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**Assessment of the exposure pathway in the uptake and distribution of  
americium and cesium in cuttlefish (*Sepia officinalis*) at different stages of  
its life cycle**

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18

19 **ABSTRACT:** Laboratory radiotracer experiments were performed to study the uptake,  
20 assimilation and retention of americium ( $^{241}\text{Am}$ ) and cesium ( $^{134}\text{Cs}$ ) by the common cuttlefish  
21 *Sepia officinalis*. Uptake and loss kinetics of the radionuclides were measured following  
22 exposure through sediments, seawater and food at different stages of the animal's life cycle.  
23 Sediment was found to be a minor uptake pathway for both radionuclides in juveniles.  
24 Following a short seawater exposure, cuttlefish accumulated  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , but only to  
25 limited extent (whole-body CF < 2). Among the cuttlefish organs, branchial hearts and their  
26 appendages displayed the highest degree of uptake for  $^{241}\text{Am}$  (CF = 42 and 16, respectively),  
27 but these tissues contained low percentage of total  $^{241}\text{Am}$  due to their relatively small  
28 contribution to whole organism weight. The major fraction of incorporated radionuclides was  
29 associated with muscular tissues (viz. 65 and 82% of total  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively).  
30 Whole-body loss of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  was relatively rapid ( $T_{b1/2}$  = 14 and 6 d, respectively).  
31 After dietary exposure, around 60% and 30 % of ingested  $^{241}\text{Am}$  was assimilated into the  
32 tissues of juvenile and adult cuttlefish, respectively. However, assimilated  $^{241}\text{Am}$  was more  
33 strongly retained in adults than in juveniles ( $T_{b1/2}$  = 28 vs 5 d, respectively), suggesting that  
34 different mechanisms govern  $^{241}\text{Am}$  elimination at both ages. Ingested  $^{134}\text{Cs}$  was assimilated  
35 to a similar extent in juveniles (29%) and adults (23%), but the depuration rate was 4 times  
36 faster in adults. Our results strongly suggest that these two radionuclides followed different  
37 excretion pathways, and that the mechanisms can vary with age for a given radionuclide.

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39 Key words: Accumulation; Biokinetics; Cephalopods; Radionuclides; Retention

## INTRODUCTION

Contamination of marine waters by radionuclides is a major concern in coastal areas which receive radioactive inputs from industries, accidents, and fallout from nuclear weapon testing. Surveys estimating concentrations of such radionuclides in water or sediments are often complemented by biomonitoring programs, and marine mussels have been used as biological monitors for radionuclides and heavy metals (Goldberg 1975, Goldberg et al 1978, Goldberg et al 1983, Goldberg & Bertine 2000). However, previous studies on the trophic transfer of trace elements and radionuclides have shown that herbivores such as mussels do not assimilate to any extent transuranic elements ingested with their food (e.g., Fowler 1982, Fisher et al. 1983, Warnau et al. 1996). Nevertheless this aspect has been little studied in higher trophic levels which are also used in many contaminant surveys. Therefore, there is a need to determine the bioaccumulation potential of marine carnivorous species for such elements.

Previous investigations with cephalopods have shown that these carnivorous species do bioaccumulate radionuclides in their tissues that can at times reach high levels (Suzuki et al. 1978, Guary et al. 1981, Yamada et al. 1999); however, little information is available on the pathways and rates of accumulation and retention of these radionuclides (Suzuki et al. 1978, Guary & Fowler 1982). Two long-lived radionuclides,  $^{241}\text{Am}$  and  $^{137}\text{Cs}$  (and  $^{134}\text{Cs}$ ), are present in fallout and also commonly found in nuclear wastes. The objective of our study was to examine the biokinetics of uptake and loss in cephalopods of these two contrasting radionuclides (particle-reactive americium and soluble cesium in sea water) in order to establish their bioaccumulation rates, tissue distribution and retention times as a function of (1) the uptake pathway, and (2) the life stage of the organism. The common cuttlefish *Sepia officinalis* was selected as a model species, and experimental exposures to these two

radionuclides via seawater, food and sediment were studied in both juvenile and adult individuals.

## MATERIAL AND METHODS

### Experimental organisms

Eggs of the common cuttlefish (*Sepia officinalis* L.) were obtained from cultured adults and were maintained in an aquarium with flowing seawater until hatching. Newly hatched juveniles ( $n = 25$ ;  $0.387 \pm 0.071$  g wet wt) were selected and used in the experiments. Adult cuttlefish ( $n = 18$ ;  $138 \pm 40$  g wet wt) were either reared in the Monaco Oceanographic Museum from hatching to one year old individuals, or collected by net fishing off Monaco ( $n = 5$ ;  $253 \pm 97$  g wet wt). All organisms were subsequently maintained in filtered seawater in constantly aerated open circuit aquaria (salinity: 36 p.s.u.; temperature:  $16.5 \pm 0.5^\circ\text{C}$ ; 12/12 h dark/light cycle) until used in the radiotracer experiments.

Prior to experimentation, adults were anaesthetised in 2% ethanol in seawater 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. In this way the same whole individual could be periodically radioanalyzed live in a small volume of sea water on the gamma well counter in order to reduce individual variability.

### Radionuclides

$^{241}\text{Am}$  [ $t_{1/2} = 433$  yr] and  $^{134}\text{Cs}$  [ $t_{1/2} = 2$  yr] purchased from Amersham, UK, as nitrate and chloride salts, respectively, were used to trace americium and cesium biokinetics. The use of  $^{134}\text{Cs}$  also served as an analogue for tracing the longer-lived  $^{137}\text{Cs}$  with a  $t_{1/2}$  of 33 yr. Stock solutions were prepared in their respective solutions (0.1 N) to obtain radioactivities which would allow using spikes of only a few microliters (typically 10 to 20  $\mu\text{L}$ ).

## **<sup>241</sup>Am and <sup>134</sup>Cs uptake via sediments**

Sediments (2.5 kg dry wt) from the North Sea (Audresselles, Pas-de-Calais, France) were spiked for 4 d with <sup>241</sup>Am and <sup>134</sup>Cs tracer using the rolling jar method (Murdoch et al. 1997). Both <sup>241</sup>Am and <sup>134</sup>Cs are rapidly adsorbed onto sediments typically reaching near equilibrium after approximately 1 day (Carroll 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 radionuclide concentration. Juvenile cuttlefish (n = 9) were exposed for 29 d in a 20-L plastic aquarium containing 3 L of natural seawater running over a 4 cm layer of spiked sediment. The level of sea water was maintained low in order to minimise the movements required for feeding and to maximise the contact time with sediments. During the experiment, all juvenile cuttlefish were fed twice daily with brine shrimp *Artemia salina* and were periodically  $\gamma$ -counted live in a well counter to follow the radionuclide uptake kinetics over the 29 d. At the end of the uptake experiment, 3 individuals were dissected to determine the distribution of the radionuclides among digestive gland, cuttlebone and remaining tissues (including other organs).

## **<sup>241</sup>Am and <sup>134</sup>Cs uptake via seawater and subsequent loss**

Newborn (n = 8) and adult (n = 5) cuttlefish were placed for 36 h and 8 h, respectively, in 70-L glass aquaria containing seawater spiked with <sup>241</sup>Am and <sup>134</sup>Cs (nominal activity: 6 kBq L<sup>-1</sup> each). Cuttlefish were then radioanalyzed and transferred to another 70-L aquarium supplied with natural flowing seawater. Juvenile cuttlefish were fed *A. salina* twice daily and were periodically  $\gamma$ -counted to follow radionuclide loss kinetics over 29 d. At the end of the loss period, 4 juveniles were dissected to determine the radionuclide distribution among digestive gland, cuttlebone and remaining tissues.

During the loss phase, adults were fed daily with soft parts of the mussel *Mytilus galloprovincialis*. Three adults were dissected after 8 h and the remaining two were dissected after 6 d of 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 sack, digestive gland, kidneys, mantle skin, mantle muscle, head and cuttlebone were separated, weighed, and their radionuclide content measured.

#### **<sup>241</sup>Am and <sup>134</sup>Cs accumulation from food**

To prepare radiolabelled food, mussels (*M. galloprovincialis*) and brine shrimp (*A. salina*) were exposed for 7 d in plastic aquaria containing 4 L of natural seawater spiked with <sup>241</sup>Am and <sup>134</sup>Cs (nominal activity: 6 kBq L<sup>-1</sup> each). Radiolabelled seawater was renewed daily and the organisms were subsequently used as food for newborn (brine shrimp) and adult (mussels) cuttlefish.

For identification purposes, each individual juvenile cuttlefish (n = 8) was enclosed in a separate compartment allowing free circulation of seawater in a 70-L aquarium. After 1 h of ingesting radiolabelled brine shrimp, each individual was immediately  $\gamma$ -counted and replaced in the aquarium. From that time on cuttlefish were fed twice daily with non-contaminated *A. salina* and periodically  $\gamma$ -counted to determine radiotracer loss kinetics and assimilation efficiency. Throughout the depuration period (29 d), feces were removed 3 times per day to reduce possible indirect contamination by radiotracer recycling through leaching from the feces. 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 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.**

Radioactivity was measured using a high-resolution  $\gamma$ -spectrometry system consisting of three 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, Intertechnique). The detectors were calibrated with appropriate standards for each of the counting geometries used, and measurements were corrected for background and physical decay of the radionuclides. 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 the extremely small dissected organs. Counting times ranged from 10 min to 1 h for whole cuttlefish, mussels and brine shrimp, and from 10 min to 48 h for the dissected organs and tissues. Following the relatively short counting periods in the containers of sea water, none of the organisms displayed any obvious abnormal or stressed behaviour when returned to their aquaria.

#### **Data and statistical analyses.**

Uptake of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  from sediments and seawater was expressed, respectively, as whole-body transfer factors (TF) and concentration factors (CF) over time ( $\text{Bq g}^{-1}$  wet wt organism divided by the time-integrated  $\text{Bq g}^{-1}$  in sediments (TF) or seawater (CF). Radionuclide 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:

$$A_t = A_0 e^{-k t},$$



where  $A_t$  and  $A_0$  are remaining activities (%) at time  $t$  (d) and 0, respectively, or by a 2-component exponential model:

$$A_t = A_{0s} e^{-k_s t} + A_{0l} e^{-k_l t},$$

where the 's' subscript refers to a short-lived component (s component) and the 'l' subscript refers to a long-lived component (l component) (Whicker & Schultz 1982, Warnau et al. 1996). The exponential model showing the best fit (based on calculation of the determination coefficients,  $R^2$ , and examination of the residuals) was selected.

The parameter  $k$  allows calculating of the radionuclide biological half-life (d) using the following equation:

$$T_{b1/2} = \ln 2/k.$$

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 radionuclide distribution among cuttlefish tissues and organs were tested for significance by the G procedure (adapted from the log-likelihood ratio test) for 2xk 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 HSD Tukey's multiple comparison test. The significance level for statistical analyses was always set at  $\alpha = 0.05$ .

## RESULTS

### Sediment exposure

Regular measurements of  $^{241}\text{Am}$  concentration in sediment did not show any significant variation during the experimental time course ( $14.5 \pm 1.8 \text{ Bq g}^{-1}$  wet wt) while  $^{134}\text{Cs}$  activities decreased from  $12.4 \pm 0.1$  to  $7.0 \pm 0.4 \text{ Bq g}^{-1}$  wet wt.

Very low  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  activities were recorded in juveniles cuttlefish even after 29 d of exposure, and transfer factors (TF) were lower than 0.5 for both elements. Dissection of 3 individuals after 29 d of exposure showed that for both radionuclides the digestive gland contained the highest proportion of the whole-body burden, i.e.  $47 \pm 28 \%$  of  $^{241}\text{Am}$  and  $49 \pm 12 \%$  of  $^{134}\text{Cs}$  (Table 1).

### **Seawater exposure**

Regular monitoring of the radionuclide concentrations in seawater allowed calculation of time-integrated radioactivities, viz.  $6.4 \pm 0.3$  and  $8.6 \pm 0.7 \text{ kBq L}^{-1}$  for  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively.

**Juveniles.** The whole-body activities measured after 36 h exposure in spiked seawater were  $38 \pm 10$  and  $37 \pm 1 \text{ Bq g}^{-1}$  wet wt for  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively, giving relatively low mean calculated whole-body CFs of  $6 \pm 2$  and  $4 \pm 1$  for these radionuclides.

Following transfer to non-contaminated seawater, loss kinetics of  $^{241}\text{Am}$  in juvenile cuttlefish were best fitted by a single-component exponential model whereas loss of  $^{134}\text{Cs}$  was best described by a two-component model (Figs 1A and 1B; Table 2). Loss kinetics were characterised by a biological half-life ( $T_{b/2}$ ) of 2 wk for  $^{241}\text{Am}$  and 1 wk for  $^{134}\text{Cs}$ .

At the end of the depuration period,  $^{134}\text{Cs}$  was mainly associated with the digestive gland of the young cuttlefish ( $61 \pm 4\%$  of whole-body activity) whereas  $^{241}\text{Am}$  was principally retained in the remaining tissues ( $61 \pm 13\%$ ) (Table 1). The lowest fraction of both radiotracers was found in the cuttlebone ( $< 15\%$  of the total activity).

**Adults.**  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  activities recorded in whole-body as well as 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  $^{241}\text{Am}$  were found in the branchial hearts and their appendages ( $264 \pm 85$  and  $103 \pm 66 \text{ Bq g}^{-1}$  wet wt, respectively). In the case of  $^{134}\text{Cs}$ , the

branchial hearts, their appendages, gills and digestive tract displayed the highest activities, ranging from 9 to 13 Bq g<sup>-1</sup> wet wt.

When considering the tissue distribution of the radionuclides, muscle and skin of adults (i.e. the sum of the mantle muscles, skin and head) contained the highest proportion of <sup>241</sup>Am and <sup>134</sup>Cs, viz. 68 and 85%, respectively (Table 3). A somewhat lesser <sup>241</sup>Am fraction was found in the branchial hearts and digestive gland (10 ± 2% for both tissues). The radionuclide distribution among the tissues did not vary significantly (G test, p > 0.05) between the beginning and the end of the depuration period (Table 3).

### **Food exposure**

In these experiments, juveniles (n = 8) were fed *ad libitum* radiolabelled adult brine shrimp for 1 h and adult cuttlefish (n = 18) ingested a total of 123 radiolabelled mussels during a 2-h feeding period. Immediately after feeding, all cuttlefish were γ-counted for determination of their radionuclide content.

**Juveniles.** The loss kinetics of ingested <sup>241</sup>Am and <sup>134</sup>Cs were best fitted by a 2-component exponential model composed of one rapid loss component followed by a single slow component (Figs 1C and 1D; Table 2). The short-lived component was derived from 40% and 70% of the initially ingested <sup>241</sup>Am and <sup>134</sup>Cs activities, respectively (Table 2), and was characterised by a T<sub>b/2s</sub> < 1 d for both radionuclides. The long-lived component, which represents the fraction of the radionuclides actually absorbed by cuttlefish, displayed a T<sub>b/2l</sub> of 5 d for <sup>241</sup>Am and 66 d for <sup>134</sup>Cs (Table 2). The same long-lived component allowed estimation of the assimilation efficiencies (AE) of the ingested nuclides. Results showed that <sup>241</sup>Am was readily assimilated in juveniles with an AE of 60% whereas the AE of <sup>134</sup>Cs was much lower, viz. 29% (Table 2). Dissections performed 29 d after feeding indicated that the highest proportion of remaining activity of both nuclides occurred in the digestive gland (ca. 60% of the whole-body activity; Table 1).

**Adults.** The loss kinetics of both radionuclides ingested with food by adult cuttlefish were best described by a 2-component exponential model. As shown Figs 1E and 1F and in Table 2, 69 and 78% of the ingested activity of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively, were rapidly lost with a  $T_{b/2s}$  of 4 and 13 h, respectively. The assimilated fraction of ingested  $^{241}\text{Am}$  was much lower in adults than in juveniles (AE = 31 vs 60%) but was lost at a slower rate from adults with a  $T_{b/2l}$  of 28 d compared to 5 d in juveniles. For  $^{134}\text{Cs}$ , AEs were nearly similar for both age groups (AE = 23 vs 29% in adults and juveniles, respectively), however the radionuclide was depurated much faster in adults ( $T_{b/2l} = 16$  d) than in juveniles ( $T_{b/2l} = 66$  d). The tissue distribution of ingested radionuclides was determined on several occasions after feeding (Table 4). At the end of the depuration period, both  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  were predominantly distributed in the digestive gland (viz. 98 and 54%, respectively). The distribution of  $^{241}\text{Am}$  among tissues remained unchanged for 29 d of observation; in contrast, some significant changes were observed for  $^{134}\text{Cs}$  (G-test,  $p \leq 0.01$ ). For example, the proportion of  $^{134}\text{Cs}$  activity decreased in the muscular tissues (mantle muscles and head), whereas between 1 and 18 days of excretion it increased in the digestive gland (Table 4).

## DISCUSSION

Cephalopods are an important resource of marine food and are fished and consumed in large quantities all around the world (Amaratunga 1983). Hence, the intake of contaminants such as radionuclides by humans through cephalopod consumption is a matter of potential concern. Cephalopods have been reported to concentrate natural and anthropogenic radionuclides such as  $^{210}\text{Po}$ ,  $^{210}\text{Pb}$ ,  $^{137}\text{Cs}$ , and  $^{239+240}\text{Pu}$  in their tissues (e.g. Smith et al. 1984, Finger & Smith 1987, Yamada et al. 1999); however, little is known about the behaviour of radionuclides in these higher trophic level molluscs. To the best of our knowledge, only two species of cephalopods, viz. the octopus *Octopus vulgaris* and the squid *Doryteuthis bleekeri*, have been

used experimentally for investigating Am, Cs, and Pu biokinetics (Suzuki et al. 1978, Guary & Fowler 1982). These works were limited to measuring the uptake from seawater (i.e. Suzuki et al. 1978) or used a less than optimal experimental approach such as injecting the prey with radionuclides for the feeding experiments (Guary & Fowler 1982).

Cephalopods are found in a great variety of habitats from coastal waters to very deep ocean environments, with some living in direct contact with bottom sediments and others experiencing different environments during their life cycle (e.g. demersal species becoming temporarily pelagic during migration). Therefore, there is an obvious need to specifically determine 1) the uptake and retention of radionuclides at different stages of the life cycle of cephalopods, and 2) to assess the relative importance of the different pathways of exposure to radionuclides (sediments, seawater and food). In this context, the common cuttlefish *Sepia officinalis* appeared to be a good model cephalopod for such experiments as it spends part of its time buried in the sediment and is easy to rear and manipulate under laboratory conditions.

After a 1 month exposure to  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  through sediments, juvenile cuttlefish still exhibited very low transfer factors ( $\text{TF} < 0.5$ ), indicating that direct contamination due to burying into sediments is a minor uptake pathway for these radionuclides in cephalopods. The occurrence of a substantial fraction of both nuclides in internal tissues (viz. digestive gland and cuttlebone), which have no direct contact with the sediment, suggests that both radionuclides were progressively translocated from the tissues in direct contact with sediment and pore water to the digestive gland and, to a lesser extent, the cuttlebone (see Table 1). Such a translocation of elements to the cuttlebone was observed in a previous study on bioaccumulation of Cd in *S. officinalis* (Bustamante et al. 2002).

Following an acute contamination of adults via seawater, activities recorded in the whole cuttlefish suggest that they do not efficiently accumulate  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  directly from the dissolved phase. Indeed, both elements displayed low whole-body CFs ( $\text{CF} = 2$  for  $^{241}\text{Am}$  and

296 CF = 1 for  $^{134}\text{Cs}$ ). Nevertheless the 8 hr acute contamination time was relatively short and  
 297 CFs are likely to be higher after a longer period of exposure. Activities of  $^{134}\text{Cs}$  measured in  
 298 the different organs and tissues were all of the same order of magnitude. In contrast, for  
 299  $^{241}\text{Am}$  the organs involved in respiration (the branchial hearts, their appendages and the gills)  
 300 and digestion (digestive gland) displayed higher activities compared to other body  
 301 compartments (see Table 3). However, in terms of their relative distribution in the whole  
 302 body, both radionuclides were mainly located in muscular tissues which represent the main  
 303 fraction (viz. 75%) of the total body weight: muscles and head contained 65% and 82% of the  
 304 total  $^{241}\text{Am}$  and  $^{134}\text{Cs}$ , respectively. A longer exposure (14 d) of *Octopus vulgaris* to  $^{137}\text{Cs}$  in  
 305 sea water gave a similar distribution (i.e. 88%) of the radioisotope in the edible parts (Suzuki  
 306 et al. 1978). In contrast, a 15-d exposure of the same species in seawater spiked with  $^{241}\text{Am}$   
 307 resulted in only ca. 20% of the retained radioactivity being found in the muscular parts with  
 308 most of the  $^{241}\text{Am}$  concentrated in the branchial hearts and their appendages (Guary & Fowler  
 309 1982). In our experiments with *S. officinalis*, these tissues contained low percentages of the  
 310 total  $^{241}\text{Am}$ , most probably because of the short duration of the exposure in sea water.  
 311 Nevertheless, even after 8 hours they significantly concentrated the radionuclide with CFs as  
 312 high as 42 in the branchial hearts and 16 in the appendages.

313 Both field and laboratory investigations with cephalopods have demonstrated the ability of  
 314 branchial hearts to concentrate transuranic elements to fairly high levels (Guary et al. 1981,  
 315 Guary & Fowler 1982). This ability could be related to the presence of polyhedral cells  
 316 containing granular, Fe-rich, pigment concretions (adenochromes) (e.g., Fox & Updegraff  
 317 1943, Nardi & Steinberg 1974). The affinity of  $^{241}\text{Am}$  for adenochromes in the branchial  
 318 hearts has been demonstrated using autoradioradiographic techniques (Miramand & Guary  
 319 1981); however, adenochromes have not been found in the appendages of the branchial hearts  
 320 (Nardi & Steinberg 1974), an observation which suggests that they serve as an excretion  
 321 pathway for  $^{241}\text{Am}$  rather than as storage sites.

322 Following exposure of juveniles in contaminated seawater, subsequent  $^{241}\text{Am}$  and  $^{134}\text{Cs}$   
323 elimination over a one month period displayed a one- and a two-component exponential loss  
324 model, respectively. Whole-body loss was relatively rapid for both nuclides with a mean  $T_{b1/2}$   
325 of 14 and 6 d, respectively. After 29 d of depuration, residual  $^{241}\text{Am}$  was mainly located in the  
326 remaining tissues (comprising the branchial hearts) of juveniles. However, as the juvenile  
327 branchial heart was not fully developed, additional work is needed to examine its role as a  
328 preferential storage organ such as occurs in adults.

329 In the case of dietary exposure,  $31 \pm 3\%$  of the ingested  $^{241}\text{Am}$  was assimilated into the tissues  
330 of adult cuttlefish, whereas in contrast  $^{241}\text{Am}$  was absorbed to a much greater extent in  
331 juveniles ( $\text{AE} = 60 \pm 10\%$ ). This difference between AEs could be due to differences in  
332 efficiency of digestion between juveniles and adults, since digestive metabolism is thought to  
333 decrease with age in cephalopods (Mangold 1989). More likely, however, the difference could  
334 also be partly due to variations in the bioavailability of  $^{241}\text{Am}$  in the food used for juveniles  
335 (brine shrimp) compared to that ingested by adults (i.e. mussels). Indeed, different storage  
336 mechanisms in prey can determine metal bioavailability to higher trophic levels (Wallace &  
337 Lopez 1997, Wallace & Luoma 2003, Seebaugh et al. 2005), which in a similar fashion could  
338 lead to different proportions of transferable  $^{241}\text{Am}$ . Overall, such very high AEs for  $^{241}\text{Am}$  in  
339 the common cuttlefish are rather unique whereas in herbivorous bivalves, many crustaceans,  
340 echinoids, and fish, assimilation of particle-reactive transuranic elements is typically very low  
341 (e.g. Fowler et al., 1976; Pentreath 1977, 1981; Fisher et al., 1983; Carvalho and Fowler,  
342 1985; Warnau et al., 1996). Such a difference could be related to the organism's feeding  
343 regime since cephalopods are strict carnivores. For instance, unexpected high AEs (up to  
344 60%) of plutonium have also been found in carnivorous crustaceans, viz. the crabs *Carcinus*  
345 *maenas* and *Cancer pagurus* (Fowler and Guary, 1977). Hence, the contribution of the  
346 radionuclide from the trophic pathway is very likely to be strongly enhanced in certain  
347 carnivorous invertebrates.

348 Once assimilated,  $^{241}\text{Am}$  was retained to a much greater degree in adults, with a half-life  
 349 approximately 6 times longer than in juveniles (i.e. 28 d vs 5 d), which suggests that different  
 350 processes govern  $^{241}\text{Am}$  elimination/retention in the two age groups. In other molluscs such as  
 351 mussels,  $^{241}\text{Am}$  has been reported to be strongly retained in the digestive gland (Bjerregaard et  
 352 al. 1985, Fisher & Teyssié 1986), a finding which is in agreement with our own observations.  
 353 Indeed, after 29 d of depuration, the major fraction of residual  $^{241}\text{Am}$  was in the digestive  
 354 gland, with a much higher fraction in adults than in juvenile cuttlefish (98% vs 59%). In the  
 355 digestive gland of *Octopus vulgaris*, Guary & Fowler (1982) reported that  $^{241}\text{Am}$  is likely  
 356 associated with the cellular waste products such as brown bodies. Considering this hypothesis  
 357 together with our experimental observations, the longer retention of  $^{241}\text{Am}$  observed in adult  
 358 *S. officinalis* could be due to a more rapid turnover of digestive cells in juveniles, thus  
 359 resulting in a higher  $^{241}\text{Am}$  excretion rate.

360 In contrast to  $^{241}\text{Am}$ , ingested  $^{134}\text{Cs}$  was assimilated to a similar extent in juveniles (29%) and  
 361 adults (23%), and the depuration rate constant was 4 times higher in adults resulting in a  
 362 significantly much shorter  $^{134}\text{Cs}$  half-life in adults (16 d) than in juveniles (66 d) (Table 2).  
 363 The longer retention time of  $^{134}\text{Cs}$  in juveniles is difficult to explain since, for certain  
 364 transition elements (Ag, Cd, Co and Zn) previously investigated in cuttlefish (Bustamante et  
 365 al. 2002, 2004) as well for  $^{241}\text{Am}$  (our study), early juveniles displayed shorter retention half-  
 366 times than adults. The main difference in tissue distribution of  $^{134}\text{Cs}$  between adults and  
 367 juveniles was the higher proportion present in the cuttlebone ( $22 \pm 21\%$  in juveniles vs  $2 \pm 0\%$   
 368 in adults; see Tables 1 and 4). This higher skeleton-associated fraction is most likely tightly  
 369 bound and hence results in the high retention half-time observed. Although, our results clearly  
 370 indicate that  $^{134}\text{Cs}$  does not follow the same excretion pathway as  $^{241}\text{Am}$ , the above  
 371 interpretation should be considered with caution since to the best of our knowledge,  
 372 calcareous skeletons have not been shown to act as a particularly efficient sink for cesium in  
 373 contrast with other elements such as, e.g.,  $^{241}\text{Am}$  or Pb (see e.g. Grillo et al. 1981, Warnau et



al. 1998). Furthermore, in our feeding experiment the very low activities measured in minute organs such as juvenile cuttlebone were frequently associated with low counting accuracy, which in turn can lead to a rather poor estimation of radioactivities and hence radionuclide distribution (as indicated by the elevated SD value of the cuttlebone-associated fraction of  $^{134}\text{Cs}$ ). Clearly, further study is needed to better understand the observed differences in the fate of  $^{134}\text{Cs}$  and  $^{241}\text{Am}$  once taken up in young and adult cephalopod tissues.

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Captions to Figure

Fig. 1. *Sepia officinalis*. Whole-body loss kinetics of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  (% of remaining activity; mean  $\pm$  SD):

(A, B) 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) juvenile cuttlefish previously fed radiolabelled brine shrimp (n = 8 from day 0 to 22 and n = 5 on day 29);

(E, F) adult cuttlefish previously fed radiolabelled mussels (n = 18 on day 0, n = 15 from day 1 to 18, n = 12 from day 19 to 29).

Parameters for the best fitting equations are given in Table 3.

**Table 1.** *Sepia officinalis*. Distribution (%; mean  $\pm$  SD) of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  among three body compartments of juvenile cuttlefish (1) after a 29-d exposure to spiked sediments, (2) after a 29-d depuration following a 36-h exposure to spiked seawater, and (3) after a 29-d depuration following ingestion of spiked food (brine shrimp).

Exposure pathway	N	Body compartment		
		Digestive gland	Cuttlebone	Remaining tissues
<b>1. Sediments (29-d exposure)</b>	3			
$^{241}\text{Am}$		49 $\pm$ 12	12 $\pm$ 3	39 $\pm$ 15
$^{134}\text{Cs}$		47 $\pm$ 28	17 $\pm$ 4	36 $\pm$ 24
<b>2. Seawater (29-d depuration)</b>	4			
$^{241}\text{Am}$		27 $\pm$ 13	13 $\pm$ 0	61 $\pm$ 13
$^{134}\text{Cs}$		61 $\pm$ 4	5 $\pm$ 0	34 $\pm$ 4
<b>3. Feeding (29-d depuration)</b>	5			
$^{241}\text{Am}$		59 $\pm$ 23	12 $\pm$ 10	29 $\pm$ 16
$^{134}\text{Cs}$		60 $\pm$ 27	22 $\pm$ 21	18 $\pm$ 14

**Table 2.** *Sepia officinalis*. Parameters of the equations best fitting the whole-body loss kinetics of  $^{241}\text{Am}$  and  $^{134}\text{Cs}$  in cuttlefish previously exposed to the radionuclides via different pathways: (1) juveniles previously exposed for 36 h via seawater; (2) juveniles previously fed radiolabelled brine shrimp; (3) adults previously fed radiolabelled mussels.

O and T: one- and two-exponential loss equations, respectively;  $A_{ol}$ : assimilation efficiency (AE); ASE: asymptotic standard error;  $R^2$ : determination coefficient; p: probability of the model adjustment.

Pathway	Model	$A_{os}$ (ASE)	$\lambda_s$ (ASE)	$T_{b1/2s}$ (days)	$A_{ol}$ (ASE)	$\lambda_l$ (ASE)	$T_{b1/2l}$ (days)	$R^2$	p
<b>1. Loss in juveniles after seawater exposure</b>									
$^{241}\text{Am}$	O	87.7 (2.8)	0.048 (0.005)	14	-	-	-	0.96	< 0.001
$^{134}\text{Cs}$	T	74.6 (7.1)	1.015 (0.163)	0.7	25.6 (6.8)	0.114 (0.036)	6.1	0.97	< 0.001
<b>2. Loss in juveniles after a single feeding on brine shrimp</b>									
$^{241}\text{Am}$	T	39.6 (10.5)	1.282 (0.654)	0.5	60.3 (10.1)	0.137 (0.029)	5.1	0.95	< 0.001
$^{134}\text{Cs}$	T	70.3 (4.4)	0.972 (0.153)	0.7	29.2 (3.6)	0.011 (0.008)	66	0.98	< 0.001
<b>3. Loss in adults after a single feeding on mussels</b>									
$^{241}\text{Am}$	T	68.6 (3.8)	4.125 (3.683)	0.17	31.4 (2.5)	0.025 (0.009)	28	0.95	< 0.001
$^{134}\text{Cs}$	T	77.6 (4.4)	1.310 (0.197)	0.53	22.5 (3.7)	0.045 (0.019)	16	0.95	< 0.001



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

Tissue	% wet wt	<sup>241</sup> Am					<sup>134</sup> Cs				
		Accumulation (8 h)			Depuration (6 d)		Accumulation (8 h)			Depuration (6 d)	
		CF	Activity	%	Activity	%	CF	Activity	%	Activity	%
Branchial heart appendages	0.03 $\pm$ 0.004	16	103 $\pm$ 66	< 1	56	< 1	1	9 $\pm$ 2	< 1	1	< 1
Branchial hearts	0.10 $\pm$ 0.02	42	264 $\pm$ 85	3 $\pm$ 0	203	3	2	13 $\pm$ 1	< 1	2	< 1
Gills	2.3 $\pm$ 0.3	7	42 $\pm$ 14	10 $\pm$ 2	11	4	1	10 $\pm$ 2	4 $\pm$ 0	2	2
Digestive tract	2.6 $\pm$ 0.6	2	15 $\pm$ 5	4 $\pm$ 2	4	2	1	10 $\pm$ 1	4 $\pm$ 1	1	1
Genital tract	3.6 $\pm$ 1.0	1	9 $\pm$ 5	3 $\pm$ 1	2	1	< 1	4 $\pm$ 1	2 $\pm$ 0	< 1	4
Ink sack	0.6 $\pm$ 0.2	2	12 $\pm$ 1	1 $\pm$ 0	7	1	1	7 $\pm$ 3	1 $\pm$ 0	2	< 1
Skin	6.4 $\pm$ 2.1	1	6 $\pm$ 4	3 $\pm$ 1	3	2	< 1	4 $\pm$ 2	3 $\pm$ 0	< 1	4
Digestive gland	4.3 $\pm$ 1.2	3	22 $\pm$ 16	10 $\pm$ 2	28	11	< 1	3 $\pm$ 2	2 $\pm$ 1	1	1
Kidney	0.07 $\pm$ 0.07	2	13 $\pm$ 5	< 1	4	< 1	1	8 $\pm$ 5	< 1	1	< 1
Muscle	35 $\pm$ 2	1	7 $\pm$ 2	26 $\pm$ 4	10	52	1	6 $\pm$ 1	36 $\pm$ 3	2	55
Head	40 $\pm$ 1	1	9 $\pm$ 3	39 $\pm$ 1	4	23	1	7 $\pm$ 2	46 $\pm$ 3	1	32
Cuttlebone	5.1 $\pm$ 0.6	< 1	2 $\pm$ 1	1 $\pm$ 1	2	1	< 1	1 $\pm$ 1	1 $\pm$ 1	< 1	< 1
Whole cephalopod	100	2	10 $\pm$ 3	100	11	100	1	6 $\pm$ 2	100	3	100

**Table 4.** *Sepia officinalis*. Radionuclide distribution among tissues (%; mean  $\pm$  SD, n = 3) of adult cuttlefish 1, 18, and 29 d after a single feeding on radiolabelled mussels.

Body compartments	1 d		18 d		29 d	
	$^{241}\text{Am}$	$^{134}\text{Cs}$	$^{241}\text{Am}$	$^{134}\text{Cs}$	$^{241}\text{Am}$	$^{134}\text{Cs}$
Branchial heart appendages	< 1	6 $\pm$ 9	< 1	1 $\pm$ 0	< 1	2 $\pm$ 0
Branchial hearts	3 $\pm$ 0	1 $\pm$ 0	< 1	2 $\pm$ 1	< 1	1 $\pm$ 1
Gills	1 $\pm$ 1	3 $\pm$ 2	< 1	2 $\pm$ 1	< 1	2 $\pm$ 1
Digestive tract	1 $\pm$ 1	3 $\pm$ 1	< 1	6 $\pm$ 0	< 1	9 $\pm$ 1
Genital tract	< 1	2 $\pm$ 1	< 1	9 $\pm$ 1	< 1	10 $\pm$ 6
Ovary	< 1	1 $\pm$ 1	< 1	3 $\pm$ 1	< 1	5 $\pm$ 2
Ink sack	< 1	1 $\pm$ 0	< 1	1 $\pm$ 0	< 1	2 $\pm$ 1
Skin	< 1	1 $\pm$ 0	< 1	2 $\pm$ 1	< 1	2 $\pm$ 0
Digestive gland	89 $\pm$ 7	31 $\pm$ 6	97 $\pm$ 1	57 $\pm$ 8	98 $\pm$ 0	54 $\pm$ 12
Kidney	< 1	1 $\pm$ 0	< 1	4 $\pm$ 1	< 1	2 $\pm$ 0
Muscle	6 $\pm$ 8	22 $\pm$ 3	1 $\pm$ 0	6 $\pm$ 5	< 1	5 $\pm$ 2
Head	2 $\pm$ 1	28 $\pm$ 6	1 $\pm$ 0	6 $\pm$ 5	1 $\pm$ 0	6 $\pm$ 2
Cuttlebone	< 1	1 $\pm$ 0	< 1	2 $\pm$ 1	< 1	2 $\pm$ 0

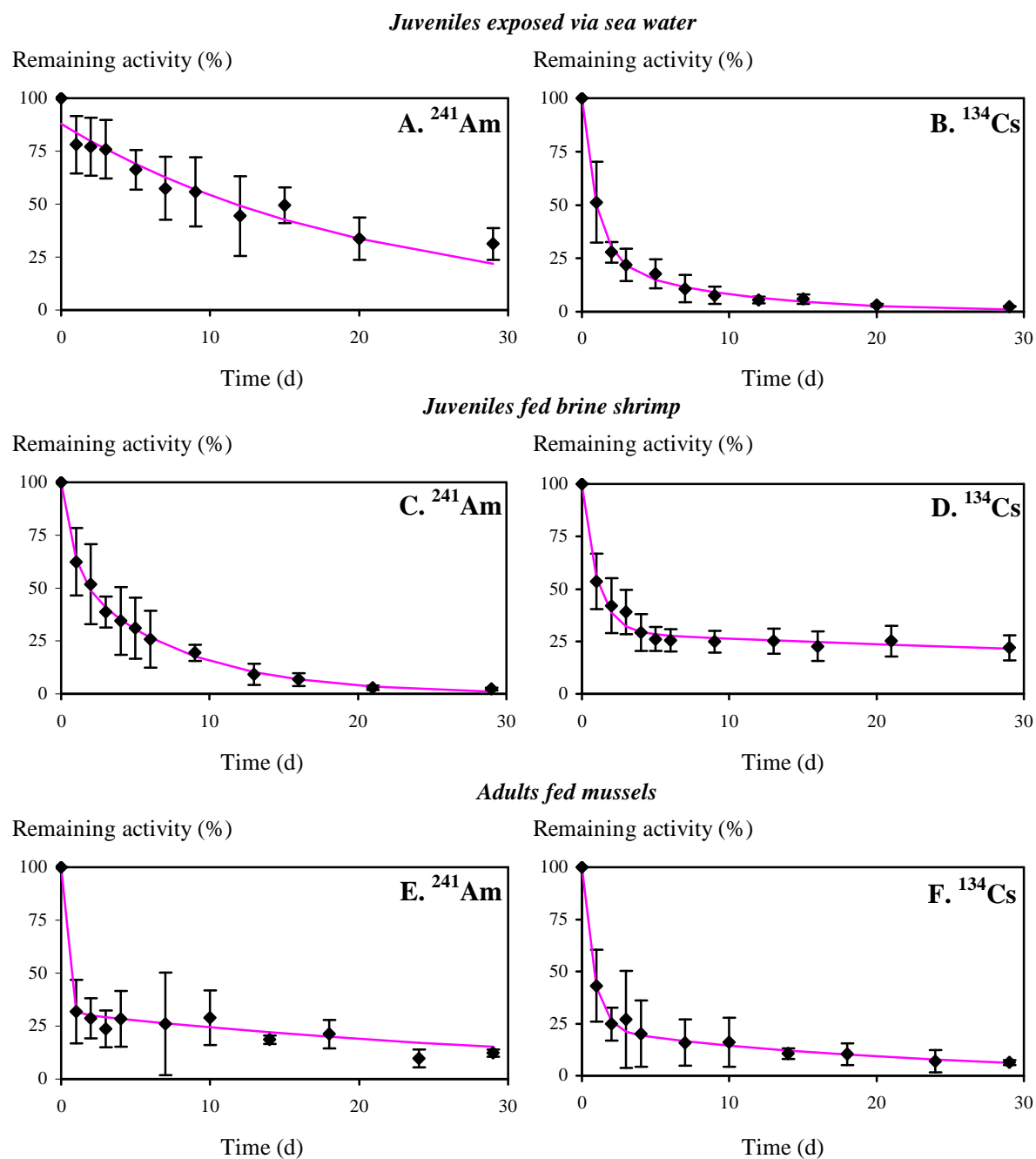


Fig. 1