia officinalis* (Cephalopoda). A comparative study by flameless atomic absorption and electron microscopy. Marine Biology 1978;47:391-399.

Smith JD, Plues L, Heyraud M, Cherry RD. Concentrations of the elements Ag, Al, Ca, Cd, Cu, Fe, Mg, Mn, Pb and Zn and the radionuclides ^{210} Pb and ^{210} Po in the digestive gland of the squid *Nototodarus gouldi*. Marine Environmental Research 1984;13:55-68.

Tanaka T, Hayashi Y, Ishizawa M. 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 (Basel) 1983;39:746-748.

Ünlü MY, Fowler SW. Factors affecting the flux of arsenic trough the mussel *Mytilus galloprovincialis*. Marine Biology 1976;51:209-219.

Van Weers AW. Uptake and loss of ^{65}Zn and ^{60}Co by the mussel *Mytilus edulis* L. In: Radioactive contamination of the marine environment, Vienna: International Atomic Energy Agency; 1973. p. 385-401.

Wolf G, Verheyen E, Vlaeminck A, Lemaire J, Decleir W. Respiration of *Sepia officinalis* during embryonic and early juvenile life. Marine Biology 1985;90:35-39.

Yim M, Boucaud-Camou E. Etude cytologique du développement post-embryonnaire de la glande digestive de *Sepia officinalis* L (Mollusque, Céphalopode). Archives d'Anatomie Microscopique et de Morphologie Expérimentale 1980;69(2):6-79

Table 1 Comparison of elemental concentrations (µg.g^{-1} dry weight) of Orchard-leaves standard, SRM 1571 (National Bureau of Standards), copepods homogenate, MA-A-1 and fish flesh homogenate, MA-A-2 (International Agency of Atomic Energy) obtained in present study with certified values.

Standard	Ag	Cd	Co	Cu	Fe	Pb	V	Zn
Orchard leaves								
Present study	-	0.10 ± 0.05	0.17 ± 0.04	10 ± 1	272 ± 14	38 ± 2	0.5 ± 0.1	22 ± 6
Certified values	-	0.11 ± 0.02	(0.2)	12 ± 1	300 ± 20	45 ± 3	(0.6)	25 ± 3
MA-A-1								
Present study	0.2 ± 0.1	0.73 ± 0.06	0.12 ± 0.02	6.9 ± 0.4	61 ± 4	2.0 ± 0.5	-	161 ± 1
Certified values	0.33 ± 0.06	0.75 ± 0.03	0.12 ± 0.01	7.6 ± 0.2	60 ± 2	2.1 ± 0.3	-	158 ± 2
MA-A-2								
Present study	0.12 ± 0.01	0.07 ± 0.01	0.09 ± 0.04	3.4 ± 0.7	65 ± 5	0.43 ± 0.14	-	35 ± 4
Certified values	0.10± 0.01	0.066 ± 0.004	0.08 ± 0.01	4.0 ± 0.1	54 ± 1	0.58 ± 0.07	-	33 ± 1

() : recommended values

Table 2. Heavy metal concentrations (Mean ± SD; µg g-1 dwt) in whole *Sepia officinalis* sampled in the bay of Seine at different stage of its life cycle. n: number of pool analysed. ( ): number of individuals in each pool. ML: mantle length; NM: not measured.

Life stage Mantle length (mm)	Estimated age	n	Ag	Cd	Co	Cu	Fe	Pb	V	Zn
Eggs		4 (25)								
Eggshell	-	4 (25)	0.07 ± 0.01	0.59 ± 0.13	1.75 ± 0.26	14 ± 2	398 ± 64	1.38 ± 0.35	3.43 ± 0.59	69 ± 2
Embryos	-	4 (25)	0.16 ± 0.04	0.76 ± 0.17	0.25 ± 0.05	31 ± 1	14 ± 8	< 0.10	< 0.10	68 ± 3
Yolk	-	1 (25)	0.22	0.40	NM	19	11	< 0.10	< 0.10	50
Hatchlings										
ML = 10 mm	0 days	1 (25)	1.80	0.50	NM	60	80	< 0.10	< 0.10	100
Juveniles										
ML = 10 ± 1 mm	7 days	3 (25)	1.82 ± 0.35	0.44 ± 0.14	NM	63 ± 2	107 ± 16	< 0.10	< 0.10	100 ± 3
ML = 18 ± 3 mm	15 days	4 (8)	2.40 ± 0.63	1.32 ± 0.15	NM	77 ± 6	196 ± 51	< 0.10	< 0.10	119 ± 5
ML = 33 ± 4 mm	1 month	3 (4)	1.42 ± 0.20	1.25 ± 0 .31	NM	73 ± 3	38 ± 8	< 0.10	< 0.10	113 ± 4
ML = 59 ± 6 mm	2 months	4 (4)	3.04 ± 0.29	0.71 ± 0.09	NM	67 ± 4	23 ± 6	< 0.10	0.6 ± 0.14	113 ± 3
Immature adults										
ML = 133 ± 19 mm	12 months	3 (5)	1.82 ± 0.49	2.03 ± 0.07	NM	104 ± 3	41 ± 9	0.65 ± 0.05	0.47 ± 0.15	145 ± 7
Mature adults										
ML = 215 ± 5 mm	18 months	3 (1)	0.60 ± 0.10	1.10 ± 0.10	NM	70 ± 1	30 ± 5	0.20 ± 0.10	0.50 ± 0.20	156 ± 6

Table 3: Heavy metal concentrations (Mean ± SD; µg g^{-1} dry wt) in the digestive gland of *Sepia officinalis* sampled in the bay of Seine at different stage of its life cycle. n: number of pool analysed. ( ): number of individuals in each pool. ML: mantle length.

Mantle length (mm)	Age (estimation)	n	Ag	Cd	Co	Cu	Fe	Pb	V	Zn
Juveniles										
10 ± 1	7 days	3 (25)	10 ± 1	2.0 ± 0.1	0.3 ±0.1	175 ± 5	90 ± 10	< 0.1	0.3 ± 0.2	240 ± 5
18 ± 3	15 days	4 (8)	18 ± 7	11 ± 1	1.3 ± 0.3	400 ± 10	160 ± 10	< 0.1	0.8 ± 0.1	400 ± 10
33 ± 4	1 month	3 (4)	14 ± 1	15 ± 3	2.6 ± 0.5	450 ± 10	210 ± 10	0.5 ± 0.2	1.6 ± 0.4	500 ± 10
59 ± 6	2 months	4 (4)	35 ± 7	7.5 ± 0.2	3.5 ± 0.5	450 ± 20	110 ± 10	0.5 ± 0.2	2.5 ± 0.7	400 ± 10
Immature										
133 ± 19	12 months	3 (5)	19 ± 7	21.6 ± 1.5	6.8 ± 0.9	760 ± 80	340 ± 30	2.4 ± 0.5	2.9 ± 0.1	770 ± 40
Mature adults										
215 ± 5	18 monthss	3 (1)	13 ± 2	25 ± 5	10 ± 2	600 ± 10	390 ± 10	2.2 ± 0.5	3.3 ± 0.1	1400 ± 500

Table 4. Percentage distribution (Mean ± SD; %) of heavy metals in the digestive gland of *Sepia officinalis* sampled in the bay of Seine at different stage of its life cycle. n: number of pool analysed. ( ): number of individuals in each pool.

Life stage Mantle length (mm)	Estimated age	n	Proportion of whole wt (%)	Ag	Cd	Cu	Fe	Pb	V	Zn
Juveniles										
ML = 10 ± 1 mm	7 days	3 (25)	10 ± 2	55 ± 7	49 ± 4	29 ± 6	9 ± 3	< 10	18 ± 10	20 ± 3
ML = 18 ± 3 mm	15 days	4 (8)	8 ± 1	61 ± 8	72 ± 2	43 ± 2	7 ± 2	< 10	17 ± 5	28 ± 1
ML = 33 ± 4 mm	1 month	3 (4)	7 ± 1	76 ± 3	88 ± 5	48 ± 1	48 ± 5	< 10	49 ± 18	34 ± 2
ML = 59 ± 6 mm	2 months	4 (4)	8 ± 1	93 ± 1	82 ± 5	57 ± 4	46 ± 3	28 ± 2	38 ± 19	34 ± 1
Immature adults										
ML = 133 ± 19 mm	12 months	3 (5)	8 ± 1	80 ± 9	87 ± 6	59 ± 6	68 ± 10	30 ± 7	54 ± 20	43± 1
Mature adults										
ML = 215 ± 5 mm	18 months	3 (1)	3 ± 1	54 ± 16	75 ± 5	42 ± 8	48 ± 5	31 ± 14	20 ± 15	40 ± 2

Table 5: Heavy metal concentrations (Mean ± SD; µg g^{-1} dry wt) in the cuttlebone of *Sepia officinalis* sampled in the bay of Seine at different stage of its life cycle. n: number of pool analysed. ( ): number of individuals in each pool.

Mantle length (mm)	Age (estimation)	n	Ag	Cd	Cu	Fe	Pb	V	Zn
Hatching		1 (25)	0.03 ± 0.10	0.08 ± 0.02	38 ± 6	21± 2	0.6 ± 0.3	< 0.5	30 ± 2
Juveniles									
10 ± 1	7 days	3 (25)	0.7 ± 0.2	0.1 ± 0.1	25 ± 6	28 ± 3	< 0.1	< 0.5	75 ± 5
18 ± 3	15 days	4 (8)	0.7± 0.2	0.13± 0 .02	14 ± 4	25 ± 1	< 0.1	< 0.5	75 ± 5
33 ± 4	1 month	3 (4)	0.10± 0.02	0.06± 0.01	12± 8	28 ± 1	< 0.1	< 0.5	75 ± 5
59 ± 6	2 months	4 (4)	0.10± 0.02	0.08± 0.01	9 ± 1	23 ± 3	< 0.1	< 0.5	80 ± 5
Immature									
133 ± 19	12 months	3 (5)	0.08± 0.02	0.22± 0.09	8 ± 2	15 ± 3	0.9 ± 0.1	< 0.5	116 ± 7
Mature adults									
215 ± 5	18 monthss	3 (1)	0.08 ± 0.02	0.03± 0.01	5 ± 1	25 ± 2	0.3 ± 0.2	< 0.5	150 ± 20

Table 6. Percentage distribution (Mean ± SD; %) of heavy metals in the cuttlebone of *Sepia officinalis* sampled in the bay of Seine at different stage of its life cycle. n: number of pool analysed. ( ): number of individuals in each pool.

Mantle length (mm)	Estimated age	n	Proportion of whole wt (%)	Ag	Cd	Cu	Fe	Pb	V	Zn
Hatching		1 (25)	13 ± 8	4.0 ± 0.1	1.3 ± 0.4	14 ± 7	24 ± 16	14 ± 3	< 10	8 ± 4
Juveniles										
10 ± 1	7 days	3 (25)	8 ± 1	3.1 ± 0.6	1.9 ± 0.4	3.2 ± 0.9	1.6 ± 1.2	< 10	< 10	5.7 ± 0.6
18 ± 3	15 days	4 (8)	8 ± 1	2.8 ± 1.6	0.8 ± 0.1	1.7 ± 0.3	1.2 ± 0.3	< 10	< 10	5.5 ± 0.6
33 ± 4	1 month	3 (4)	8 ± 1	0.9 ± 0.6	0.5 ± 0.3	1.8 ± 1.2	6.7 ± 1.5	< 10	< 10	7.3 ± 1.2
59 ± 6	2 months	4 (4)	8 ± 1	0.4 ± 0.3	1.6 ± 0.2	1.5 ± 0.6	13 ± 3.8	< 10	< 10	10 ± 1.4
Immature										
133 ± 19	12 months	3 (5)	14 ± 1	0.7 ± 0.4	1.5 ± 0.6	1.0 ± 0.3	5.4 ± 1.6	<10	< 10	11 ± 1.0
Mature adults										
215 ± 5	18 months	3 (1)	20 ± 2	2.2 ± 0.1	0.7 ± 0.1	1.3 ± 1.0	16 ± 4.0	<10	< 10	19 ± 4.0

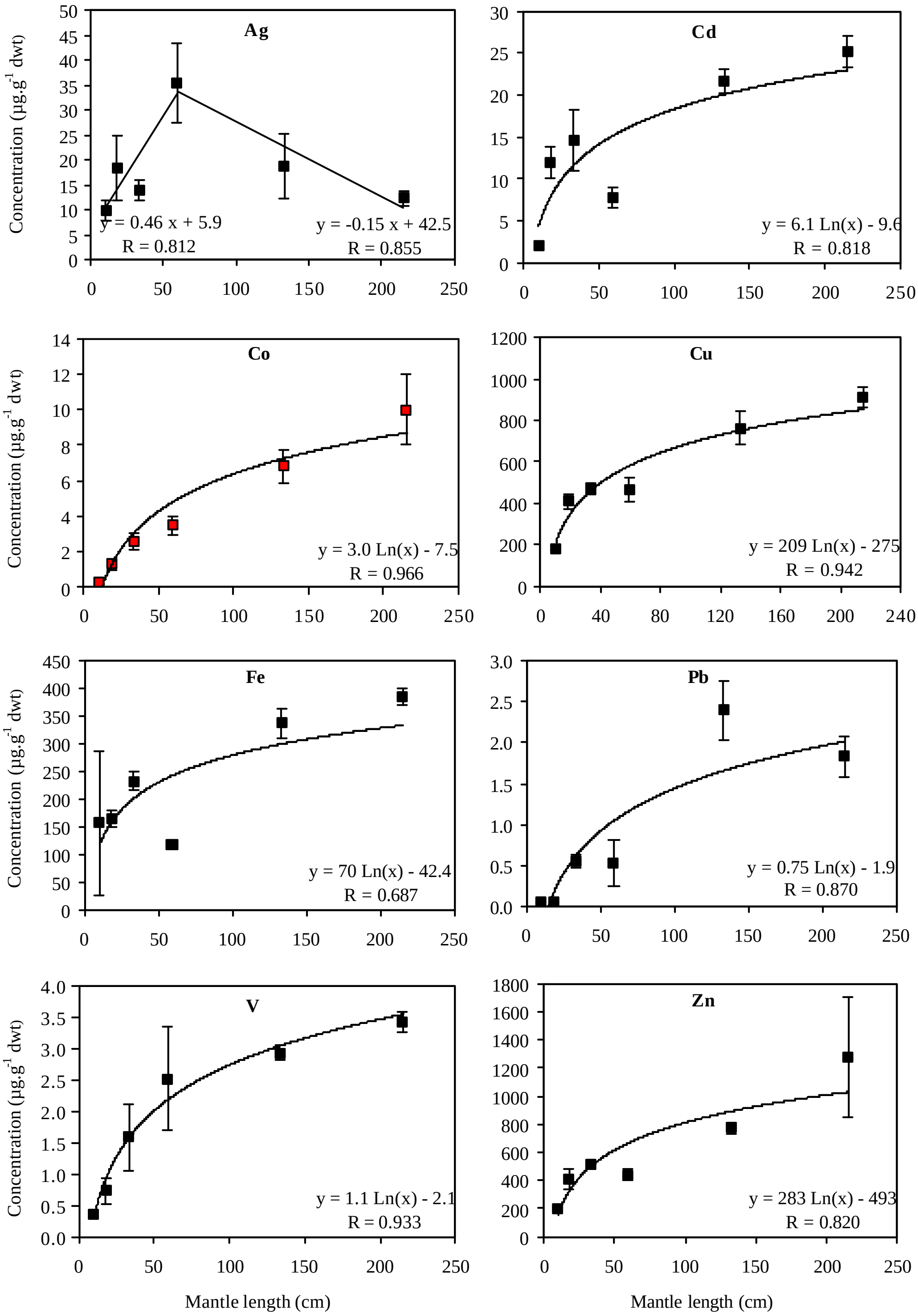

Figure 1. Variations of metal concentrations (µg g^{-1} dry wt) in the digestive gland of *Sepia officinalis* in relation with the mantle length.

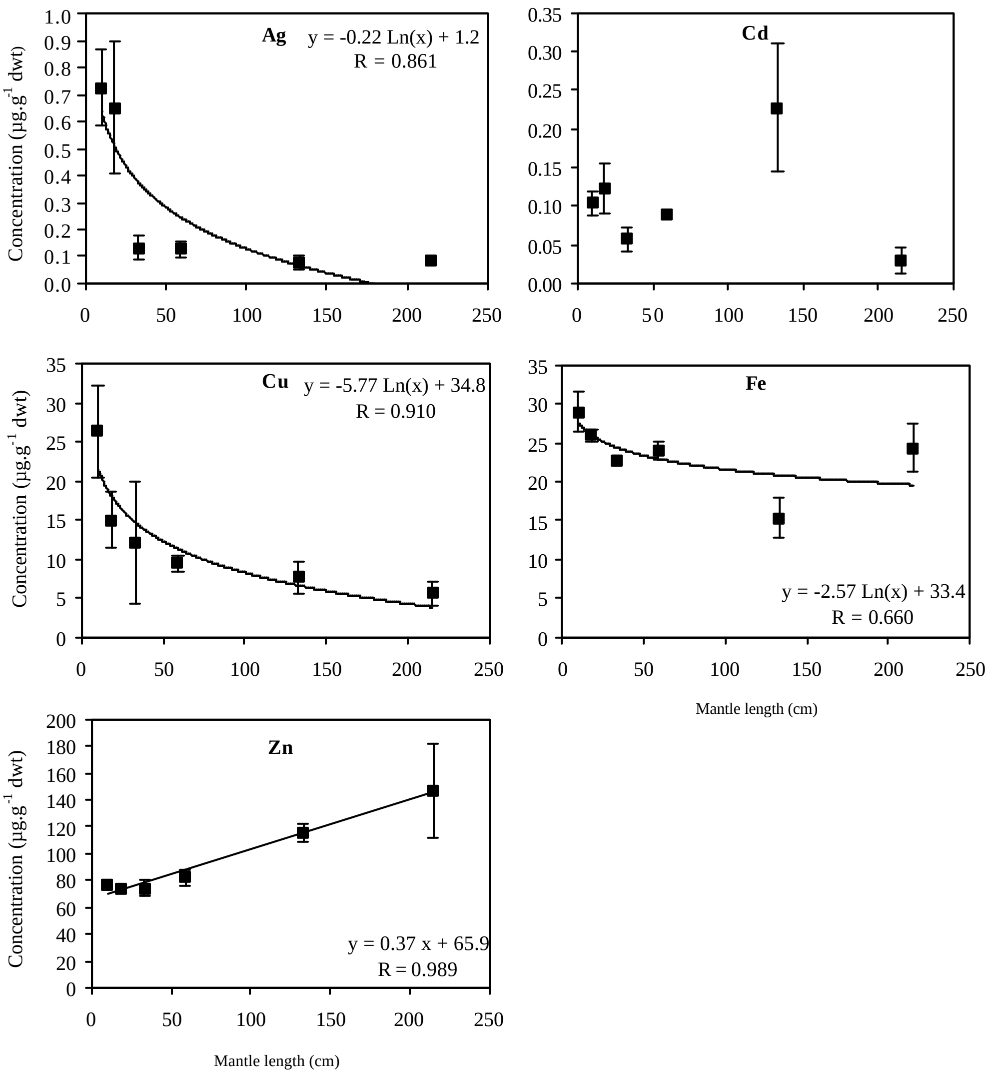

Figure 2. Variations of metal concentrations (µg g^{-1} dry wt) in the cuttlebone of *Sepia officinalis* in relation with the mantle length.

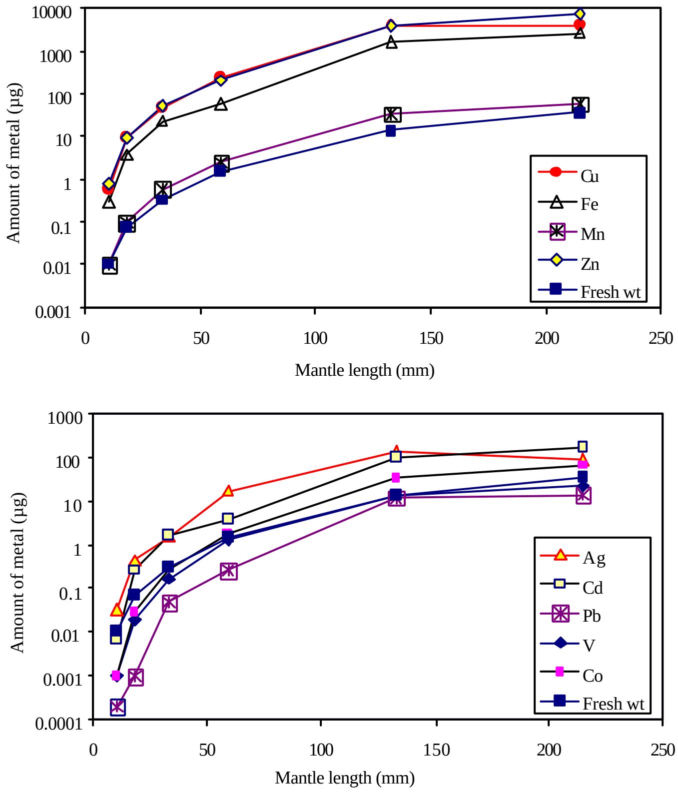

Figure 3. Amounts of metal (µg) contained in the digestive gland of *Sepia officinalis* in relation with the mantle length during the life cycle in the Bay of Seine

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Various studies have shown the ability of cephalopods to concentrate large amounts of trace elements within their tissues. Among cephalopod tissues, the digestive gland has been previously shown to have a key function in metal storage and detoxification. Up to date these processes have been mainly studied for Cd, which has been shown to be mainly associated to cytolosic compounds in the digestive gland of these cephalopods (Bustamante et al. 2002a). Therefore, a significant fraction of Cd could be likely detoxificated by association with cytolosic proteins of the same type of metallothioneins as reported for different squid species (Tanaka et al. 1983; Finger & Smith, 1987; Castillo & Maita, 1991). Nevertheless, there might be other detoxification processes of this metal in cephalopods as the ratio of Cd associated to cytolosic compounds decreases when the concentration of total Cd increases in the digestive gland (Bustamante et al. 2002a). Despite an important number of studies (Rocca, 1969; Martin & Flegal, 1975; Tanaka et al. 1983, Smith et al. 1984; Finger & Smith, 1987; Bustamante et al. 1998; 2002ab, 2004), such processes are still not well understood for Cd whereas some other elements as Pb and V have not yet been studied.

Furthermore, the dynamic of the bioaccumulation processes of metals during the whole-life cycle of these molluscs have never been investigated. In that aim, cuttlefish was chosen as a cephalopod model and accumulation of metals has been studied at different stage of its life cycle, from the embryonic development in the eggs to the mature pre-spawning adults. However, the behaviour of metals in cuttlefish individuals from the field was rather complex to explain probably because of its way of life with coast to open sea migrations which implies transitions between contaminated and clean environments. After this study, experimental investigations using radiotracers have been done meanwhile to provide and validate several hypothesis of this work (Bustamante et al 2002b, 2004).

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The concentrations of toxic metals within the eggs remained very low until the end of the embryogenesis and were in the same range than in the vitellus of early spawned eggs. It

Therefore, eggshell seems to constitute a protective barrier limiting or hindering the incorporation of waterborne metals into the eggs during the development of the embryo. This barrier seems to be particularly effective in the case of Pb and V since the concentrations measured in the embryos were very weak, respectively under 0.1 and 0.5 µg g^{-1} dry wt, which corresponds to the detection limit of the analytical technique. The high concentrations of Pb and V but also of Fe and Co in the eggshell suggest that these metals are adsorbed at the surface but do not penetrate into the egg. This observation is in good agreement with the results obtained with radio-labelled metals such as ^{65}Zn, ^{109}Cd and ^{57}Co (Bustamante et al. 2002b; 2004), which were associated in more than 96% to the eggshell after 11 days of exposure to these radio-isotopes. This mechanism would allow limiting the access of potentially toxic elements into the eggs during the development of the embryos. However, such a protective effect could be limited for some metals as it is suggested by the higher Ag and Cu concentrations in embryos compared to those in the eggshell (Table 2).

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Previous experimental investigations with ^{110m}Ag have shown a completely different behaviour for this metal, more than 40% being associated to the embryos after 11 d of exposure (Bustamante et al. 2004). Therefore, it is likely that Ag can permeate the eggshell, which could have a major importance in contaminated coastal environment such as the bay of Seine. Indeed, this area constitutes an important laying ground for common cuttlefish in the English Channel and is subjected to high Ag arrivals by the Seine River, as shown by the measurements carried out in mussels by the National Observation Network, implemented in France by IFREMER (RNO, 2001). The strong toxicity of Ag with respect to the larvae of marine invertebrates is well known (e.g. Calabrese et al. 1973, Martin et al. 1981). Silver incorporation into the eggs during the incubation could potentially lead to toxic or teratogenic effects on the embryos, potentially influencing the recruitment and ultimately, which could be reflected on the number of captures of cuttlefish in this area (Boucaud-Camou, 1988; Denis & Robin, 2001). Therefore, further investigation on the permeation of the eggshell to Ag and on its likely effects in the embryogenesis of cuttlefish should be carried out.

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. This agrees with the experimental results for ^{65}Zn showing elevated concentration factors of juveniles after 36h exposure to

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by the juveniles highlights the major importance of the dissolved pathway in this process and confirms the outstanding rate of bioaccumulation of this metal by the juveniles, which displayed very high CF

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a short exposure period in radiolabelled ^{110m}Ag seawater

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On the contrary, the concentrations of Cd, Pb and V do not change in the whole organisms at this stage, Pb and V concentrations remaining below the detection limit (Table 2). The incorporation of these metals from seawater seems then to be limited in cuttlefish, which is also consistent with previous results for cephalopods for Cd (Bustamante et al. 2002) or for other marine invertebrates for V (Miramand et al. 1980, 1981, 1998).

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, suggesting that food is not the major pathway of uptake for the juveniles

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arising either from the seawater or from the feeding regime.