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**Uptake, transfer and distribution of silver and cobalt in tissues of the common cuttlefish *Sepia officinalis* at different stages of its life cycle**

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**ABSTRACT:** Three pathways of exposure (sediment, seawater and food) were examined to determine transfer of  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  in juvenile cuttlefish *Sepia officinalis*. Additional experiments were conducted on adult cuttlefish and their eggs/embryos in order to assess bioaccumulation patterns at different stages of the organism's life cycle. Eggs, juveniles and adults readily accumulated both Ag and Co from seawater. In eggs, both metals were predominantly adsorbed onto the capsule membrane ( $\geq 60\%$  for Ag and  $\geq 99\%$  for Co), indicating that the latter may act as an effective shield to limit exposure of embryos to soluble metals. Adult cuttlefish incorporated waterborne radiotracers mainly in their muscular tissues ( $\geq 60\%$  of the whole-body burden); subsequent metal retention was greater for Co (biological half-life,  $T_{b/2} = 34$  d) than for Ag ( $T_{b/2} = 7$  d). Turnover of Co ingested with food was much more rapid in juveniles ( $T_{b/2} = 5$  d) than in adults ( $T_{b/2} = 990$  d), suggesting that the functional maturation of the digestive gland was not complete in the juveniles. With ingested Ag, retention was roughly similar for juveniles and adults ( $T_{b/2} = 13$  and 9 d, respectively). Transfer from sediments was negligible for Co and Ag. Regardless of the exposure pathway, the digestive gland of juveniles and adults contained the major fraction of incorporated metal either following uptake or after depuration. This observation demonstrates that substantial metal transfer takes place from several organs to the digestive gland, and further highlights the major role this organ plays in metal storage and detoxification processes in these cephalopods.

**KEY WORDS:** Metal; Radiotracer; Bioaccumulation; Digestive gland; Cephalopods

## INTRODUCTION

Interest in the bioaccumulation of trace elements in cephalopods stems mainly from their high trophic position in the marine food web, and from their role as important prey for marine mammals, seabirds and fish (Clarke 1996, Croxall & Prince 1996, Klages 1996, Smale 1996). Furthermore, heavy metal levels in cephalopods, which are extensively fished and consumed by humans, are also of direct concern to public health. Various studies have investigated the concentrations and tissue distribution of several trace metals in cephalopods (e.g. Martin & Flegal 1975, Miramand & Guary 1980, Miramand & Bentley 1992, Bustamante et al. 2000, Ishihashi et al. 2001); however, most have been focused on essential trace elements or specific organs such as the digestive gland, the branchial hearts or the gills (see Miramand & Bentley 1992). Owing to the high feeding rates characteristic of cephalopods, food has often been cited as the main uptake pathway for explaining the high concentrations of metals found in cephalopod tissues (e.g. Martin & Flegal 1975, Bustamante et al. 1998, Gerpe et al. 2000). However, there are very few published studies that determine the assimilation efficiencies and depuration rate constants of trace metals in cephalopods (Suzuki et al. 1978, Guary & Fowler 1982, Ueda et al. 1985, Bustamante et al. 2002a). These parameters are necessary in order to assess the degree of retention of waterborne and dietary metals and to assess the relative importance of both pathways. In addition, benthic cephalopods live in direct contact with the substratum, and many swimming species such as cuttlefish spend long periods buried into sediment (Mangold 1989). Therefore sediments should also be considered as a possible pathway for metal transfer to cephalopods.

Ag and Co have been reported to be concentrated, sometimes to a very large extent, in cephalopods (Martin & Flegal 1975, Ueda et al. 1979); however, little is known about the metabolism of these 2 heavy metals. The digestive gland appears to contain most of the body

burden of Ag and Co in squid (Ishihashi et al. 2001), in octopus and cuttlefish (Miramand & Bentley 1992) and in nautilus (Bustamante et al. 2000). A large proportion (>60%) of the Ag in digestive gland is located in the cytosolic fraction of the cells (Tanaka et al. 1983). To the best of our knowledge, no data are available in the literature regarding pathway-specific bioaccumulation (viz., water vs food bioaccumulation) of Ag and Co in these organisms. Therefore, the present work has focused on uptake and loss kinetics of Ag and Co in a cephalopod exposed via different routes in order to better characterise their bioaccumulation rates, the subsequent tissue distribution and their retention times depending on the uptake pathway. The common cuttlefish *Sepia officinalis* was selected as an experimental model, and contamination via seawater, food and sediment was studied in eggs/embryos, juvenile and adult cuttlefish.

## MATERIAL AND METHODS

Experimental organisms. Eggs of the common cuttlefish *Sepia officinalis* L. were obtained from cultured adults and maintained in an aquarium with flowing seawater until hatching. These newly hatched juveniles (n = 25;  $0.387 \pm 0.071$  g wet wt) were then used in the experiments. Adult cuttlefish (n = 18;  $138 \pm 40$  g wet wt) were reared in the Oceanographic Museum (Principality of Monaco) from hatching to 1 yr old, or collected by net fishing off Monaco (n = 5;  $253 \pm 97$  g wet wt). Prior to any experimentation, adults were anaesthetised in seawater containing 2% ethanol for making biometric measurements, sex determination and for the insertion of a numbered plastic tag into the mantle fin to identify each animal during the experiments.

**Radiotracers.** Radiotracers  $^{110m}\text{Ag}$  [ $t_{1/2} = 250.4$  d] and  $^{57}\text{Co}$  [ $t_{1/2} = 271.8$  d] were purchased from Amersham as nitrate and chloride salts, respectively. Stock solutions were prepared in

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their respective acids (0.1 N) to obtain radioactivities which would allow the use of spikes measuring only a few  $\mu\text{l}$  (typically 10 to 20  $\mu\text{l}$ ).

**Ag and Co uptake in *Sepia officinalis* exposed via sediments.** Sediments (2.5 kg dry wt) from the North Sea (Audresselles, Pas-de-Calais, France) were spiked for 4 d with  $^{110\text{m}}\text{Ag}$  and  $^{57}\text{Co}$  using the rolling jar method (Murdoch et al. 1997). Before initiating the experiment, radiolabelled sediments were held in flowing seawater overnight in order to leach weakly bound radiotracer. Sediments (50 g wet wt) were sampled at fixed intervals during the experiment to check for possible variations in radiotracer concentrations. Juvenile cuttlefish ( $n = 9$ ) were exposed for 29 d in a 20 l plastic aquarium (constantly aerated open circuit aquarium; salinity: 36 psu; temperature:  $16.5 \pm 0.5^\circ\text{C}$ ; 12:12 h dark:light cycle) containing ca. 3 l of natural seawater running over a 4 cm layer of spiked sediments. The depth of seawater was maintained at a low level in order to minimise the movements required for feeding, and to maximise the time of contact with sediments. During the experiment, all juvenile cuttlefish were fed twice daily with *Artemia salina* and were periodically  $\gamma$ -counted to follow the radiotracer uptake kinetics over the 29 d. At the end of the uptake experiment, 3 individuals were dissected to determine the distribution of the radiotracers among digestive gland, cuttlebone and remainder (rest of the organs).

**Ag and Co uptake in *Sepia officinalis* exposed via seawater and subsequent loss.**

**Eggs/embryos:** Cuttlefish eggs were placed in a 70 l glass aquarium containing natural filtered (0.45  $\mu\text{m}$ ) seawater spiked with 6 kBq  $^{110\text{m}}\text{Ag l}^{-1}$  (57 pM) and 6 kBq  $^{57}\text{Co l}^{-1}$  (0.68 pM) for 11 d (constantly aerated closed circuit; salinity: 36 psu; temperature:  $16.5 \pm 0.5^\circ\text{C}$ ; 12:12 h light:dark cycle). Radiotracers and seawater were renewed daily to maintain seawater quality and radiotracer concentrations as constant as possible. Radiotracer activities in seawater were checked daily (before and after seawater renewals). During the experiment,

$^{110m}\text{Ag}$  and  $^{57}\text{Co}$  radioactivities were measured in the eggs on Days 1, 2, 3, 6, 9, and 11. At each time, 3 eggs were dissected to determine the distribution of the radiotracers among the capsule membrane, peri-embryonary liquid and embryo.

**Juveniles and adults:** juvenile ( $n = 8$ ) and adult ( $n = 5$ ) cuttlefish were placed for 36 and 8 h, respectively, in 70 l glass aquaria containing seawater spiked with  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  ( $6 \text{ kBq l}^{-1}$  each). Cuttlefish were then radioanalyzed and transferred to another 70 l aquarium supplied with natural flowing seawater (open circuit with constant aeration; seawater renewal:  $20 \text{ l h}^{-1}$ ; salinity: 36 psu; temperature:  $16.5 \pm 0.5^\circ\text{C}$ ; 12:12 h light:dark cycle). Juvenile cuttlefish were fed *Artemia salina* twice daily and were periodically  $\gamma$ -counted to follow radiotracer loss kinetics over 29 d. At the end of the loss period, 4 juveniles were dissected to determine the radiotracer distribution among digestive gland, cuttlebone and remainder.

Adults were fed daily with soft parts of the mussel *Mytilus galloprovincialis*. Three adults were dissected after 8 h and the 2 remaining ones were dissected after 6 d depuration. For each individual, the branchial heart appendages, branchial hearts, gills, digestive tract (after removal of the gut contents), genital tract, ovary or testes, ink sac, digestive gland, kidneys, mantle skin, mantle muscle, head and cuttlebone were separated, weighed, and their radiotracer content measured.

**Ag and Co bioaccumulation in *Sepia officinalis* from food.** Different prey were selected to match as closely as possible to the size of the natural food of juveniles and adult cuttlefish (Mangold 1989). To prepare radiolabelled food, mussels *Mytilus galloprovincialis* and brine shrimp *Artemia salina* were exposed for 7 d in plastic aquaria containing 4 l of natural seawater spiked with  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  ( $6 \text{ kBq l}^{-1}$  each). Radiolabelled seawater was renewed daily and the organisms were subsequently used as food for juvenile (brine shrimp) and adult (mussels) cuttlefish.

Juvenile cuttlefish ( $n = 8$ ) were held in a 70 l glass aquarium (open circuit with constant aeration; seawater renewal:  $20 \text{ l h}^{-1}$ ; salinity: 36 psu; temperature:  $16.5 \pm 0.5^\circ\text{C}$ ; 12:12 h light:dark cycle). For identification purposes, each individual was enclosed in a separate compartment allowing free circulation of seawater. After 1 h of ingesting radiolabelled brine shrimp, each individual was immediately  $\gamma$ -counted. From that time on, cuttlefish were fed twice daily with non-contaminated *Artemia salina* and regularly  $\gamma$ -counted to determine radiotracer loss kinetics and assimilation efficiency. Throughout the depuration period (29 d), faeces were removed 3 times per day to reduce possible indirect contamination by radiotracer recycling through leaching from the faeces. At the end of the depuration period, 5 juveniles were dissected to determine the radiotracer distribution in their tissues.

Adult cuttlefish ( $n = 18$ ) were held in a 3000 l aquarium (open circuit with constant aeration; seawater renewal:  $300 \text{ l h}^{-1}$ ; salinity: 36 psu; temperature:  $16.5 \pm 0.5^\circ\text{C}$ ; 12:12 h light/dark cycle) and fed soft parts of the previously labelled mussels for 2 h. Immediately after ingestion, each individual was  $\gamma$ -counted and the same procedure was followed as for the juveniles. In addition, 3 adult cuttlefish were dissected at each counting time to determine the radiotracer distribution among their organs and tissues.

**Radioanalyses.** Radioactivities of the tracers were measured using a high-resolution  $\gamma$ -spectrometry system consisting of coaxial Ge (N- or P-type) detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyser and a computer with spectra analysis software (Interwinner). The detectors were calibrated with appropriated standards for each of the counting geometries used and measurements were corrected for background and physical decay of the radiotracers. Counting times were adapted to obtain relative propagated errors less than 5%. However, in a few cases, this counting precision could not be obtained even after 48 h of counting, due to the very low activity in extremely small organs. Counting times



ranged from 10 min to 1 h for whole cuttlefish, mussel and brine shrimp, and from 10 min to 48 h for the dissected organs and tissues.

**Data and statistical analyses.** Uptake of  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  from seawater and sediments was expressed, respectively, as change in whole-body concentration factors (CF) and transfer factors (TF) over time ( $\text{Bq g}^{-1}$  wet wt organism divided by the time-integrated  $\text{Bq g}^{-1}$  in seawater, CF, or sediments, TF). Uptake kinetics in the eggs were described using a single-component first-order kinetic model (eq. 1):

$$\text{CF}_t = \text{CF}_{\text{ss}} (1 - e^{-k t}) \quad (1)$$

where  $\text{CF}_t$  and  $\text{CF}_{\text{ss}}$  are concentration factors at time  $t$  (d) and steady state, respectively, and  $k$  is the depuration rate constant ( $\text{d}^{-1}$ ) (Whicker & Schultz 1982).

Radiotracer loss was expressed in terms of percentage of remaining radioactivity over time, i.e. radioactivity at time  $t$  divided by initial radioactivity measured in the organisms at the beginning of the depuration period. Loss kinetics were described either by a single-component exponential model (Eq. 2) or by a 2-component exponential model (Eq. 3):

$$A_t = A_0 e^{-k t} \quad (2)$$

$$A_t = A_{0s} e^{-k_s t} + A_{0l} e^{-k_l t} \quad (3)$$

where  $A_t$  and  $A_0$  are the remaining activities (%) at time  $t$  (d) and 0, respectively; the 's' subscript refers to a short-lived component (s component) and the 'l' subscript refers to a long-lived component (l component) (Hubbell et al. 1965, Whicker & Schultz 1982). The exponential model showing the best fit (based on calculation of the determination coefficients,  $R^2$ , and examination of the residuals) was selected. In the case of radiotracer loss following ingestion of spiked food, the long-lived 'l' component is a model of the loss of the radiotracer fraction that is actually assimilated by the organism. Thus, the constant  $A_{0l}$  is an estimate of

the fraction of radioactivity (or metal) assimilated from food, i.e. the assimilation efficiency (AE) (Hubbell et al. 1965, Reinfelder et al. 1998).

Depuration rate constant  $k$  allows the calculation of the radiotracer biological half-life ( $d$ ) using the following equation:

$$T_{b/2} = \ln 2/k \quad (4).$$

Constants of the models and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation, respectively, using the non-linear curve-fitting routines in the Statistica 5.1 Software. Changes in radiotracer distribution among cuttlefish tissues were tested for significance by the  $G$ -procedure (adapted from the log-likelihood ratio test) for  $2 \times k$  contingency tables (Zar 1996). Changes in % of radioactivity in a single tissue during the depuration period were tested by 1-way ANOVA (after arcsin-transformation of data) followed by the Tukey's multiple comparison test. The significance level for statistical analyses was always set at  $\alpha = 0.05$ .

## RESULTS

### Sediment exposure

Regular monitoring of radiotracer activities in sediment showed no significant variation during the experimental time course; time-integrated radioactivities were  $14.7 \pm 1.2$  Bq g wet wt<sup>-1</sup> for <sup>110m</sup>Ag and  $45.5 \pm 3.4$  Bq g wet wt<sup>-1</sup> for <sup>57</sup>Co. Uptake of <sup>110m</sup>Ag in juveniles cuttlefish displayed linear kinetics during the experiment period, with TF reaching 1 after 29 d of exposure (**Fig. 1A**). In contrast, very low <sup>57</sup>Co activities were recorded in the same individuals, with maximum TF values of 0.013 (Fig. 1B). Dissection of 3 individuals after 29 d of exposure showed that, for both metals, the digestive gland contained the highest proportion of the whole body burden, i.e.  $57 \pm 2\%$  of <sup>110m</sup>Ag and  $79 \pm 0.3\%$  of <sup>57</sup>Co (**Table 1**).

### Seawater exposure

Regular monitoring of radiotracer activities in seawater allowed calculation of time-integrated radioactivities, viz.  $8.3 \pm 0.9$  and  $5.8 \pm 0.3$  kBq l<sup>-1</sup> for <sup>110m</sup>Ag and <sup>57</sup>Co, respectively.

#### Eggs

Despite the relatively short experimental exposure (11 d), the uptake of both radiotracers clearly displayed saturation kinetics (**Fig. 2**). Estimated steady-state concentration factors (CF<sub>ss</sub>) in the eggs were  $28.6 \pm 1.5$  for <sup>110m</sup>Ag and  $33.1 \pm 1.7$  for <sup>57</sup>Co. During the experiment, the greatest proportion of both radiotracers was associated with the egg capsule membrane, viz. 57 to 84% for <sup>110m</sup>Ag and  $\geq 99\%$  for <sup>57</sup>Co (**Fig. 3**).

#### Juveniles

The activities measured in juveniles after 36 h of exposure via seawater were  $2660 \pm 1040$  and  $131 \pm 24$  Bq g wet wt<sup>-1</sup> for <sup>110m</sup>Ag and <sup>57</sup>Co, respectively. Mean calculated CF were elevated, viz.  $320 \pm 125$  and  $23 \pm 4$  for <sup>110m</sup>Ag and <sup>57</sup>Co, respectively. Following transfer to non-contaminated seawater, loss kinetics of both radiotracers in juvenile cuttlefish were best described by a single-component model (**Fig. 4A,B Table 2**). Loss kinetics were characterised by  $T_{b/2}$  of 1 wk for <sup>110m</sup>Ag and 5 wk for <sup>57</sup>Co. At the end of the depuration period, both radiotracers were mainly associated with the digestive gland of the young cuttlefish (69 to 78% of whole-body activity), while the lowest fraction was deposited in the cuttlebone (<1% of the total activity) (Table 1).

#### Adults

<sup>110m</sup>Ag and <sup>57</sup>Co activities recorded in the different organs and tissues of adult cuttlefish after 8 h of exposure and corresponding CFs are presented in **Table 3**. The highest activities of <sup>110m</sup>Ag were found in the gills ( $1253 \pm 465$  Bq g<sup>-1</sup> wet wt) followed by branchial hearts and skin ( $631 \pm 398$  and  $335 \pm 135$  Bq g wet wt<sup>-1</sup>, respectively). In the case of <sup>57</sup>Co, the digestive

gland contained the highest activities, followed by branchial hearts and kidney ( $86 \pm 77$ ,  $70 \pm 12$ , and  $66 \pm 30$  Bq g wet wt<sup>-1</sup>, respectively). When considering the tissue distribution of the radiotracer activities, muscular tissues of adults (i.e. sum of the mantle muscles, skin and head) were found to contain the highest proportion of <sup>110m</sup>Ag and <sup>57</sup>Co, 66 and 60%, respectively (Table 3). The second highest fraction was found in the gills (25% for <sup>110m</sup>Ag) and in the digestive gland (20% for <sup>57</sup>Co).

The radiotracer distribution among the tissues varied greatly between the beginning and the end of the depuration period (Table 3). Indeed, the fraction of <sup>110m</sup>Ag and <sup>57</sup>Co increased significantly over time ( $p < 0.001$ ) in the digestive gland (from 5 to 61% and from 20 to 64%, respectively). In contrast, tissues in direct contact with seawater generally exhibited a lower % of the radiotracer after depuration; this was particularly evident for <sup>110m</sup>Ag in the gills, head and skin ( $p \leq 0.005$ ). It is noteworthy that radioactivity measured in the digestive gland increased from the beginning to the end of the depuration period (factor of 14 for <sup>110m</sup>Ag and 3 for <sup>57</sup>Co).

### Exposure via the food chain

In these experiments, juveniles ( $n = 8$ ) were fed radiolabelled adult brine shrimp ad libitum for 1 h and adult cuttlefish ( $n = 18$ ) ingested a total of 123 radiolabelled mussels within 2 h. After feeding, the cuttlefish with their ingested prey were immediately  $\gamma$ -counted for determination of total radiotracer contents (**Table 4**).

#### Juveniles

The loss kinetics of ingested <sup>110m</sup>Ag fitted a 2-component exponential model composed of a rapid- and a slow-loss compartment while loss kinetics of <sup>57</sup>Co was best described by a single component model (Fig. 4C,D, Table 2). The short-lived compartment contained 34% of the initially ingested <sup>110m</sup>Ag activity (Table 2) and was characterised by a  $T_{b1/2s}$  of 1 d; the long-lived component, representing the proportion of radiotracer actually absorbed by the

individuals, had a  $T_{b1/2l}$  of 13 d.  $^{57}\text{Co}$  was lost with a  $T_{b1/2}$  of 5 d (Table 2). Results showed that both radiotracers were readily assimilated in juveniles with assimilation efficiencies (AE) of 67% for  $^{110\text{m}}\text{Ag}$  and 99% for  $^{57}\text{Co}$  (Table 2). Dissections performed 29 d after ingestion indicated that the highest proportion of remaining activity of both tracers occurred in the digestive gland ( $\geq 79\%$ ; Table 1).

#### Adults

The loss kinetics of both  $^{110\text{m}}\text{Ag}$  and  $^{57}\text{Co}$  ingested with food were best described by a 2-component exponential model in adults. As shown in Fig. 4E,F and in Table 2, 81 and 57% of the ingested activity of  $^{110\text{m}}\text{Ag}$  and  $^{57}\text{Co}$ , respectively, were rapidly lost with  $T_{b1/2s}$  of 6 and 13 h. The assimilated fraction of ingested  $^{110\text{m}}\text{Ag}$  and  $^{57}\text{Co}$  was much lower in adults than in juveniles (AE = 19 vs 67% for  $^{110\text{m}}\text{Ag}$  and 43 vs 99% for  $^{57}\text{Co}$ ). Assimilated  $^{57}\text{Co}$  was strongly retained within the tissues of adult cuttlefish; during the observation period, the estimated  $T_{b1/2l}$  was 990 d (Table 2).

The tissue distribution of ingested radiotracers was determined on several occasions after feeding (**Table 5**). At the end of the depuration period, both  $^{110\text{m}}\text{Ag}$  and  $^{57}\text{Co}$  were predominantly found in the digestive gland (i.e. 58 and 95%, respectively). The distribution of  $^{57}\text{Co}$  among tissues remained unchanged for 29 d of observation. In contrast, some significant changes were observed for  $^{110\text{m}}\text{Ag}$  ( $G$ -test,  $p \leq 0.01$ ); for example, the proportion of  $^{110\text{m}}\text{Ag}$  activity decreased in the digestive tract and muscular tissues (mantle muscles and head), while it increased dramatically in the genital tract and ovaries (Table 5).

## DISCUSSION

Published literature regarding Ag and Co concentrations in cephalopod tissues only concerns data from a limited number of field studies (e.g. Martin & Flegal 1975, Ueda et al. 1979, Miramand & Bentley 1992, Bustamante et al. 2000, Ishiashi et al. 2001). Cephalopods have

been reported to concentrate Ag and Co, sometimes to high levels, with concentrations up to  $84 \mu\text{g Ag g dry wt}^{-1}$  in the digestive gland of squid and  $70 \mu\text{g Co g dry wt}^{-1}$  in the branchial hearts of octopus (Martin & Flegal 1975, Ueda et al. 1979). Field data also suggest that the digestive gland plays an important role in the bioaccumulation of these trace metals. Indeed, this organ contains more than 80% of the total body burden of Ag and Co in the octopus *Eledone cirrhosa* and in the cuttlefish *Sepia officinalis* from the English Channel (Miramand & Bentley 1992), and in the nautilus *Nautilus macromphalus* from New Caledonia (Bustamante et al. 2000). However, virtually no information is available about the relative importance of the uptake pathways for these elements.

Many cephalopods like the common cuttlefish *Sepia officinalis* have rapid growth rates, implying intense metabolism and high food intake requirements. Hence, most of them are active predators and have high digestion efficiencies (Boucher-Rodoni et al. 1987). Therefore, food most probably constitutes an important source of trace element uptake for cephalopods. This has been demonstrated experimentally for a few metals and radionuclides; e.g. Cd, Zn,  $^{241}\text{Am}$  and  $^{237}\text{Pu}$  (Guary & Fowler 1982, Ueda et al. 1985, Koyama et al. 2000, Bustamante et al. 2002a). Nevertheless, uptake of Ag and Co from seawater cannot be neglected, since the whole organism is in close and permanent contact with the ambient medium. In addition, cuttlefish spend most of the daylight period buried into sediment; hence transfer of heavy metals from the sediment should also be considered as a potential uptake pathway.

Juvenile cuttlefish exposed to spiked sediments for 1 mo accumulated Ag following linear uptake kinetics, suggesting that a steady-state in uptake would take a very long time to achieve under natural conditions. Moreover, uptake of Co from sediment was very low ( $\text{TF} \leq 0.013$ ). Examination of the tissue distribution of both accumulated metals suggests that an efficient translocation occurs from the tissues in direct contact with sediment to the digestive gland, but not to the cuttlebone.

During embryonic development, Ag and Co were efficiently taken up from seawater. However, most of the metals taken up remained associated with the capsule membrane of the eggs. This membrane thus acts as an efficient shield protecting the embryo against direct metal exposure. Similar observations have been reported previously for Cd and Zn (Bustamante et al. 2002a). However, for Ag, the capsule-membrane shielding capacity appears to be limited, since after 6 d Ag was also found in relatively high concentrations within the embryos. In molluscs, it has been established that Ag is not highly toxic for adults (for a review, see Wood et al. 2002), whereas embryos and larvae are very sensitive to the metal (Calabrese et al. 1973, Martin et al. 1981). Such a functional shield for these toxic metals could be an important advantage for organisms that live and reproduce in coastal areas, which are often heavily contaminated by metals. For example, this could be the case in the bay receiving the outflow from the Seine River, where an important population of cuttlefish migrate in spring for mating and breeding. Indeed, very high inputs of Ag in the Bay of Seine were shown to result in high accumulation in the tissues of marine organisms such as the scallop *Pecten maximus* and the whelk *Buccinum undatum* (Miramand unpubl. data). Therefore, cephalopod ontogenic development could be affected under such particular environmental conditions.

After a short exposure to radiolabelled seawater (viz. 8 h), adult cuttlefish displayed high whole-body activities for both Ag and Co, indicating that both metals are rapidly accumulated from seawater. However, Ag appears to be more bioavailable to cuttlefish than Co, since Ag displayed 6 fold higher CFs. Following seawater exposure, the respiratory tissues, i.e. gills and branchial hearts, displayed the highest  $^{110\text{m}}\text{Ag}$  activities, whereas digestive gland, kidney and branchial hearts concentrated Co to a greater extent (Table 3). However, in terms of body burden, radiotracers were mainly found in tissues which are in direct contact with seawater (gills and skin accounted for 44% of the whole-body activity of  $^{110\text{m}}\text{Ag}$ ) or which represent the main proportion of the total body weight (muscle and head contained 47% of the total

$^{110m}\text{Ag}$  and 50% of the total  $^{57}\text{Co}$ ). Interestingly, the cuttlebone contained the lowest activity among tissues and was the only compartment that always displayed a CF less than one. Similar results ( $\text{CF} \leq 0.1$ ) have been found for Cd and Zn in the cuttlebone of *Sepia officinalis* (Bustamante et al. 2002a).

After 6 d of depuration in clean, running seawater, the tissues in direct contact with seawater contained significantly less  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  than at the beginning of the depuration period; gills and skin contained only 10% of the total  $^{110m}\text{Ag}$ , and the muscular parts contained 24% of the total radioactivity for both radiotracers (Table 3). These tissues also contained the lowest activities, indicating that previously incorporated tracers were rapidly lost and/or redistributed. In contrast, the digestive gland displayed higher activities of  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  (14 and 3 times higher than at the beginning of the depuration period, respectively) and contained more than 60% of the whole body activity for both radiotracers, indicating an important internal metal translocation to the digestive gland. Therefore, this organ appears to be the main target organ for both Ag and Co. Due to its stronger retention capacity compared to other tissues, the digestive gland could act as a long-term biological indicator organ for Ag and Co contamination.

Branchial hearts also showed a specific affinity for Co as their  $^{57}\text{Co}$  activity increased by a factor of 3 during the 6 d of depuration. These observations are consistent with those made for  $^{60}\text{Co}$  in the branchial hearts of the octopus *Octopus vulgaris* (Nakahara et al. 1979). In addition, Nakahara et al. (1979) suggested that the increase in  $^{60}\text{Co}$  in branchial hearts was due to transfer of the nuclide from other parts of the body.

When juveniles were exposed in seawater, subsequent  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  release followed a single exponential loss model. Whole-body loss was rapid for  $^{110m}\text{Ag}$  but relatively slow for  $^{57}\text{Co}$  ( $T_{b1/2}$ : 1 vs 5 wk). After 29 d of depuration, residual radioactivity of both Ag and Co was mainly located in the digestive gland, suggesting that, like in adults, metal translocations



occur from other tissues to the digestive gland. Efficiency of the translocation processes for Ag and Co might be responsible of such different retention times noted in cuttlefish.

In the case of dietary exposure, less than 20% of ingested Ag was assimilated into the tissues of adult cuttlefish. In contrast, Ag was absorbed to a much greater extent in juveniles (AE up to 67%). Such a radically different degree of Ag assimilation might be due to differences in digestive metabolism between juvenile and adult cuttlefish (Mangold 1989). In contrast, assimilated Ag was rapidly depurated with similar half-lives in juveniles and adults (i.e. 13 vs 9 d, respectively) which suggests that similar processes govern Ag elimination at both ages. In this context, it appears that difference in AE could also be related to variation in Ag bioavailability due to the different food types ingested by juveniles and adults (brine shrimp vs mussels). Indeed, different metal storage strategies in mussels and brine shrimp could partially control bioavailability owing to different physico-chemical speciation of the metals in the prey (e.g. Reinfelder & Fisher 1991, Wallace & Lopez 1997).

The digestive gland clearly plays a major role in the storage of Ag in juveniles and adult cuttlefish. Indeed, regardless of the exposure route, the digestive gland retained most of the metal incorporated. Bivalves such as Pectinidae and Ostreidae have been shown to accumulate very high levels of Ag in their tissues and to store the contaminant mainly as  $\text{Ag}_2\text{S}$ , a stable, non-toxic form of Ag (Martoja et al. 1989, Berthet et al. 1992). This detoxified form remains sequestered in the amoebocytes and in the basement membranes of various bivalve organs (Berthet et al. 1990, 1992). The occurrence of sulphhydryl-rich proteins such as metallothioneins have been found in the cephalopod digestive gland (Tanaka et al. 1983, Bustamante et al. 2002b). Histochemical and microanalytical investigations in the digestive gland of the cuttlefish *Sepia officinalis* have shown that Ag is associated with Cu and Zn in spherules accumulated in the basal cells, and these spherules are composed of metallothionein-like proteins (Martoja & Marcaillou 1993). Metalloproteins play a major role in the homeostasis of Cu and Zn and are well known for their capacity to bind Ag, Cd and Hg

(see e.g. Cosson et al. 1991, George & Olsson 1994). In the digestive gland of the squid *Todarodes pacificus*, ca. 60% of the whole-body burden of Ag is associated mainly with the low molecular weight cytosolic proteins (mol. wt < 20 kDa) (Tanaka et al. 1983).

Ingested Co was assimilated to a greater extent in juveniles (99%) than in adults (57%) (Table 2). Furthermore, depuration rate constants were very different between both stages, resulting in very different half-lives, viz. 5 d for juveniles and 990 d for adults. Thus, Co appears to be depurated and/or detoxified by completely different processes in juveniles and adults. Such a difference is difficult to explain, since Co is an essential element and has been shown to be easily redistributed to branchial hearts and digestive gland following seawater exposure to the metal (Nakahara et al. 1979, present study). In fact, the very elevated whole-body retention capacity of assimilated Co by adult cuttlefish concerned exclusively the digestive gland, which contained from 91 to 95% of the total Co body burden. Thus, once incorporated into the adult digestive gland, assimilated Co ingested with prey can be considered as definitively sequestered. The lower retention efficiency for Co in juvenile cuttlefish could be due to the incomplete development of the digestive gland in early juveniles, as has been suggested for assimilated Cd (Bustamante et al. 2002a). However, although incompletely mature, the digestive gland also plays a role in the storage of Co in juveniles, given that this organ contained 79% of the whole-body burden of Ag after 29 d of loss. Further investigations are needed to better understand the metabolism of Co during the life cycle of cephalopods.

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Captions to Figs.

Fig. 1. *Sepia officinalis*. Whole-body uptake kinetics of (A)  $^{110m}\text{Ag}$  and (B)  $^{57}\text{Co}$  in juvenile cuttlefish exposed to radiolabelled sediments (transfer factor, TF; mean  $\pm$  SD, n = 9 from Day 0 to 22, and n = 3 on Day 29)

Fig. 2. *Sepia officinalis*. Whole-body uptake kinetics of (A)  $^{110m}\text{Ag}$  and (B)  $^{57}\text{Co}$  in cuttlefish eggs exposed for 11 d to dissolved radiotracers in seawater (concentration factor, CF; mean  $\pm$  SD, n = 3)

Fig. 3. *Sepia officinalis*. Distribution (%; mean values) of (A)  $^{110m}\text{Ag}$  and (B)  $^{57}\text{Co}$  among the cuttlefish egg compartments at different times during their exposure via seawater

Fig. 4. *Sepia officinalis*. Whole-body loss kinetics of  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  (% of remaining activity; mean  $\pm$  SD): (A, B) in juvenile cuttlefish previously exposed to spiked seawater for 36 h (n = 8 from Day 0 to 20 and n = 4 on Day 29); (C, D) in juvenile cuttlefish previously fed radiolabelled brine shrimp *Artemia salina* (n = 8 from Day 0 to 22 and n = 5 on Day 29); (E, F) in adult cuttlefish previously fed radiolabelled mussels *Mytilus galloprovincialis* (n = 18 on Day 0, n = 15 from Day 1 to 18, n = 12 from Day 19 to 29). Parameters of the best fitting equations are given in Table 2

Table 1. *Sepia officinalis*. Radiotracer distribution (%; mean  $\pm$  SD) among 3 body compartments of juvenile cuttlefish (a) after a 29 d exposure to spiked sediments, (b) after a 29 d depuration following a 36 h exposure to spiked seawater, and (c) after a 29 d depuration following ingestion of spiked food (brine shrimp)

Exposure pathway	n	Body compartment		
		Digestive gland	Cuttlebone	Remainder
<b>(a) Sediments</b>	3			
$^{110m}\text{Ag}$		57 $\pm$ 2	0.5 $\pm$ 0.4	43 $\pm$ 3
$^{57}\text{Co}$		79 $\pm$ 0.3	1.8 $\pm$ 0.6	19 $\pm$ 1
<b>(b) Seawater</b>	4			
$^{110m}\text{Ag}$		69 $\pm$ 11	0.5 $\pm$ 0.1	31 $\pm$ 11
$^{57}\text{Co}$		78 $\pm$ 1	0.4 $\pm$ 0.1	22 $\pm$ 1
<b>(c) Feeding</b>	5			
$^{110m}\text{Ag}$		83 $\pm$ 5	0.9 $\pm$ 0.7	16 $\pm$ 4
$^{57}\text{Co}$		79 $\pm$ 6	0.8 $\pm$ 0.8	20 $\pm$ 6

Table 3. *Sepia officinalis*. Concentration factors (CF), radiotracer activities (Bq g wet wt<sup>-1</sup>; mean ± SD) and tissue distribution of radioactivity (%; mean ± SD) in adult cuttlefish after 8 h of exposure via seawater (n = 3) and after 6 d of depuration (n = 2)

Mis en forme

Tissue	% wet wt	<sup>110m</sup> Ag					<sup>57</sup> Co				
		Accumulation (8 h)			Depuration (6 d)		Accumulation (8 h)			Depuration (6 d)	
		CF	Activity	%	Activity	%	CF	Activity	%	Activity	%
Branchial heart appendages	0.03 ± 0.004	27 ± 24	229 ± 182	<1	51	<1	4.7 ± 1.2	27 ± 7	<1	81	<1
Branchial hearts	0.10 ± 0.02	76 ± 48	631 ± 398	<1	316	<1	12 ± 2	70 ± 12	<1	222	2
Gills	2.3 ± 0.3	150 ± 56	1253 ± 465	25 ± 6	175	3	5.1 ± 1.9	29 ± 11	4 ± 1	7	1
Digestive tract	2.6 ± 0.6	11 ± 3	89 ± 25	2 ± 1	134	4	4.7 ± 0.9	27 ± 5	4 ± 1	20	5
Genital tract	3.6 ± 1.0	4.6 ± 1.8	39 ± 15	1 ± 0	52	1	6.7 ± 8.1	39 ± 47	10 ± 13	4	1
Ink sac	0.6 ± 0.2	10 ± 8	85 ± 69	<1	26	<1	3.2 ± 0.7	19 ± 4	1 ± 0	3	<1
Skin	6.4 ± 2.1	40 ± 16	335 ± 135	19 ± 4	144	7	3.5 ± 2.3	21 ± 14	7 ± 2	6	3
Digestive gland	4.3 ± 1.2	17 ± 14	142 ± 120	5 ± 2	2054	61	15 ± 13	86 ± 77	20 ± 10	280	64
Kidney	0.07 ± 0.07	17 ± 12	145 ± 100	<1	19	<1	11 ± 5	66 ± 30	<1	27	<1
Muscle	35 ± 2	7.3 ± 3.7	61 ± 31	18 ± 2	43	13	1.6 ± 0.5	9 ± 3	19 ± 1	3	9
Head	40 ± 1	9.8 ± 4.3	82 ± 36	29 ± 6	30	11	2.5 ± 0.7	15 ± 4	34 ± 5	5	15
Cuttlebone	5.1 ± 0.6	0.3 ± 0.4	3 ± 3	<1	1	<1	0.4 ± 0.3	2 ± 2	1 ± 0	1	1
Whole cephalopod	100	19 ± 4	112 ± 42	100	120	100	2.9 ± 0.8	17 ± 5	100	13	100

Table 2. *Sepia officinalis*. Parameters of the equations describing the loss kinetics of  $^{110m}\text{Ag}$  and  $^{57}\text{Co}$  in whole cuttlefish previously exposed to the radiotracers via different pathways: (a) juveniles previously exposed for 36 h via seawater; (b) juveniles previously fed radiolabelled brine shrimp *Artemia salina*; (c) adults previously fed radiolabelled mussels *Mytilus galloprovincialis*. O and T: 1- and 2-exponential loss equations, respectively; ASE: asymptotic standard error; R<sup>2</sup>: determination coefficient; p: probability of the model adjustment. For abbreviation definitions, see ‘Data and statistical analyses’

Pathway	Model	$A_{0s}$ (ASE)	$k_s$ (ASE)	$T_{b1/2s}$ (d)	$A_{0t}$ (ASE)	$k_t$ (ASE)	$T_{b1/2t}$ (d)	R <sup>2</sup>	p
<b>(a) Loss in juveniles after seawater</b>									
$^{110m}\text{Ag}$	O	100.8 (2.5)	0.103 (0.006)	6.7	–	–	–	0.97	<0.001
$^{57}\text{Co}$	O	93.3 (2.0)	0.020 (0.003)	34	–	–	–	0.98	<0.001
<b>(b) Loss in juveniles after a single feeding on brine shrimp</b>									
$^{110m}\text{Ag}$	T	33.8 (7.3)	0.668 (0.237)	1.0	67.2 (6.4)	0.053 (0.009)	13.2	0.82	<0.001
$^{57}\text{Co}$	O	98.8 (3.1)	0.128 (0.100)	5.4	–	–	–	0.86	<0.001
<b>(c) Loss in adults after a single feeding on mussels</b>									
$^{110m}\text{Ag}$	T	80.7 (3.7)	2.683 (0.667)	0.26	19.3 (3.1)	0.078 (0.027)	8.9	0.94	<0.001
$^{57}\text{Co}$	T	56.9 (4.9)	1.290 (0.325)	0.54	43.2 (3.5)	0.001 (0.007)	990	0.66	<0.001

