Journal of Experimental Marine Biology and Ecology
Volume 331, Issue 2 , 18 April 2006, Pages 198-207
http://dx.doi.org/10.1016/j.jembe.2005.10.018

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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Abstract:
Laboratory radiotracer experiments were performed to study the uptake, assimilation and retention of americium (^{241}Am) and cesium (^{134}Cs) by the common cuttlefish *Sepia officinalis*. Uptake and loss kinetics of the radionuclides were measured following exposure through sediments, seawater and food at different stages of the animal's life cycle. Sediment was found to be a minor uptake pathway for both radionuclides in juveniles. Following a short seawater exposure, cuttlefish accumulated ^{241}Am and ^{134}Cs, but only to a limited extent (whole-body CF < 2). Among the cuttlefish organs, branchial hearts and their appendages displayed the highest degree of uptake for ^{241}Am (CF = 42 and 16, respectively), but these tissues contained low percentage of total ^{241}Am due to their relatively small contribution to whole organism weight. The major fraction of incorporated radionuclides was associated with muscular tissues (viz. 65% and 82% of total ^{241}Am and ^{134}Cs, respectively). Whole-body loss of ^{241}Am and ^{134}Cs was relatively rapid ($T_{b½}$ = 14 and 6 days, respectively). After dietary exposure, around 60% and 30% of ingested ^{241}Am was assimilated into the tissues of juvenile and adult cuttlefish, respectively. However, assimilated ^{241}Am was more strongly retained in adults than in juveniles ($T_{b½}$ = 28 vs. 5 days, respectively), suggesting that different mechanisms govern ^{241}Am elimination at both ages. Ingested ^{134}Cs was assimilated to a similar extent in juveniles (29%) and adults (23%), but the depuration rate was four times faster in adults. Our results strongly suggest that these two radionuclides follow different excretion pathways and that the mechanisms can vary with age for a given radionuclide.

Keywords: Accumulation; Biokinetics; Cephalopods; Radionuclides; Retention

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

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ABSTRACT: Laboratory radiotracer experiments were performed to study the uptake, assimilation and retention of americium (^{241}Am) and cesium (^{134}Cs) by the common cuttlefish *Sepia officinalis*. Uptake and loss kinetics of the radionuclides were measured following exposure through sediments, seawater and food at different stages of the animal's life cycle. Sediment was found to be a minor uptake pathway for both radionuclides in juveniles. Following a short seawater exposure, cuttlefish accumulated ^{241}Am and ^{134}Cs, but only to limited extent (whole-body CF < 2). Among the cuttlefish organs, branchial hearts and their appendages displayed the highest degree of uptake for ^{241}Am (CF = 42 and 16, respectively), but these tissues contained low percentage of total ^{241}Am due to their relatively small contribution to whole organism weight. The major fraction of incorporated radionuclides was associated with muscular tissues (viz. 65 and 82% of total ^{241}Am and ^{134}Cs, respectively). Whole-body loss of ^{241}Am and ^{134}Cs was relatively rapid ($Tb_{1/2}$ = 14 and 6 d, respectively). After dietary exposure, around 60% and 30 % of ingested ^{241}Am was assimilated into the tissues of juvenile and adult cuttlefish, respectively. However, assimilated ^{241}Am was more strongly retained in adults than in juveniles ($Tb_{1/2}$ = 28 *vs* 5 d, respectively), suggesting that different metabolic processes govern ^{241}Am elimination at both ages. Ingested ^{134}Cs was assimilated to a similar extent in juveniles (29%) and adults (23%), but the depuration rate was 4 times slower in adults. Our results strongly suggest that these two radionuclides followed different excretion pathways, and that the mechanisms can vary with age for a given radionuclide.

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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 chemicals in water or sediments are generally complemented by biomonitoring programs and marine mussels are often 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 to any extent assimilate 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 routes and rates of accumulation and retention of these radionuclides (Suzuki et al. 1978, Guary & Fowler 1982). Two long-lived radionuclides, the particle-reactive ^{241}Am and the soluble ^{134}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 of these two contrasting radionuclides in cephalopods in order to establish their bioaccumulation rates, tissue distribution and retention times depending on (1) the uptake pathway, and (2) the life stage of the organism. The common cuttlefish *Sepia officinalis* was selected as an experimental model, and exposures to these two radionuclides via seawater, food and sediment were studied in 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; the newly hatched juveniles (n = 25; 0.387 ± 0.071 g wet wt) were then used in the experiments. Adult cuttlefish (n = 18; 138 ± 40 g wet wt) were reared in the Monaco Oceanographic Museum from hatching to one year old organisms, or collected by net fishing off Monaco (n = 5; 253 ± 97 g wet wt). All organisms were maintained in filtered seawater in constantly aerated open circuit aquaria (salinity: 36 p.s.u.; temperature: 16.5 ± 0.5°C; 12/12 h dark/light cycle).

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.

Radionuclides

^{241}Am [$t_{½}$ = 433 yr] and ^{134}Cs [$t_{½}$ = 2 yr] purchased from Amersham, UK, as nitrate and chloride salts, respectively, were used to trace americium ad cesium biokinetics. 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 µL).

^{241}Am and ^{134}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 ^{241}Am and ^{134}Cs 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 radionuclide

concentration. Juvenile cuttlefish (n = 9) were exposed for 29 d in a 20-L plastic aquarium containing ca. 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 γ-counted 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).

^{241}Am and ^{134}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 ^{241}Am and ^{134}Cs (nominal activity: 6 kBq L^{-1} 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 γ-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.

^{241}Am and ^{134}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 ^{241}Am and ^{134}Cs (nominal activity: 6 kBq L^{-1} 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 γ-counted. From that time on cuttlefish were fed twice daily with non-contaminated *A. salina*, and regularly γ-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 γ-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 γ-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

140 decay of the radionuclides. Counting times were adapted to obtain relative propagated errors
141 less than 5%. However, in a few cases, this counting precision could not be obtained even
142 after 48 h of counting due to the very low activity in extremely small organs. Counting times
143 ranged from 10 min to 1 h for whole cuttlefish, mussels and brine shrimp, and from 10 min to
144 48 h for the dissected organs and tissues.
145
146 **Data and statistical analyses.**
147 Uptake of ^{241}Am and ^{134}Cs from sediments and seawater was expressed, respectively, as
148 whole-body transfer factors (TF) and concentration factors (CF) over time (Bq g^{-1} wet wt
149 organism divided by the time-integrated Bq g^{-1} in sediments –TF– or seawater –CF–).
150 Radionuclide loss was expressed in terms of percentage of remaining radioactivity over time,
151 i.e. radioactivity at time t divided by initial radioactivity measured in the organisms at the
152 beginning of the depuration period. Loss kinetics were described either by a single-component
153 exponential model:
154 $$A_t = A_0\, e^{-k\,t},$$
155 where A_t and A_0 are remaining activities (%) at time t (d) and 0, respectively, or by a 2-
156 component exponential model:
157 $$A_t = A_{0s}\, e^{-k_s\,t} + A_{0l}\, e^{-k_l\,t},$$
158 where the 's' subscript refers to a short-lived component (s component) and the 'l' subscript
159 refers to a long-lived component (l component) (Whicker & Schultz 1982, Warnau et al.
160 1996). The exponential model showing the best fit (based on calculation of the determination
161 coefficients, R^2, and examination of the residuals) was selected.
162 Parameter k allows the calculation of the radionuclide biological half-life (d) using the
163 following equation:
164 $$T_{b½} = \ln 2/k.$$

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

Very low ^{241}Am and ^{134}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 nuclides, the digestive gland contained the highest proportion of the whole-body burden, i.e. 47 ± 28 % of ^{241}Am and 49 ± 12 % of ^{134}Cs (Table 1).

Seawater exposure

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

190 **Juveniles.** The whole-body activities measured after 36 h of exposure in spiked seawater
191 were 38 ± 10 and 37 ± 1 Bq g^{-1} wet wt for ^{241}Am and ^{134}Cs, respectively, giving relatively low
192 mean calculated whole-body CFs of 6 ± 2 and 4 ± 1 for these radionuclides.
193 Following transfer to non-contaminated seawater, loss kinetics of ^{241}Am in juvenile cuttlefish
194 were best fitted by a single-component exponential model whereas loss of ^{134}Cs was best
195 described by a two-component model (Figs 1A and 1B; Table 2). Loss kinetics were
196 characterised by a biological half-life ($T_{b½}$) of 2 wk for ^{241}Am and 1 wk for ^{134}Cs.
197 At the end of the depuration period, ^{134}Cs was mainly associated with the digestive gland of
198 the young cuttlefish (61 ± 4% of whole-body activity) whereas ^{241}Am was mainly retained in
199 the remaining tissues (61 ± 13%) (Table 1). The lowest fraction of both radiotracers was
200 found in the cuttlebone (< 15% of the total activity).
201 **Adults.** ^{241}Am and ^{134}Cs activities recorded in whole-body as well as in the different organs
202 and tissues of adult cuttlefish after 8 h of exposure and corresponding CFs are presented in
203 Table 3. The highest activities of ^{241}Am were found in the branchial hearts and their
204 appendages (264 ± 85 and 103 ± 66 Bq g^{1} wet wt, respectively). In the case of ^{134}Cs, the
205 branchial hearts, their appendages, the gills and the digestive tract displayed the highest
206 activities, ranging from 9 to 13 Bq g^{-1} wet wt.
207 When considering the tissue distribution of the radionuclides, muscle and skin of adults (i.e.
208 the sum of the mantle muscles, skin and head) contained the highest proportion of ^{241}Am and
209 ^{134}Cs, viz. 68 and 85%, respectively (Table 3). A somewhat lesser ^{241}Am fraction was found
210 in the branchial hearts and digestive gland (10 ± 2% for both tissues). The radionuclide
211 distribution among the tissues did not vary significantly (G test, $p > 0.05$) between the
212 beginning and the end of the depuration period (Table 3).
213

Food exposure

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 during a 2-h feeding. Immediately after feeding, all cuttlefish were γ-counted for determination of their radionuclide content.

Juveniles. The loss kinetics of ingested ^{241}Am and ^{134}Cs were best fitted by a 2-component exponential model composed of one rapid loss component followed by one slow component (Figs 1C and 1D; Table 2). The short-lived component was derived from 40% and 70% of the initially ingested ^{241}Am and ^{134}Cs activities, respectively (Table 2) and was characterised by a $T_{b½s}$ < 1 d for both radionuclides. The long-lived component, which represents the fraction of the radionuclides actually absorbed by cuttlefish, displayed a $T_{b½l}$ of 5 d for ^{241}Am and 66 d for ^{134}Cs (Table 2). The same long-lived component allowed estimation of the assimilation efficiencies (AE) of the ingested nuclides. Results showed that ^{241}Am was readily assimilated in juveniles with AE of 60% whereas the AE of ^{134}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}Am and ^{134}Cs, respectively, were rapidly lost with a $T_{b½s}$ of 4 and 13 h, respectively. The assimilated fraction of ingested ^{241}Am was much lower in adults than in juveniles (AE = 31 *vs* 60%) but was lost at a slower rate with a $T_{b½l}$ of 28 d compared to 5 d in juveniles. For ^{134}Cs, AEs were nearly similar at both ages (AE = 23 *vs* 29% in adults and juveniles, respectively) but the radionuclide was lost much faster in adults ($T_{b½l}$ = 16 d) than in juveniles ($T_{b½l}$ = 66 d).

239 The tissue distribution of ingested radionuclides was determined on several occasions after
240 feeding (Table 4). At the end of the depuration period, both ^{241}Am and ^{134}Cs were
241 predominantly distributed in the digestive gland (viz. 98 and 54%, respectively). The
242 distribution of ^{241}Am among tissues remained unchanged for 29 d of observation; in contrast,
243 some significant changes were observed for ^{134}Cs (G-test, $p = 0.01$). For example, the
244 proportion of ^{134}Cs activity decreased in the muscular tissues (mantle muscles and head)
245 whereas between 1 and 18 days of excretion it increased in the digestive gland (Table 4).
246
247 **DISCUSSION**
248
249 Cephalopods are an important resource of marine food and are fished and consumed in large
250 quantities all around the world (Amaratunga 1983). The intake of contaminants such as
251 radionuclides by humans through cephalopod consumption is therefore a matter of potential
252 concern. Cephalopods have been reported to concentrate natural and anthropogenic
253 radionuclides such as ^{210}Po, ^{210}Pb, ^{137}Cs, and $^{239+240}$Pu in their tissues (e.g. Smith et al. 1984,
254 Finger & Smith 1987, Yamada et al. 1999); however, little is known about the metabolism of
255 radionuclides in these higher trophic level molluscs. To the best of our knowledge, only two
256 species of cephalopods, viz. the octopus *Octopus vulgaris* and the squid *Doryteuthis bleerkeri*
257 have been investigated experimentally for Am, Cs, and Pu (Suzuki et al. 1978, Guary &
258 Fowler 1982). These works were limited to seawater uptake (i.e. Suzuki et al. 1978) or used a
259 less than optimal experimental approach such as injecting the prey with radionuclides for the
260 feeding experiments (Guary & Fowler 1982).
261 Overall, cephalopods are found in a great variety of habitats from coastal waters to very deep
262 ocean environments, some live in direct contact with bottom sediments, and others experience
263 different environments during their life cycle (e.g. demersal species becoming temporarily
264 pelagic during migration). Therefore, there is a further need to 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 for such experiments as it spends part of its time buried in the sediment and is easy to rear under laboratory conditions.

After 1 month of exposure to ^{241}Am and ^{134}Cs through sediments, juvenile cuttlefish still exhibited very low transfer factors (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 to the digestive gland and, to a lesser extent, to the cuttlebone (see Table 1). Such a translocation to the cuttlebone was observed in a previous study on bioaccumulation of Cd in *S. officinalis* (Bustamante et al. 2002).

Following a short contamination of adults via seawater, activities recorded in the whole cuttlefish suggest that they do not efficiently accumulate ^{241}Am and ^{134}Cs directly from the dissolved phase. Indeed, both elements displayed low whole-body CFs (CF = 2 for ^{241}Am and CF = 1 for ^{134}Cs). Activities of ^{134}Cs measured in the different organs and tissues were all of the same order of magnitude. In contrast, for ^{241}Am the organs involved in respiration (the branchial hearts, their appendages and the gills) and digestion (digestive gland) displayed higher activities compared to others body compartments (see Table 3). However, in terms of their relative distribution in the whole body, both radionuclides were mainly found in muscular tissues which represent the main fraction (viz. 75%) of the total body weight: muscles and head contained 65% and 82% of the total ^{241}Am and ^{134}Cs, respectively. A longer exposure (14 d) of octopus *Octopus vulgaris* to ^{137}Cs in water gave a similar distribution (i.e.

88%) of the radioisotope in the edible parts (Suzuki et al. 1978). In contrast, a 15-d exposure of the same species in seawater spiked with ^{241}Am resulted in only ca. 20% of the retained radioactivity being found into the muscular parts with ^{241}Am mainly being concentrated in the branchial hearts and their appendages (Guary & Fowler 1982). In our experiments with *S. officinalis*, these tissues contained low percentages of the total ^{241}Am, most probably because of the short duration of the experiment. Nevertheless, they significantly concentrated the radionuclide with CF reaching 42 in the branchial hearts and 16 in the appendages.

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

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

In the case of dietary exposure, 31 ± 3% of the ingested ^{241}Am was assimilated into the tissues of adult cuttlefish, whereas, in contrast, ^{241}Am was absorbed to a much greater extent in juveniles (AE = 60 ± 10%). This difference between AEs could be due to difference in

317 efficiency of digestion between juveniles and adults, since digestive metabolism is thought to
318 decrease with age in cephalopods (Mangold 1989). More likely, however, the difference could
319 also be due partly to variations in the bioavailability of ^{241}Am in the food used for juveniles
320 (brine shrimp) compared to that used for adults (mussels). Indeed, different storage
321 mechanisms in prey can determine metal bioavailability to higher trophic levels (Wallace &
322 Lopez 1997), which can lead to different proportions of transferable ^{241}Am. Overall, such
323 very high AEs for ^{241}Am in the common cuttlefish are rather unique whereas in herbivorous
324 bivalves, many crustaceans, echinoids, and fish assimilation of particle-reactive transuranic
325 elements is typically very low (e.g. Fowler et al., 1976; Penthreat 1977, 1981; Fisher et al.,
326 1983; Carvalho and Fowler, 1985; Warnau et al., 1996). Such a difference could be related to
327 the feeding regime as cephalopods are strict carnivores. For instance, unexpected high AEs
328 (up to 60%) of plutonium have also been found in carnivorous crustaceans, viz. the crabs
329 *Carcinus maenas* and *Cancer pagurus* (Fowler and Guary, 1977). Hence, the contribution of
330 the trophic pathway is very likely to be strongly enhanced in certain carnivorous
331 invertebrates.
332 Once assimilated, ^{241}Am was retained to a much greater degree in adults, with a half-life 6
333 times longer than in juveniles (i.e. 28 d *vs* 5 d), which suggests that different processes govern
334 ^{241}Am elimination/retention at the two life stages. In other molluscs such as mussels, ^{241}Am
335 has been reported to be strongly retained in the digestive gland (Bjerregaard et al. 1985,
336 Fisher & Teyssié 1986), a finding which is in agreement with our own observations. Indeed,
337 after 29 d of depuration, the major fraction of residual ^{241}Am was in the digestive gland, with
338 a much higher fraction was in adults than in juvenile cuttlefish (98% *vs* 59%). In the digestive
339 gland of the octopus *O. vulgaris*, Guary & Fowler (1982) reported that ^{241}Am is likely
340 associated with the cellular waste products such as brown bodies. Considering this hypothesis
341 together with our experimental observations, the longer retention of ^{241}Am observed in adult

342 *S. officinalis* could be due to a more rapid turnover of digestive cells in juveniles, thus leading
343 to a higher ^{241}Am excretion rate.
344 In contrast to ^{241}Am, ingested ^{134}Cs was assimilated to a similar extent in juveniles (29%) and
345 adults (23%) and depuration rate constant was 4 times higher in adults, resulting in a
346 significantly much shorter ^{241}Am half-life in adults (16 d) than in juveniles (66 d) (Table 2).
347 The longer retention time of ^{134}Cs in juveniles is difficult to explain since, for certain
348 transition elements (Ag, Cd, Co and Zn) previously investigated in cuttlefish (Bustamante et
349 al. 2002, 2004) as well for ^{241}Am (our study), early juveniles displayed shorter retention times
350 than adults. The main difference in tissue distribution of ^{134}Cs between adults and juveniles
351 was the higher proportion present in the cuttlebone (22 ± 21% in juveniles *vs* 2 ± 0% in
352 adults; see Tables 1 and 4). This higher skeleton-associated fraction is most likely tightly
353 bound and hence results in the high retention time observed. Although, our results clearly
354 indicate that ^{134}Cs would not follow the same excretion pathway as ^{241}Am, the above
355 interpretation should be considered with caution since to the best of our knowledge,
356 calcareous skeletons are not documented to act as a particularly efficient sink for cesium in
357 contrast with other elements such as e.g. ^{241}Am or Pb (see e.g. Grillo et al. 1981, Warnau et al.
358 1998). Furthermore, in this feeding experiment the very low activities measured in minute
359 organs such as juvenile cuttlebone were frequently associated with low counting accuracy,
360 which in turn can lead to a rather poor estimation of radioactivities and hence radionuclide
361 distribution (as indicated by the elevated SD value of the cuttlebone-associated fraction of
362 ^{134}Cs). Clearly, further study is needed to better understand the differences observed in the
363 fate of ^{134}Cs and ^{241}Am once taken up in young and adult cephalopod tissues.
364
365 ***Acknowledgements.*** We thank N. Tevenin and P. Gilles (Musée Océanographique, Monaco)
366 for providing us with the organisms. We are also grateful to E. Boucaud-Camou (Université
367 de Caen, France) for her advice on cuttlefish rearing. MW is an Honorary Research Associate

of the National Fund for Scientific Research (NFSR, Belgium). The Marine Environment Laboratory operates under a bipartite agreement between the International Atomic Energy Agency and the Government of the Principality of Monaco.

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488 Captions to Figure

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491 Fig. 1. *Sepia officinalis*. Whole-body loss kinetics of ^{241}Am and ^{134}Cs (% of remaining
492 activity; mean ± SD):
493 (A, B) juvenile cuttlefish previously exposed to spiked seawater for 36 h (n = 8 from day 0 to
494 20 and n = 4 on day 29);
495 (C, D) juvenile cuttlefish previously fed radiolabelled brine shrimp (n = 8 from day 0 to 22
496 and n = 5 on day 29);
497 (E, F) adult cuttlefish previously fed radiolabelled mussels (n = 18 on day 0, n = 15 from day
498 1 to 18, n = 12 from day 19 to 29).
499 Parameters of the best fitting equations are given in Table 3.

Table 1. *Sepia officinalis*. Distribution (%; mean ± SD) of ^{241}Am and ^{134}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
1. Sediments (29-d exposure)	3			
^{241}Am		49 ± 12	12 ± 3	39 ± 15
^{134}Cs		47 ± 28	17 ± 4	36 ± 24
2. Seawater (29-d depuration)	4			
^{241}Am		27 ± 13	13 ± 0	61 ± 13
^{134}Cs		61 ± 4	5 ± 0	34 ± 4
3. Feeding (29-d depuration)	5			
^{241}Am		59 ± 23	12 ± 10	29 ± 16
^{134}Cs		60 ± 27	22 ± 21	18 ± 14

Table 2. *Sepia officinalis*. Parameters of the equations best fitting the whole-body loss kinetics of ^{241}Am and ^{134}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; ASE: asymptotic standard error; R^2: determination coefficient; p: probability of the model adjustment.

Pathway	Model	A_{0s} (ASE)	ls (ASE)	$T_{b1/2s}$ (days)	A_{0l} (ASE)	ll (ASE)	$T_{b1/2l}$ (days)	R^2	p
1. Loss in juveniles after seawater exposure									
^{241}Am	O	87.7 (2.8)	0.048 (0.005)	14	-	-	-	0.96	< 0.001
^{134}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
2. Loss in juveniles after a single feeding on brine shrimp									
^{241}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}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
3. Loss in adults after a single feeding on mussels									
^{241}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}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^{-1} wet wt; 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).

Tissue	% wet wt	^{241}Am					^{134}Cs				
		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	16	103 ± 66	< 1	56	< 1	1	9 ± 2	< 1	1	< 1
Branchial hearts	0.10 ± 0.02	42	264 ± 85	3 ± 0	203	3	2	13 ± 1	< 1	2	< 1
Gills	2.3 ± 0.3	7	42 ± 14	10 ± 2	11	4	1	10 ± 2	4 ± 0	2	2
Digestive tract	2.6 ± 0.6	2	15 ± 5	4 ± 2	4	2	1	10 ± 1	4 ± 1	1	1
Genital tract	3.6 ± 1.0	1	9 ± 5	3 ± 1	2	1	< 1	4 ± 1	2 ± 0	< 1	4
Ink sack	0.6 ± 0.2	2	12 ± 1	1 ± 0	7	1	1	7 ± 3	1 ± 0	2	< 1
Skin	6.4 ± 2.1	1	6 ± 4	3 ± 1	3	2	< 1	4 ± 2	3 ± 0	< 1	4
Digestive gland	4.3 ± 1.2	3	22 ± 16	10 ± 2	28	11	< 1	3 ± 2	2 ± 1	1	1
Kidney	0.07 ± 0.07	2	13 ± 5	< 1	4	< 1	1	8 ± 5	< 1	1	< 1
Muscle	35 ± 2	1	7 ± 2	26 ± 4	10	52	1	6 ± 1	36 ± 3	2	55
Head	40 ± 1	1	9 ± 3	39 ± 1	4	23	1	7 ± 2	46 ± 3	1	32
Cuttlebone	5.1 ± 0.6	< 1	2 ± 1	1 ± 1	2	1	< 1	1 ± 1	1 ± 1	< 1	< 1
Whole cephalopod	100	2	10 ± 3	100	11	100	1	6 ± 2	100	3	100

Table 4. *Sepia officinalis*. Radionuclide distribution among tissues (%; mean ± 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}Am	^{134}Cs	^{241}Am	^{134}Cs	^{241}Am	^{134}Cs
Branchial heart appendages	< 1	6 ± 9	< 1	1 ± 0	< 1	2 ± 0
Branchial hearts	3 ± 0	1 ± 0	< 1	2 ± 1	< 1	1 ± 1
Gills	1 ± 1	3 ± 2	< 1	2 ± 1	< 1	2 ± 1
Digestive tract	1 ± 1	3 ± 1	< 1	6 ± 0	< 1	9 ± 1
Genital tract	< 1	2 ± 1	< 1	9 ± 1	< 1	10 ± 6
Ovary	< 1	1 ± 1	< 1	3 ± 1	< 1	5 ± 2
Ink sack	< 1	1 ± 0	< 1	1 ± 0	< 1	2 ± 1
Skin	< 1	1 ± 0	< 1	2 ± 1	< 1	2 ± 0
Digestive gland	89 ± 7	31 ± 6	97 ± 1	57 ± 8	98 ± 0	54 ± 12
Kidney	< 1	1 ± 0	< 1	4 ± 1	< 1	2 ± 0
Muscle	6 ± 8	22 ± 3	1 ± 0	6 ± 5	< 1	5 ± 2
Head	2 ± 1	28 ± 6	1 ± 0	6 ± 5	1 ± 0	6 ± 2
Cuttlebone	< 1	1 ± 0	< 1	2 ± 1	< 1	2 ± 0

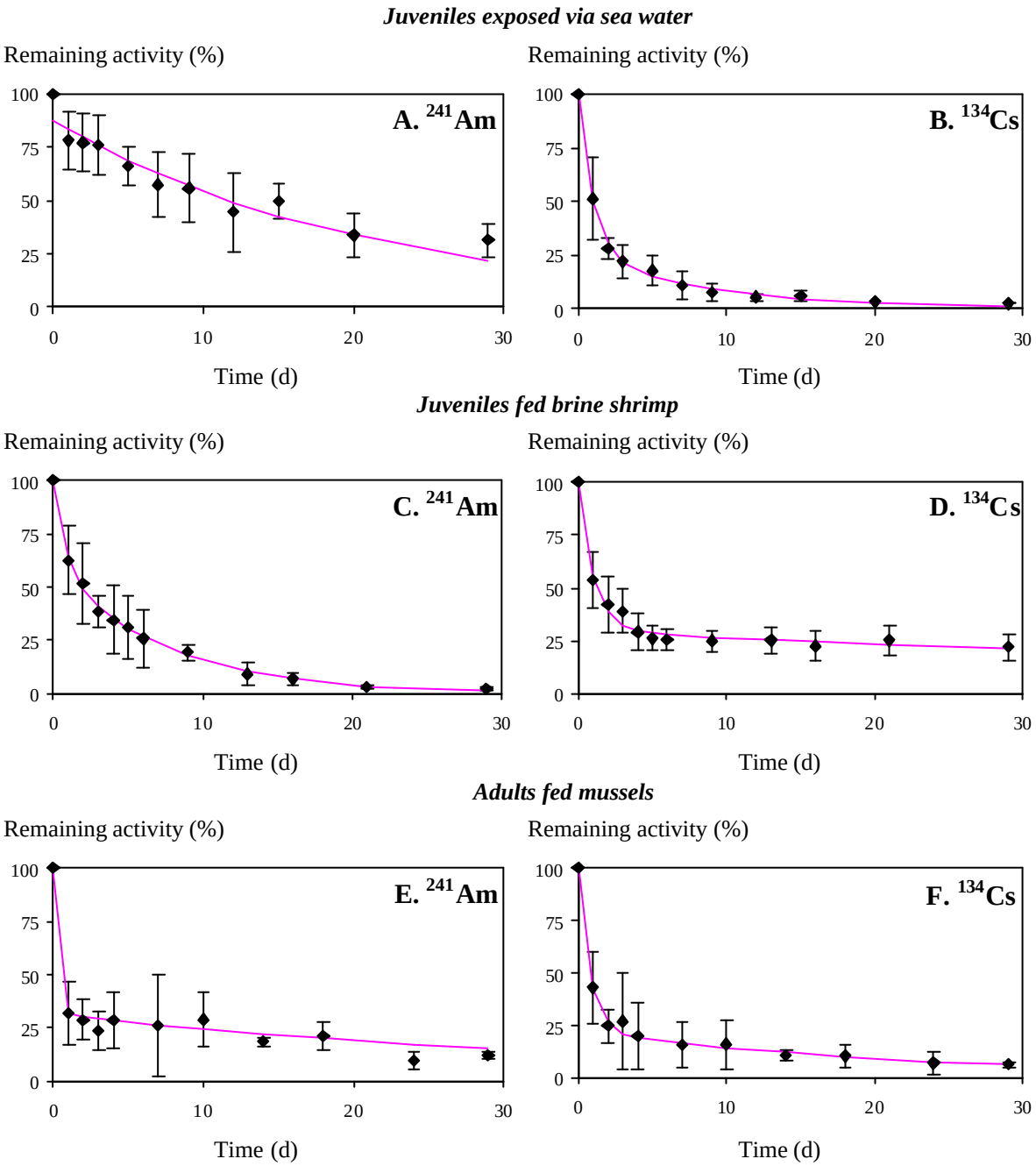
Juveniles exposed via sea water
Remaining activity (%)
A. 241Am
B. 134Cs
Time (d)
Juveniles fed brine shrimp
C. 241Am
D. 134Cs
Adults fed mussels
E. 241Am
F. 134Cs
0
25
50
75
100
10
20
30

Fig. 1