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BIOKINETICS OF CADMIUM AND ZINC ACCUMULATION AND DEPURATION AT DIFFERENT STAGES IN THE LIFE CYCLE OF THE CUTTLEFISH *SEPIA OFFICINALIS*

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ABSTRACT: Bioaccumulation of ^{109}Cd and ^{65}Zn by the cuttlefish *Sepia officinalis* was studied at different stages of its life cycle, i.e. in embryos, juveniles and adults, following exposures via sea water, sediments and food. Cuttlefish eggs efficiently accumulated both elements from sea water with bioconcentration factors reaching 46 for ^{109}Cd and 79 for ^{65}Zn after 11 days of exposure. Most of the radiotracers was found in the capsule membrane of the eggs, demonstrating that the capsule acts as a shield to protect embryos against metals. Juveniles and adults efficiently bioconcentrated both radiotracers from sea water, with the muscular tissues containing 62% of the total ^{109}Cd and 84% of the total ^{65}Zn. Loss kinetics followed a single exponential function for ^{65}Zn while for ^{109}Cd, loss was best described by a double exponential model. Biological half-lives for elimination were ca. 2 months for both elements. After 29 days of depuration in uncontaminated sea water, 76 to 87% of the radiotracers were found in the digestive gland. For both elements the dissolved phase can be considered as a significant source of accumulation. In an experiment with radiolabelled sediments, transfer factors were very low, even after 29 days of exposure. Food chain transfer experiments demonstrated that both juveniles and adults assimilated ^{109}Cd and ^{65}Zn very efficiently. Moreover, loss of ingested radiotracers was much slower than elimination of ^{109}Cd and ^{65}Zn taken up from sea water, indicating a very strong retention of dietary Cd and Zn by juvenile as well as by adult cuttlefish. As with direct uptake from sea water, ingested radiotracers were mainly found in the digestive gland with fractions reaching 82% for ^{65}Zn and 97% for ^{109}Cd after 29 days of depuration. These tracer experiments indicate that (1) food is likely the primary pathway for Cd and Zn bioaccumulation in *Sepia officinalis* and (2) the digestive gland plays a major role in the subsequent storage and presumed detoxification of these elements regardless of the uptake pathway.

Key words : Cadmium ; Zinc ; Bioaccumulation ; Depuration ; Cephalopods ; *Sepia officinalis*; Radiotracers.

INTRODUCTION

Cephalopods constitute a class of marine molluscs which are found in a great variety of habitats from coastal waters to very deep ocean environments. They have also been found to live under extreme conditions such as near hydrothermal vents (e.g. the hydrothermal octopus *Vulcanoctopus hydrothermalis*, Gonzalez et al. 1998). Cephalopods are benthic (e.g. octopus), nectobenthic (cuttlefish), neritic and pelagic (squids), and it follows that they are a primary food source for many marine predators such as marine mammals or seabirds (Clarke 1996, Croxall & Prince 1996, Smale 1996).

Whatever their morphological and evolutionary diversity and heterogeneity in their geographic distribution, cephalopods have in common the ability to concentrate Cd to extremely high levels (Martin & Flegal 1975, Miramand & Guary 1980, Finger & Smith 1987, Miramand & Bentley 1992, Bustamante et al. 1998a, 2000). Cephalopods are therefore a potential threat for higher trophic levels. Indeed, their predators are well known to display high Cd concentrations as well (Bustamante et al. 1998b). The reason for such a high Cd bioaccumulation capacity is still poorly understood. Thus, the primary objective of the present work was to investigate biokinetics of Cd uptake and elimination in cephalopods in order to better characterize their bioaccumulation, tissue distribution and retention capacity.

The common cuttlefish *Sepia officinalis* was selected as a model to study Cd transfer in cephalopods via sediments, sea water and food. Furthermore, bioaccumulation was followed at different stages of the life cycle of *S. officinalis*, *viz.* in embryos, early juveniles and adults, to better define the physiological basis for enhanced Cd bioconcentration.

In addition to Cd, Zn was also considered in this study. This essential element has chemical properties similar to those of Cd that, in turn, could interfere with Zn metabolism (Nieboer & Richardson 1980). Biokinetics were determined using carrier-free radiotracers in order to

measure element fluxes in real time at environmentally realistic contaminant concentrations (Fisher et al. 1991, Warnau et al. 1996).

MATERIAL AND METHODS

Organisms

Eggs, newly hatched juveniles and adult common cuttlefish (*Sepia officinalis* L.) were used in the experiments. Cuttlefish eggs were obtained from the Marine Station of Banyuls sur Mer (France) and were maintained in an aquarium until hatching. Young cuttlefish were then kept in a separate aquarium (open circuit, 20 l h^{-1} flow rate, constant aeration, 38 p.s.u., 16.5 ± 0.5 °C, 12h/12h light/dark cycle) and fed with brine shrimp (*Artemia salina*) for several days before the experiments.

In addition, two different groups of adults were investigated. The first group was reared in the Musée Océnaographique (Principality of Monaco) from hatching up to one year old organisms, and conditioned to feed on dead fish and mussels. The second group consisted of adults collected by net fishing off Monaco. They were maintained individually in aquaria for acclimation to laboratory conditions for two weeks prior to investigations. During acclimation, the wild cuttlefish were also conditioned on a diet of dead fish and mussels.

In order to maintain animals in good living conditions and to prevent cannibalism, food was provided *ad libitum* for both size classes before and during the experiments. Different food items were given to young and adult cuttlefish because of their specific biological characteristics: diet of young individuals is restricted to small, relatively soft and fleshy crustaceans while adults feed on larger crustaceans including crabs, molluscs (including cephalopods), and fish (Boucher-Rodoni et al. 1987).

Prior to any experimentation, adults were anaesthetised in sea water containing 2% ethanol in order to determine their sex, weight and mantle length. At the same time, a numbered plastic marker was inserted into the mantle fin to identify each animal.

Radiotracers and radioanalyses.

Carrier-free radiotracers, ^{65}Zn ($T_{1/2}$ = 243.9 d) and ^{109}Cd ($T_{1/2}$ =462.6 d), were purchased from Amersham, UK. Stock solutions of ^{65}Zn and ^{109}Cd were prepared in 0.1 N HCl.

Activities of the radiotracers were measured using a high-resolution γ-spectrometry system consisting of coaxial Ge N or P type detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyser and a PC employing spectral analysis software (Interwinner, Intertechnique). The absolute activities of the samples were determined by comparison with known standards of appropriate geometry and 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 some minute organs. Counting times ranged from 10 min to 1 h for whole cuttlefish, mussel and brine shrimp radioanalyses, and from 10 min to 48 h for the dissected tissues.

Experimental procedures.

Contamination of <u>Sepia officinalis</u> from sediments.

A group of nine newly hatched cuttlefish (mean wet weight ± SD: 0.327 ± 0.063 g) were placed for 29 days in a 20 l plastic aquarium (open circuit, 20 l h^{-1} flow rate, constant aeration, 38 p.s.u., 16.5 ± 0.5 °C) containing ca. 3 l of natural sea water running over a 4 cm layer of spiked sediments containing 120 ± 9 KBq ^{65}Zn g^{-1} and 49 ± 8 KBq ^{109}Cd g^{-1} wet wt over the experiment time. The height of sea water was maintained low in order to minimise the movements required for feeding and to maximise the time of contact with sediments. All cuttlefish were fed twice daily with *Artemia salina* and were periodically γ-counted to follow

the radiotracer uptake kinetics. At the end of the experiment, 3 individuals were dissected to determine the distribution of the radiotracers among digestive gland, cuttlebone and remainder (rest of the organs).

Contamination of Sepia officinalis from sea water.

Eggs. The eggs were placed in a 75 l glass aquarium containing natural sea water spiked with ^{65}Zn (6 KBq l^{-1}) and ^{109}Cd (13 KBq l^{-1}) for 11 days. Spiked activities corresponded to very low additions of stable metals in the sea water, viz. 0.3 pM Zn and 1.16 pM Cd; this allowed experimentation under simulated conditions that were environmentally realistic.

The spiked sea water was changed daily to maintain radiotracer concentrations relatively constant and to avoid any build-up of exometabolites. During the experiment, activities of the eggs were measured at days 1, 2, 3, 6, 9, and 11. At each time, 3 eggs were dissected to determine the distribution of the tracers among the capsule membrane, peri-embryonar liquid and embryo.

Juveniles. Eight newly hatched cuttlefish (mean weight ± SD: 0.460 ± 0.060 g) were placed for 36 h in a 20 l plastic aquarium containing sea water spiked with ^{65}Zn (6 KBq l^{-1}) and ^{109}Cd (13 KBq l^{-1}). After that time, radiolabelled juvenile cuttlefish were held for 29 d in clean flowing sea water (75 l glass aquarium, open circuit, 20 l h^{-1} flow rate, constant aeration, 38 p.s.u., 16.5 ± 0.5 °C). In order to facilitate the retrieval and counting of each individual during the loss experiment, juveniles were held individually in the aquaria in separate circular plastic containers (10 cm diameter, 5 cm height) covered with a plastic netting. Cuttlefish were fed twice daily with *Artemia salina* and were periodically γ-counted to follow the radiotracer loss kinetics. After 29 d of loss, four individuals were dissected to determine the distribution of the tracers among digestive gland, cuttlebone and remainder.

Adults. A group of five sexually mature male cuttlefish (mean weight ± SD: 253 ± 97 g) was held for 8 h in a 75 l glass aquarium containing sea water spiked with 20 KBq ^{65}Zn l^{-1}

and 13 KBq ^{109}Cd l^{-1}. In terms of stable metal addition, these activities corresponded to concentrations of 1 pM Zn and 1.16 pM Cd. Afterwards, the cuttlefish were radioanalyzed and transferred to a flowing sea water aquarium (3000 l, open circuit, 600 l h^{-1} flow rate, constant aeration, 38 p.s.u., 16.5 ± 0.5 °C) for 6 d. Three adults were dissected after 24 h and the remaining ones were dissected after 6 days of depuration. For each individual, the branchial heart appendages, branchial hearts, gills, digestive tract (after removal of the gut contents), genital tract, ink sack, digestive gland, kidneys, mantle skin, mantle muscle, head and cuttlebone were separated, weighed, and their radiotracer content measured.

Contamination of <u>Sepia officinalis</u> through the food.

In order to better reflect the different feeding habits of juvenile and adult cuttlefish (Boucher-Rodoni et al. 1987), two different prey were selected for the feeding experiments. Mussels (*Mytilus galloprovincialis*) and brine shrimp (*Artemia salina*) were exposed for 7 d in a plastic aquarium containing 4 l of natural sea water spiked with 6 KBq ^{65}Zn l^{-1} and 13 KBq ^{109}Cd l^{-1}. Radiolabelled sea water was changed daily and the organisms were subsequently used as food for cuttlefish.

Juveniles. Eight newly hatched cuttlefish (mean weight ± SD: 0.380 ± 0.030 g) were placed in individual plastic containers (10 cm diameter, 5 cm height), and held in a 75 l glass aquarium under the same conditions as in the previous experiments. These individuals were then fed for one hour with the previously radiolabelled *A. salina*. At the end of the feeding period, each individual was immediately γ-counted. From that time on, cuttlefish were fed twice a day with uncontaminated *A. salina* and regularly γ-counted to determine radiotracer loss kinetics and assimilation efficiency. Throughout the depuration period, faeces were removed three times per day to reduce direct contamination by recycled radiotracers leaching

from the faeces. After 29 d, five juveniles were dissected to determine body distribution of the radiotracer.

Adults. Eighteen sexually mature cuttlefish (11 females and 7 males; mean weight ± SD: 164 ± 34 g and 107 ± 23 g, respectively) were placed in a 3000 l aquarium under conditions described above and fed for two hours with the soft parts of the previously labelled mussels. Following ingestion, each individual was γ-counted and the same procedure followed as for the juveniles. In addition, three adult cuttlefish were dissected after each radioanalysis to determine the radiotracer content of their different tissues.

Data analyses.

Uptake of the radiotracers from sea water and sediments was expressed as change in concentration factors (CF) and transfer factors (TF) (CF and TF = Bq g^{-1} wet organism divided by the time-integrated Bq g^{-1} in sea water or sediments, respectively) over time. Uptake kinetics in the eggs were described using a single-component first-order kinetic model

$$CF_t = CF_{equil} (1 - e^{-kt})$$

where CF_t and CF_{equil} are concentration factors at time t (d) and steady state, respectively, and k is the rate constant (d^{-1}) (Whicker & Schultz 1982).

Radiotracer elimination was expressed in terms of percentage of remaining radioactivity, i.e. radioactivity at time t divided by initial radioactivity measured in the organisms at the beginning of the depuration period. When radiotracer loss plotted against time displayed an exponential shape, the kinetics were described either by single-component exponential model

$$A_t = A_0 e^{-\lambda t}$$

where A_t and A_0 are remaining activities (%) at time t (d) and 0, respectively, and λ is the depuration rate constant (d^{-1}) which allows the calculation of the radiotracer biological half-life ($T_{b1/2} = \ln 2/\lambda$), or by a 2-component exponential model

$$A_t = A_{0s}\, e^{-\lambda st} + A_{0l}\, e^{-\lambda lt}$$

where 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 (decision based on calculation of the determination coefficients, R^2, and examination of the residuals) was then selected.

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 Systat 5.2.1 Software (Wilkinson 1988). Changes in radiotracer distribution among cuttlefish tissues were tested for significance by the G procedure (adapted from the log-likelihood ratio test) for 2 x k contingency tables (Zar 1996). Changes in % of radioactivity in a single tissue during the depuration period were tested by one-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

Contamination from the sediments

Although cuttlefish spend most of their time on the bottom sediments, very low activities were recorded in the individuals exposed to spiked sediments, even after an exposure as long as 29 d. The transfer factors between sediment and cuttlefish remained less than 0.10 for both ^{65}Zn and ^{109}Cd (Figure 1). The heterogeneity of the results was substantial and no realistic accumulation kinetics could be calculated for this experiment.

After 29 d, the dissection of 3 individuals showed that the digestive gland contained most of the two metals studied, i.e. 75 ± 8% for ^{65}Zn and 68 ± 7% for ^{109}Cd while the remainder and cuttlebone contained respectively 23 ± 8 and 2.5 ± 0.1% for ^{65}Zn and 20 ± 6 and 11 ± 1% for ^{109}Cd.

Contamination from sea water

Eggs. The experiment was carried out for 11 d at the end of which elevated concentration factors were reached in the eggs for both ^{65}Zn (79 ± 5, m ± SD) and ^{109}Cd (46 ± 2, m ± SD). The uptake of both tracers displayed saturation kinetics (Figure 2). Most of the radiotracers (96-99% for ^{65}Zn and 99% for ^{109}Cd) was always found associated with the capsule membrane of the eggs (Figure 3).

Juveniles and adults. Due to obvious safety problems resulting from maintenance of cuttlefish in aquaria containing spiked sea water, the exposure time was shortened as much as possible. Preliminary experiments indicated that a 36 h exposure was necessary to obtain juveniles with activity levels suitable for efficient detection; a 8 h exposure was found to be sufficient for adults.

Activities (Bq g^{-1} wet wt) recorded in the different cuttlefish tissues after a 8 h exposure are given in Table 1. In the adults, ^{65}Zn exhibited the highest activities in the skin and gills (121 and 119 Bq g^{-1}, respectively) while the branchial hearts contained the highest activities of ^{109}Cd (185 Bq g^{-1}). ^{65}Zn appears to be more bioavailable to cuttlefish than ^{109}Cd since the whole body CF was 3 times higher for Zn than for Cd.

The distributions of the radiotracers in body compartments of adult and juvenile cuttlefish are presented in Tables 1 (6 d of depuration) and 2 (29 d of depuration), respectively. Following exposure, the muscles of adults (i.e. mantle muscle and skin, and the head) contained the highest fraction of both radiotracers (84% for ^{65}Zn and 62% for ^{109}Cd). The digestive gland contained the second highest proportion of ^{109}Cd (i.e. 25%). After 6 d of depuration, the global

distribution of radiotracers changed since the fraction of ^{65}Zn increased significantly in the digestive gland ($p=0.001$), and ^{109}Cd in branchial hearts ($p=0.004$) and appendages ($p=0.005$). In contrast, the tissues in direct contact with sea water generally exhibited a lower percentage of radioactivity after a 6 d depuration. This is particularly obvious in the case for ^{65}Zn in gills ($p<0.001$)and head ($p=0.007$) (see Table 1).

Loss of ^{65}Zn in juvenile cuttlefish followed a single-component exponential model (Figure 4A, Table 3) with a $T_{b1/2}$ of approx. 2 months. In contrast, loss of ^{109}Cd was best described by a two-component model (Figure 4B, Table 3). The short term component was characterised by a very short $T_{b1/2}$ (ca. 14 h) while the long-lived loss component which represented a large fraction of the ^{109}Cd content (A_{0l} = 71%) turned over with a $T_{b1/2}$ of ca. 65 days.

After 29 d in uncontaminated sea water, both tracers were found in highest proportion in the digestive gland of the young cuttlefish (from 76 to 87%) (Table 2) while their lowest proportion was found in the cuttlebone (around 1% of the total activity).

Contamination via food

Eighteen adult cuttlefish ingested 123 radiolabelled mussels in 2 hours and 8 juveniles were fed radiolabelled brine shrimp *ad libitum* for one hour. The cuttlefish and their prey were immediately γ-counted for their tracer content (Table 4).

Loss kinetics of ^{65}Zn and ^{109}Cd ingested with food displayed a 2-component exponential model for both juveniles (Figures 4C & 4D, Table 3) and adults (Figures 4E & 4F, Table 3). Regarding adults, the short-lived component involved about half the total activity in whole cuttlefish (59% for Zn and 47% for Cd) and both elements displayed very short $T_{b1/2s}$ (ca. 10 h) (Table 3). The short-lived loss component for juveniles represented a lesser fraction of radiotracer (38% for both elements) than that of adults (Table 3). The long-lived component of ^{109}Cd depuration had very low depuration rate constants (λ_l), which resulted in a very long

$T_{b1/2l}$ in both juvenile and adult cuttlefish (> 250 d). In contrast, in the young cephalopods, slow depuration rates characterising the long-lived component were noted for ^{65}Zn ($T_{b1/2}$ =173 d) while in adults, depuration rates were much faster ($T_{b1/2}$ =38 d). Young individuals incorporated metals more efficiently from brine shrimp (assimilation efficiency -AE-: 63% for Zn and 62% for Cd) than did adults ingesting mussels (AE = 59% for Zn and 47% for Cd) (Table 4).

The distribution of radioactivity in cuttlefish tissues was determined on several occasions in adults (Table 5) and only at the end of the loss period for juveniles (Table 2). At the end of the depuration period, the highest proportion of radiotracer was found in the digestive gland (i.e. 79 and 97% in adults and 82 and 91 % in juveniles for ^{65}Zn and ^{109}Cd, respectively). In adults, tissue distribution of ^{65}Zn varied significantly during the experiment (G test, $p<0.05$) (Table 5). The proportion of ^{65}Zn activity decreased in the muscular tissues (mantle muscle and cephalic parts) and increased in the digestive gland (Tukey test, $p=0.021$) suggesting a lower depuration rate in digestive gland than in other organs (Table 5). For ^{109}Cd, a G test did not indicate any significant difference among radioactivity distributions during the experiment.

DISCUSSION

The use of radiotracers in very low concentrations allows the investigation of trace metal metabolism at realistic environmentally conditions. Furthermore, these radiotracer experiments permit simultaneously investigating several elements without inter-element interferences.

During embryonic development, Zn and Cd were efficiently taken up from sea water by the eggs, with CF of 79 for ^{65}Zn and 46 for ^{109}Cd after only 11 d of exposure. However, most of the radiotracers was associated with the capsule membrane of the egg, which thus would act as an efficient shield protecting the embryo against metal direct exposure. This observation is surprising for Zn since it is an essential element for the development of the embryos. That may

in fact explain the large amounts of this metal occurring in ovaries, such as in the squid *Illex argentinus* in which the female gonad may account for 13.6% of the total Zn body burden (Gerpe et al. 2000). This suggests that eggs contain enough Zn for the needs of the embryo, and that additional metal could become toxic for the embryo. In fact Zn incorporation may also be blocked to inhibit Cd from crossing the capsule membrane, since the latter element has similar chemical properties as Zn and follows the same uptake pathways. Nevertheless, the exact mechanism causing the specific blockage of both metals in the capsule membrane of the eggs remains unknown.

Although the experimental contamination via sea water was only carried out for a short period of time, activities recorded in the whole cuttlefish suggest that they would efficiently accumulate these two elements directly from water. In other invertebrates such as echinoderms or bivalves, the major fraction of the radiotracers was found in the calcified parts, i.e. body wall of sea urchins (Warnau et al. 1996) and shell of mussels (Guary 1980, Fisher et al. 1996). Although the cuttlefish *Sepia officinalis* has a calcareous compartment -the cuttlebone- representing around ca. 5% of the total body weight, it contained less than 1% of the total radioactivity of ^{65}Zn and ^{109}Cd after sea water exposure. This may be due to the internal location of the cuttlebone which has no direct contact with ambient sea water. Thus, trace elements are distributed more homogeneously among cuttlefish tissues than in invertebrates with calcareous compartments directly in contact with sea water (bivalves) or protected by a thin epidermis (sea urchins). In *S. officinalis*, most of the tracers taken up, i.e. 84% for ^{65}Zn and 62% for ^{109}Cd, was located in the tissues in direct contact with sea water, i.e. mantle, skin of the mantle, and head.

If direct sorption of metals on the cuttlebone is not possible (it is entirely surrounded by muscular tissue), transfer and redistribution of Cd to this compartment would be expected since the cuttlebone is mainly composed of calcium carbonate. Indeed, Cd mimics Ca because of

their similar ionic radius (Huheey 1983). Therefore, due to its mineral composition, the cuttlebone of *S. officinalis* could play a storage role for some elements, including Cd. However, results presented here showed that transfer of radiotracers to the cuttlebone were very limited, even after a long depuration period, following either sea water or food exposure (Tables 2 and 5).

After 6 d in uncontaminated sea water, the two elements were found to be distributed differently among body compartments (Table 1). This allowed the determination of three distinct groups of tissues: (1) those whose percentage of total radioactivity had decreased (muscular parts and gills); (2) those in which there was an increase in the percentage of total radioactivity (i.e. digestive gland, branchial hearts and appendages); and (3) those in which the percentage of the radioactivity remained similar (cuttlebone, kidney, ink sack, genital tract and gut). The tissues of the first group might have rapid depuration rates while those of the second group -which are also known or supposed detoxification organs (Mangold et al. 1989)- would have either a longer retention capacity or would be the targets of preferential translocation of the elements from other organs. A similar distinction for the tissues has been made for the squid *Sepioteuthis lessoniana* which was exposed to stable Cd in sea water (Koyama et al. 2000); in this case the concentration of Cd in the digestive gland was still increasing several days after the beginning of depuration while loss was immediate in the gills and mantle. Koyama et al. suggested a stronger retention of the metal in the digestive gland than in other organs. Nevertheless, our results clearly demonstrated (1) the translocation of Zn to the digestive gland where the activity was more than 10 times higher after 6d of depuration, and (2) the translocation of Cd to the branchial hearts and appendages in which activities increased by a factor 5 and 4, respectively, over the same period of time (see Table 1).

After bioaccumulation from sea water, Zn and Cd were released from juvenile cuttlefish following a single component (^{65}Zn) or a 2-component (^{109}Cd) exponential loss kinetics. Whole body loss was relatively slow for both ^{65}Zn and ^{109}Cd with mean biological half-lives of

53 and 65 d, respectively. Our results are quite similar to those obtained for Zn in the octopus *Octopus vulgaris* which displayed a mean half-life of 74 d (Ueda et al. 1985). Comparison for ^{109}Cd retention with other cephalopod species is not possible as there are apparently no data available in the literature. However, similar half-lives for ^{109}Cd have been reported for other mollusc species such as the mussels *Mytilus edulis* (67-78 d) and *M. galloprovincialis* (16-60 d) (Fisher et al. 1996, Wang et al. 1996). For these bivalves, the dissolved phase is usually considered as a significant source of metal accumulation (Nolan & Dahlgaard 1991, Wang et al. 1996), since they have high filtration rates and can process large volumes of sea water. As cephalopods have lower respiration rates compared to the filtration rates of bivalves, the dissolved phase might be of lesser importance in metal uptake than it is for bivalves.

After 29 d of depuration, the residual radioactivity was mainly located in the digestive gland. Thus, the digestive gland appears to be the major storage organ for both Zn and Cd. It is also noteworthy that ^{109}Cd activities in branchial hearts were relatively elevated following the exposure to contaminated sea water (Table 1). After 6 d of loss, branchial hearts and appendages exhibited a significantly higher percentage of total ^{109}Cd than at day 0. In fact, branchial heart of cephalopods generally contains very low Cd concentrations (Miramand & Guary 1980, Miramand & Bentley 1992), even if they concentrate many elements such as copper, iron, cobalt, nickel or vanadium (Ueda et al. 1979, Nakahara et al. 1979, Miramand & Guary 1980, Miramand & Bentley 1992). Radiotracer investigations with *Octopus vulgaris* have also shown the ability of octopus to concentrate radionuclides such as americium and plutonium in the branchial hearts and appendages (Guary et al. 1981, Guary & Fowler 1982). Furthermore, ^{241}Am is eliminated only very slowly from octopus tissues (Guary & Fowler 1982). In contrast, Cd has a very short retention time in branchial hearts, which probably serve as excretory organs for this element. In fact, these organs have circulatory and excretory functions, since they are actively involved in the ultrafiltration of the haemolymph (Mangold et

al. 1989). Accordingly, Cd that is directly taken up in the cephalopod haemolymph through the gills could be excreted by these organs.

Following the dietary contamination of young and adult cuttlefish, loss kinetics were described by a 2-component exponential model. The $T_{b1/2s}$ of the elements were very short (≤ 1 d). In this case, the short-lived component would represent the unabsorbed fraction of the element which was directly eliminated with the faeces. Indeed, this loss rate corresponds quite well to the gut-residence time in both juvenile and adult *Sepia officinalis* for which digestion is typically completed in ca. 15 hrs at 20°C (Boucaud-Camou 1973). Thus, the remaining radioactivity can be considered as the absorbed fraction of the ingested dose. In adults, 41% of ^{65}Zn and 53% of ^{109}Cd from ingested mussels were readily incorporated into the tissues. These elements were absorbed to an even greater degree in juvenile cuttlefish with assimilation efficiencies (AE) of 63% for ^{65}Zn and 62% for ^{109}Cd from ingested brine shrimp. These differences between juveniles and adults could be due to differences in efficiency of digestion metabolism (Mangold 1989). However, since food type was different in experiments with juveniles (fed brine shrimp *A. salina*) and adults (fed mussels *M. edulis*), it is difficult to determine whether the differences in element AE are due to different energetic needs of juvenile and adult cuttlefish rather than to differences in element bioavailability. Indeed, different storage and/or detoxification processes occurring in mussels and brine shrimps could partially control metal bioavailability owing to the different physico-chemical speciation of the elements in the prey. In general, metals located in the soluble subcellular fraction (i.e. cytosol) are more bioavailable to higher trophic levels whereas those bound to insoluble subcellular fraction have a lower potential for transfer to predators (Reinfelder & Fisher 1991, Wallace & Lopez 1997). Likewise, it would be of interest to examine trace element assimilation efficiencies in cephalopods with respect to the subcellular location of the elements in each prey.

The biological half-life of ^{65}Zn was found to be longer in juveniles than in adults probably because the newly hatched cuttlefish incorporate large amounts of Zn in their cells to satisfy their metabolic needs. In this context, it was also expected that ^{109}Cd would have been retained more efficiently ($T_{b1/2}$ = 257 d) than an essential element such as Zn. Cd was also strongly retained in the tissues of adults, with an estimated $T_{b/2}$ tending towards infinity. These very elevated retention capacities concerned almost exclusively the digestive gland. Indeed, the digestive gland retained 91% and 97 % of the total body burden of ^{109}Cd in juvenile and adult cuttlefish, respectively. Thus, assimilated Cd that is contained in the digestive gland may be considered as actually stored. The occurrence of Cd $T_{b1/2}$ of the same time-scale order as the cuttlefish lifespan (1-2 y in the Mediterranean) (Boletzky 1983) suggests that during their evolution, cephalopods have developed a detoxification strategy favouring the storage of this toxic element instead of its elimination.

The high degree of Cd retention might thus be a result of a very efficient detoxification system which allows storing this toxic metal as one or another detoxified form. Such a detoxification process could involve precipitation or co-precipitation of metals into metal-rich granules leading to a highly stable form. This sequestering process has been observed for heavy metals in several invertebrate species (Coombs & George 1978, Brown 1982, Taylor & Simkiss 1984). However, in cephalopods, subcellular investigations have shown that Cd is mainly associated with soluble (cytosolic) compounds, but this proportion decreases when concentration of total Cd increases in the organ (Bustamante et al. in press). This is mainly due to the transfer and sequestration of Cd in the lysosomal system which is very well developed in cephalopods. Nevertheless, neither ultrastructural nor microanalytical investigations have shown the presence of any granules in the digestive gland of squids or cuttlefish (Boucaud-Camou & Boucher-Rodoni 1983, Bustamante 1998).

Cd analyses of the digestive gland of adult cuttlefish which died during collection or acclimation period indicate Cd concentrations (18.6 ± 7.3 µg g^{-1} dry wt) which are similar to those measured in the same species from the Bay of Biscay (9.4 ± 4.1 µg g^{-1} dry wt; Bustamante 1998) or the English Channel (12.9 ± 0.3 µg g^{-1} dry wt; Miramand & Bentley 1992). Given that 64% of the total Cd in the digestive gland of *S. officinalis* from the Bay of Biscay were located in the soluble fraction of this organ (Bustamante et al. in press), it is most likely that the accumulated ^{109}Cd was mainly bound to soluble compounds in the cuttlefish used in our experiments. Such an efficient detoxification system could involve cytosolic proteins such as metallothioneins as suggested by the chromatographic results obtained with different squid species (Tanaka et al. 1983, Finger & Smith 1987, Castillo & Maita 1991). But proteins with a high molecular weight (> to 70 kDa) may play an important role in Cd detoxification allowing cephalopods to accumulate high Cd levels (Tanaka et al. 1983, Finger & Smith 1987, Castillo & Maita 1991). The lower retention efficiency for Cd that was noted in juvenile cuttlefish (257 d vs infinity in adults) would result from the incomplete development of the digestive gland in early juveniles. Indeed the digestive gland is not yet fully grown (and thus not yet fully physiologically active) in juveniles, since its two segments are still separated by the anterior lobe of the inner yolk sack (Boucher-Rodoni et al. 1987). Interestingly, the structure of the digestive gland in juveniles appears to be similar to that of adults 30 days after hatching, and it becomes functional as soon as the cuttlefish begins to feed (i.e. 1-2 days after hatching). Thus, it would be of particular interest to compare the detoxification processes of Cd by cytosolic proteins in both juvenile and adult cephalopods.

In contrast to most marine invertebrates which would accumulate Cd mainly from sea water (Dahlgaard 1981, Nolan & Dahlgaard 1991, Fisher et al. 1996, Warnau et al. 1996), cephalopods like *S. officinalis* would accumulate Cd principally from food. Once incorporated, the fate of this metal is mainly controlled by the digestive gland which acts as an efficient

detoxification organ whatever the source of Cd is sea water, food, or sediment. This detoxification process occurs throughout the lifetime of the cephalopod except during embryonic development. Results for Zn were very similar to those for Cd in juveniles contaminated from sea water and from food. However, a lower retention Zn in adults could be a result of displacement of this element from the cytosolic proteins in the digestive gland by other metals such as Cd.

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Caption to figures

Figure 1. *Sepia officinalis*. Transfer factors (TF) of ^{65}Zn and ^{109}Cd over time in whole cuttlefish exposed to spiked sediments (mean transfer factor ± SD, n=9 at day 0 to 22, and n=3 at day 29).

Figure 2. *Sepia officinalis*. Uptake of ^{65}Zn and ^{109}Cd in whole eggs exposed for 11 d to radiotracer in sea water (mean concentration factor ± SD, n=3). Fitted model: $CF_t = CF_{equil}$ (1 - e^{-kt}); CF_t, CF_{equil}: concentration factors at time t (d) and steady state, respectively; k: rate constant (d^{-1}); R^2: determination coefficient; p: probability of the model adjustment.

Figure 3. *Sepia officinalis*. Radiotracer distribution (mean %) among the egg compartments at different times during the uptake phase.

Figure 4. *Sepia officinalis*. A and B: Loss of ^{65}Zn and ^{109}Cd in whole juvenile cuttlefish previously exposed to radiolabelled sea water for 36 h (mean remaining activity ± SD, n =8 at day 0 and n=4 at day 29); C and D: Loss of ^{65}Zn and ^{109}Cd in whole juvenile cuttlefish previously fed with radiolabelled *Artemia salina* (mean remaining activity ± SD, n =8 at day 0 and n=5 at day 29); E and F: Loss of ^{65}Zn and ^{109}Cd in whole adult cuttlefish previously fed with radiolabelled mussels (mean remaining activity ± SD, n =18 at day 0 and n=3 at day 29). Parameters of the equations are given in Table 3.

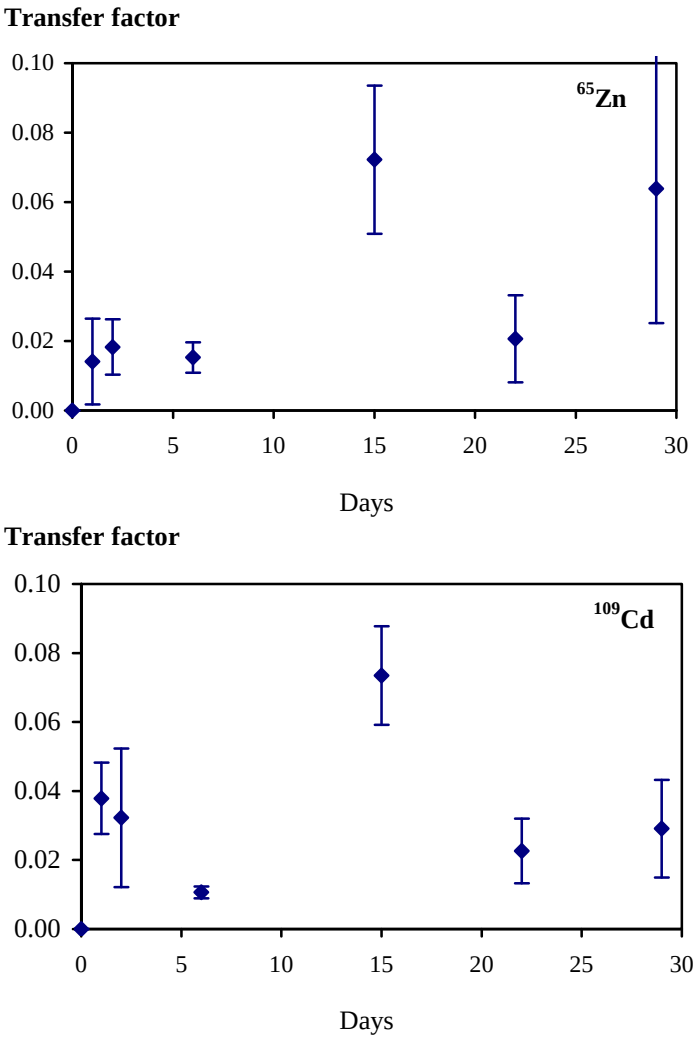
Transfer factor
0.10
0.08
0.06
0.04
0.02
0.00
65Zn
0
5
10
15
20
25
30
Days
Transfer factor
0.10
0.08
0.06
0.04
0.02
0.00
109Cd
0
5
10
15
20
25
30
Days

Figure 1

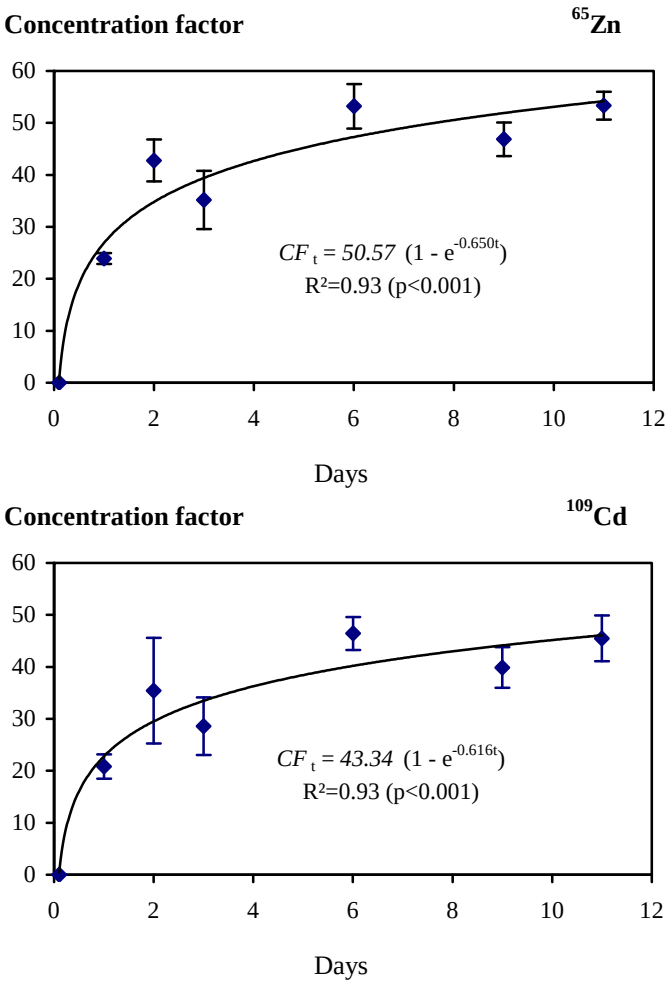
Concentration factor
65Zn
60
50
40
30
20
10
0
0
2
4
6
8
10
12
CF t = 50.57 (1 - e-0.650t)
R2=0.93 (p<0.001)
Days
Concentration factor
109Cd
60
50
40
30
20
10
0
0
2
4
6
8
10
12
CF t = 43.34 (1 - e-0.616t)
R2=0.93 (p<0.001)
Days

Figure 2

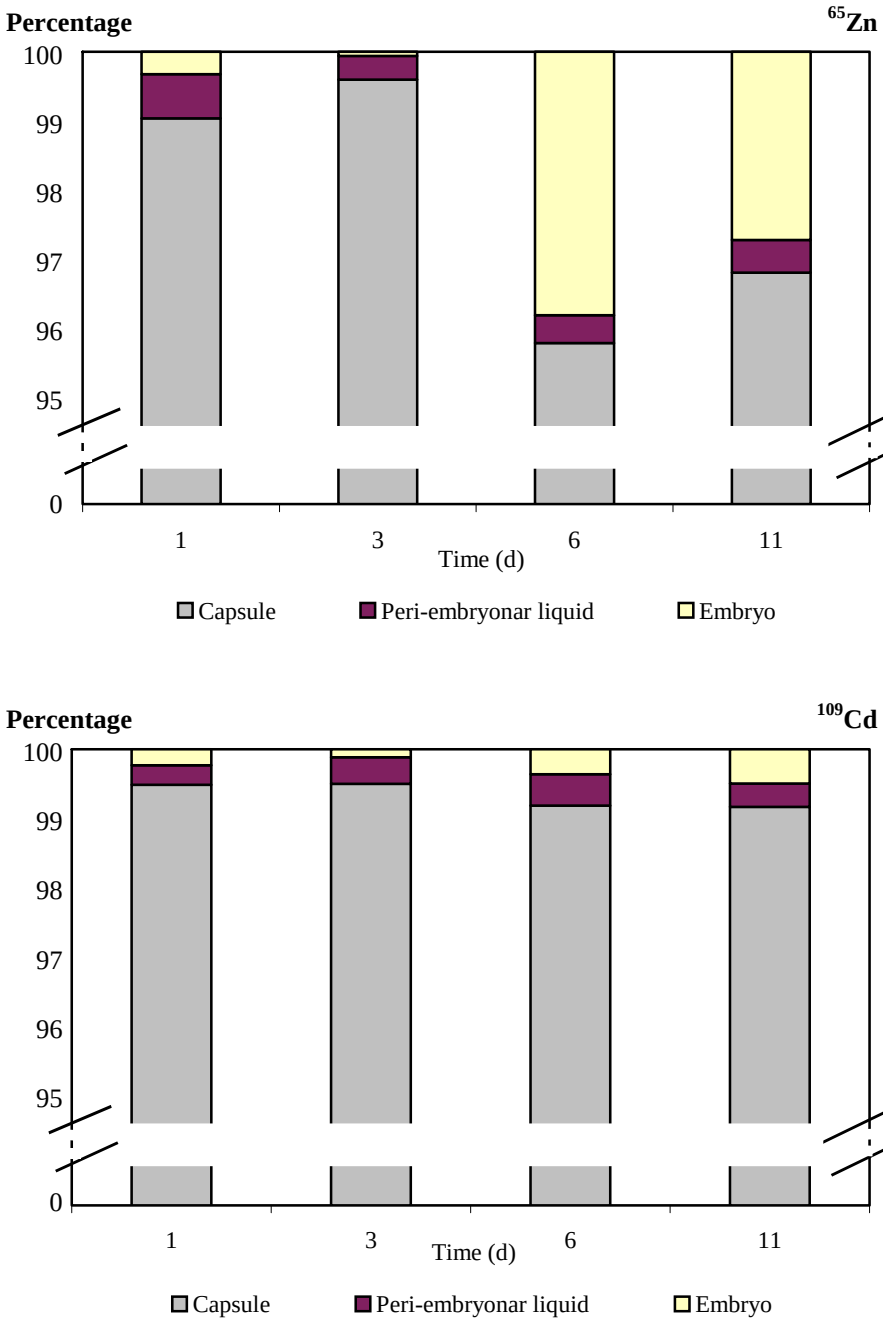
Percentage
65Zn
100
99
98
97
96
95
0
1
3
6
11
Time (d)
Capsule
Peri-embryonar liquid
Embryo
Percentage
109Cd
100
99
98
97
96
95
0
1
3
6
11
Time (d)
Capsule
Peri-embryonar liquid
Embryo

Figure 3

Table 1. *Sepia officinalis*. Concentration factor (CF), radiotracer concentrations (mean Bq g^{-1} wet wt ± SD) and distribution (mean % ± SD) in the organs of adult cuttlefish after 8 h of exposure in sea water (n=3) and after 6 d of depuration (n=2).

Tissue	% fresh weight	^{65}Zn					^{109}Cd				
		Accumulation (8 h)			Depuration (6d)		Accumulation (8 h)			Depuration (6d)	
		CF	Activity	Percentage	Activity	Percentage	CF	Activity	Percentage	Activity	Percentage
Branchial heart appendages	0.03 ± 0.004	2.2 ± 0.6	44 ± 12	< 1	47	< 1	1.8 ± 0.8	23 ± 10	< 1	129	1
Branchial hearts	0.10 ± 0.02	2.8 ± 0.9	56 ± 17	< 1	44	< 1	14.2 ± 8.8	185 ± 114	2± 2	800	13
Gills	2.3 ± 0.3	6.0 ± 2.1	119 ± 42	7 ± 0.1	51	3	1.5 ± 0.5	20 ± 7	5 ± 1	7	3
Digestive tract	2.6 ± 0.6	1.5 ± 0.5	30 ± 9	2 ± 0.3	26	2	0.6 ± 0.3	8 ± 4	2 ± 1	4	2
Genital tract	3.6 ± 1.0	0.9 ± 0.3	17 ± 6	2 ± 0.3	29	3	0.3 ± 0.2	4 ± 2	2 ± 1	2	1
Ink sack	0.6 ± 0.2	1.5 ± 1.1	29 ± 21	< 1	14	< 1	0.4 ± 0.2	5 ± 3	< 1	1	< 1
Skin	6.4 ± 2.1	6.1 ± 5.3	121 ± 105	17 ±5	50	8	0.9 ± 0.7	12 ± 9	6 ± 3	5	5
Digestive gland	4.3 ± 1.2	2.3 ± 1.8	45 ± 35	5 ± 2	536	42	4.5 ± 3.8	58 ± 49	25 ± 10	91	42
Kidney	0.07 ± 0.07	3.6 ± 1.6	72 ± 31	< 1	87	< 1	1.2 ± 0.6	16 ± 8	< 1	5	< 1
Muscle	35 ± 2	1.0 ± 0.4	20 ± 8	18 ± 2	14	13	0.3 ± 0.2	4 ± 2	14 ± 4	2	9
Head	40 ± 1	2.4 ± 0.8	48 ± 16	49 ± 4	24	26	0.7 ± 0.1	9 ± 1	42 ± 13	1	26
Cuttlebone	5.1 ± 0.6	0.1 ± 0.1	2 ± 2	< 1	8	1	0.1 ± 0.0	1 ± 0	< 1	<1	< 1
Whole cephalopod	100	3.8 ± 1.4	76 ± 27	100	65	100	1.3 ± 0.3	17 ± 4	100	13	100

Table 2. *Sepia officinalis*. Radiotracer distribution (mean % ± SD) among three body compartments of juvenile (J) and adult (A) cuttlefish exposed via sediments, sea water, and food followed by 29 d of depuration in uncontaminated sea water.

Experiment	n	Compartment		
		Digestive gland	Cuttlebone	Remainder
Sediments (J)	3			
^{65}Zn		75 ± 8	3 ± 0	23 ± 8
^{109}Cd		68 ± 7	11 ± 1	20 ± 6
Sea water (J)	4			
^{65}Zn		87 ± 3	0.6 ± 0.1	13 ± 3
^{109}Cd		76 ± 4	1.3 ± 0.5	23 ± 4
Artemia (J)	5			
^{65}Zn		82 ± 2	1.3 ± 0.9	17 ± 2
^{109}Cd		91 ± 3	1.2 ± 0.9	8 ± 3
Mussels (A)	3			
^{65}Zn		79 ± 4	2 ± 1	19 ± 2
^{109}Cd		97 ± 0	< 1	3 ± 0

Table 3. *Sepia officinalis*. Parameters of the equations describing the loss kinetics of ^{65}Zn and ^{109}Cd in whole cuttlefish previously exposed through different pathways: (1) juveniles previously exposed for 36 h to radiotracers in sea water; (2) juveniles fed labelled brine shrimp *Artemia salina*; (3) adults which received a single ration of labelled mussels *Mytilus galloprovincialis*. O and T: one- and two-component loss model, respectively; ASE: asymptotic standard error; R^2: determination coefficient; p: probability of the model adjustment. See text for other abbreviations.

Pathway	Model	A_{0s} (ASE)	λs (ASE)	$T_{b1/2s}$	A_{0l} (ASE)	λl (ASE)	$T_{b1/2l}$	R^2	p
1. Sea water exposure									
^{65}Zn	O	92.5 (2.0)	0.013 (0.002)	53	-	-	-	0.98	< 0.001
^{109}Cd	T	29.2 (6.7)	1.131 (0.530)	0.6	71.4 (4.2)	0.011 (0.005)	65	0.96	< 0.001
2. Labelled brine shrimp exposure									
^{65}Zn	T	37.5 (3.9)	1.044 (0.232)	0.7	63.2 (2.3)	0.004 (0.003)	173.3	0.98	< 0.001
^{109}Cd	T	37.8 (6.3)	0.653 (0.223)	1.1	62.3 (4.4)	0.003 (0.004)	256.7	0.96	< 0.001
3. Labelled mussel exposure									
^{65}Zn	T	59.1 (4.2)	1.940 (0.545)	0.36	40.9 (3.0)	0.018 (0.007)	38	0.95	< 0.001
^{109}Cd	T	46.7 (6.5)	1.509 (0.817)	0.46	53.1 (4.6)	-0.010 (0.006)	∞	0.91	< 0.001

Table 4. Radioactivity (Bq g^{-1} wet wt, mean ± SD) in *Mytilus galloprovincialis* soft parts (n=20) and in *Artemia salina* (n=10) used as radiolabelled food for adult and juvenile cuttlefish, respectively, and radioactivity (Bq, range) in both groups of *Sepia officinalis* fed these prey.

Radiotracer	*M. galloprovincialis* (n=20) (Bq g^{-1} wet wt)	*S. officinalis* (n=18 Adults) (Bq)	*A. salina* Bq / ind. (n=10)	*S. officinalis* (n= 8 Juveniles) (Bq)
^{65}Zn	780 ± 129	249-4284	10.92 ± 0.32	30-98
^{109}Cd	996 ± 162	117-4126	7.19 ± 0.37	19-68

Table 5. *Sepia officinalis*. Radiotracer distribution (mean % ± SD, n=3) among the tissues and organs of adult cuttlefish exposed to the radiotracers via the food after three different times of depuration in uncontaminated sea water.

Tissues	1 day		18 days		29 days	
	^{65}Zn	^{109}Cd	^{65}Zn	^{109}Cd	^{65}Zn	^{109}Cd
Branchial heart appendages	< 1	< 1	< 1	< 1	< 1	< 1
Branchial heart	< 1	1 ± 0	< 1	< 1	< 1	< 1
Gill	3 ± 0	< 1	1 ± 0	< 1	1 ± 0	< 1
Digestive tract	4 ± 1	3 ± 1	2 ± 0	2 ± 0	2 ± 0	2 ± 0
Genital tract	3 ± 0	< 1	2 ± 0	< 1	2 ± 0	< 1
Ink sack	< 1	< 1	4 ± 2	< 1	5 ± 0	< 1
Skin	4 ± 1	< 1	1 ± 0	< 1	1 ± 0	< 1
Digestive gland	45 ± 6	92 ± 2	74 ± 5	97 ± 1	79 ± 4	97 ± 0
Kidney	< 1	< 1	< 1	< 1	< 1	< 1
Muscle	16 ± 1	1 ± 0	6 ± 1	< 1	4 ± 0	< 1
Head	22 ± 5	2 ± 0	11 ± 3	1 ± 0	7 ± 1	1 ± 0
Cuttlebone	1 ± 0	< 1	1 ± 0	<1	2 ± 1	<1

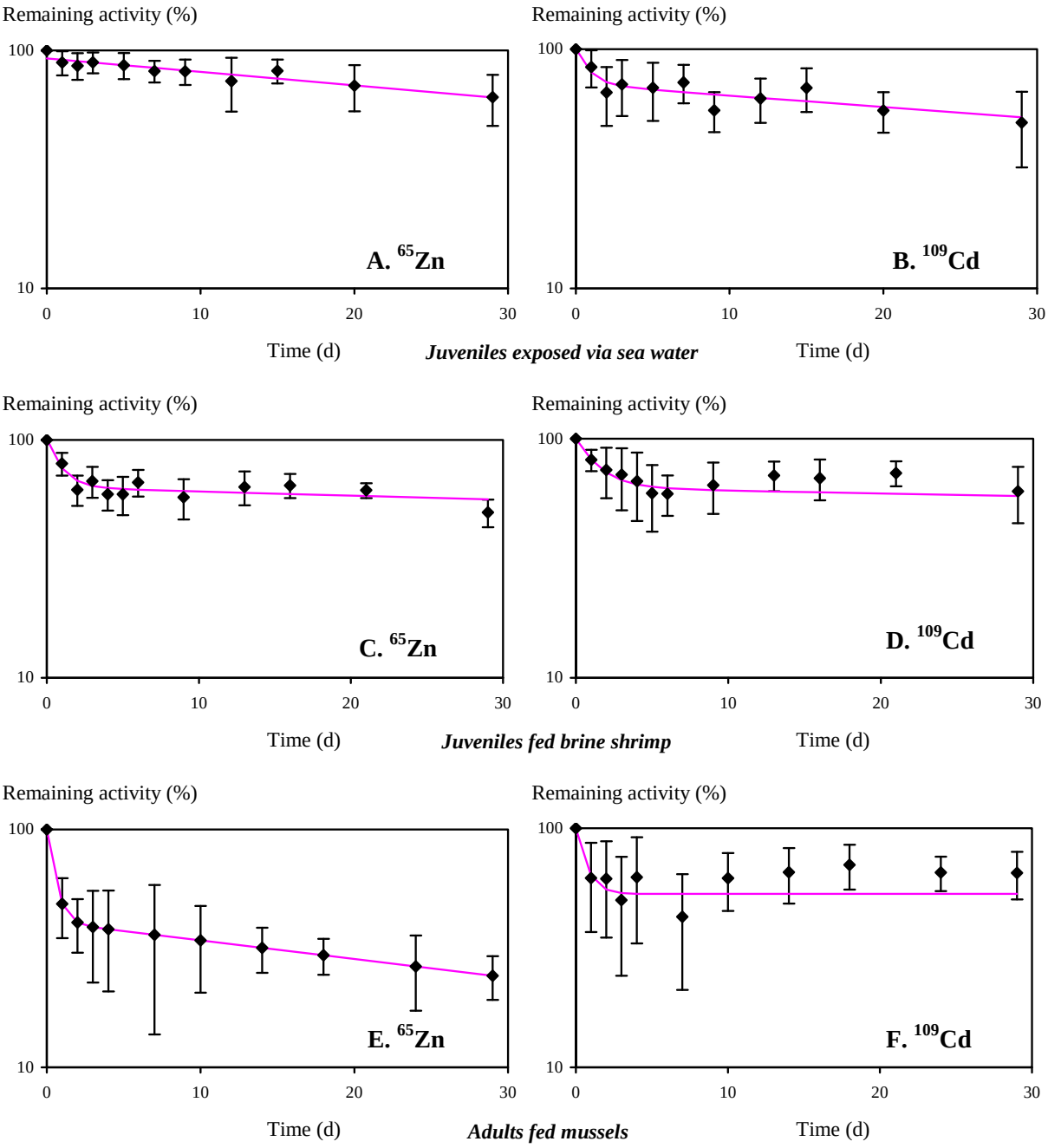
Remaining activity (%)
100
10
0
10
20
30
A. 65Zn
Time (d)
Remaining activity (%)
B. 109Cd
Time (d)
Juveniles exposed via sea water
Remaining activity (%)
C. 65Zn
Time (d)
Remaining activity (%)
D. 109Cd
Time (d)
Juveniles fed brine shrimp
Remaining activity (%)
E. 65Zn
Time (d)
Remaining activity (%)
F. 109Cd
Time (d)
Adults fed mussels

Figure 4.