Contrasting bioaccumulation and transport behaviour of two artificial radionuclides (241Am and 134Cs) in cuttlefish eggshell

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BIOACCUMULATION ET TRANSPORT DE DEUX RADIONUCLEIDES ARTIFICIELS (^{241}AM AND ^{134}CS) AU TRAVERS DE LA PAROI DES ŒUFS DE SEICHES

CONTRASTING BIOACCUMULATION AND TRANSPORT BEHAVIOUR OF TWO ARTIFICIAL RADIONUCLIDES (^{241}AM AND ^{134}CS) IN CUTTLEFISH EGGSHELL

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Key words: RADIONUCLIDES; BIOKINETICS; BIOACCUMULATION; CEPHALOPODS

ABSTRACT - Radionuclide experiments were performed to study the accumulation, retention and transport of ^{241}Am and ^{134}Cs in eggs of the common cuttlefish *Sepia officinalis*. Experiments were designed to determine the processes controlling uptake of both radionuclides following exposure via seawater. The bioaccumulation of both radionuclides displayed saturation kinetics with steady state in uptake being reached in 10 d for ^{241}Am and 6 d for ^{134}Cs. ^{241}Am was readily taken up by the eggs which reached a concentration factor (CF) of 20 after 10 d of exposure. During embryonic development, the eggshell acted as an effective shield against ^{241}Am penetration with 98% of the radionuclide associated with this membrane. In contrast, ^{134}Cs was transported across the eggshell but was not concentrated to any large extent in the eggs. The mean ^{134}Cs CF for the whole egg did not exceed 1.3 ± 0.2, indicating a poor affinity of ^{134}Cs for the embryonic tissues of cuttlefish.

Mots clés: RADIONUCLEIDES; BIOCINETIQUES; BIOACCUMULATION; CEPHALOPODES

RÉSUMÉ- Des expérimentations radioécologiques ont été conduites pour étudier l'accumulation, la rétention et le transport de l'^{241}Am et du ^{134}Cs chez les œufs de la seiche commune *Sepia officinalis*. Ces expérimentations

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ont permis de déterminer les processus contrôlant l'incorporation de ces deux radionucléides lors d'une exposition par l'eau de mer. La bioaccumulation de ces deux éléments a montré une cinétique atteignant la saturation ave un équilibre à partir du dixième jour pour l'^{241}Am et du sixième jour pour le ^{134}Cs. L'^{241}Am a été aisément incorporé par les œufs qui montraient un facteur de concentration (FC) de 20 après 10 jours d'exposition. Durant le développement embryonnaire, la paroi de l'œuf a joué un rôle de protection contre la pénétration de l'^{241}Am retenant 98% de ce radionucléide. Au contraire, le ^{134}Cs a facilement diffusé à travers de cette membrane mais ne s'est pas accumulé à de hautes concentrations dans les œufs. Le FC du ^{134}Cs pour les œufs entiers n'a pas dépassé 1.3 ± 0.2, suggérant une faible affinité de ce radionucléide pour les tissus des embryons de seiche.

INTRODUCTION

Various contaminants occur in relatively high concentrations in coastal areas which are subjected to release from agricultural, domestic and industrial activities. Increased ambient contaminant concentrations may provoke toxic effects in marine organisms which ultimately could lead to a modification of the entire ecosystem structure (Watzin & Roscigno 1997). In invertebrates, the early life stages, viz. embryos and juveniles, are very sensitive to contaminants (Calabrese *et al.* 1973, Martin *et al.* 1981, Warnau *et al.* 1996a). This is particularly obvious when the spawning and the subsequent embryonic development occur directly in seawater, thus allowing a direct contact of waterborne contaminants with the embryos and larvae. This sensitivity is believed to be reduced when the embryo develops within an egg, with the eggshell acting as a potential protective barrier to reduce or prevent the intake of contaminants from the dissolved phase.

The common cuttlefish *Sepia officinalis* spawns eggs surrounded by a proteinaceous eggshell which is known to prevent the incorporation of some non-essential metals such as Cd, Pb, or V (Bustamante *et al.* 2002, Miramand *et al.* in press). However, other toxic elements such as Ag can pass through the eggshell and become incorporated in embryonic tissues (Bustamante *et al.* 2004). Element transport selectivity in the eggshell is apparently not determined by the metabolic needs of the embryo for essential elements, since the non-biologically essential element Ag is well known for its enhanced embryotoxicity (Calabrese *et al.* 1973, Martin *et al.* 1981, Warnau *et al.* 1996a).

In general, knowledge about such selectivity remains very limited and, in particular, is lacking for certain long-lived radionuclides. Therefore, the present work aims at investigating the accumulation of two key

radioelements, ^{241}Am and ^{134}Cs, in cuttlefish eggs. These radionuclides were selected to investigate any differences in behaviour of a particle-reactive element (^{241}Am) and a biological-reactive element like ^{134}Cs which can act as an analogue for potassium. This study focuses specifically on the accumulation rates of these radionuclides in the whole eggs and their subsequent distribution among the different egg compartments.

MATERIAL AND METHODS

Biological material and radiotracer exposure

Eggs of the common cuttlefish Sepia officinalis L. were obtained from cultured adults and were maintained in an aquarium with flowing seawater (salinity: 36 p.s.u.; temperature: 16.5 ± 0.5°C; 12/12 h dark/light cycle) until used in the experiments. Approximately 35 days after spawning, the eggs were placed in a 70 L glass aquarium containing natural seawater spiked with ^{241}Am and ^{134}Cs (nominal activity: 6 kBq L^{-1} each) until hatching, i.e. for 11 d. Both radionuclides and seawater were renewed daily in order to maintain seawater quality and radionuclide concentration as constant as possible. The radionuclide activity in seawater was checked daily, before and after each seawater renewal. During the experiment, ^{241}Am and ^{134}Cs levels were measured in the eggs on days 1, 2, 3, 6, 9, and 11. At each counting time, 3 eggs were dissected to determine the partitioning of the radiotracers among the three egg compartments: eggshell, peri-embryonic fluid and embryo.

Radionuclides and radioanalyses.

^{241}Am [$t_{½}$ = 433 yrs] and ^{134}Cs [$t_{½}$ = 2 yrs] were purchased from Amersham, UK, as nitrate and chloride salts, respectively. Stock solutions were prepared in their respective solutions (0.1 N) to obtain radioactivities which would allow using spikes of 20 µL.

Radioactivity of the tracers was 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 computer with spectra analysis software (Interwinner, Intertechnique). The detectors were calibrated with appropriate standards for each of the counting geometries used. 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 smallest fractions.

Data and statistical analyses.

Uptake of ^{241}Am and ^{134}Cs from seawater was expressed as change in whole-egg concentration factors (CF) over time (CF = Bq g^{-1} wet weight (wwt) egg divided by the time-integrated Bq g^{-1} in seawater). Uptake kinetics in the eggs were described using a single-component first-order kinetic model:

$$CF_t = CF_{equil} (1 - e^{-k\,t}),$$

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

Constants of the model and their statistics were estimated by iterative adjustment 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 egg compartments 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 compartment during the accumulation period were tested by one-way ANOVA followed by the Tukey's multiple comparison test after arcsin transformation of the data (Zar 1996). The significance level for statistical analyses was routinely set at $\alpha = 0.05$.

RESULTS

Accumulation of the radionuclides

Regular monitoring of radiotracer activities in seawater allowed calculation of time-integrated radioactivities, viz. 6.4 ± 0.3 kBq L^{-1} for ^{241}Am and 8.6 ± 0.7 kBq L^{-1} for ^{134}Cs. Despite the relatively short experimental exposure (11 d), the uptake of ^{241}Am and ^{134}Cs displayed saturation kinetics (Figs 1A & 1B). Estimated steady-state concentration factors (CF_{equil}) in the eggs were 19.7 ± 3.2 and 1.33 ± 0.21 for ^{241}Am and ^{134}Cs, respectively.

Distribution among egg compartments

Table 1 shows the distribution of the nuclides among the different compartments of the eggs. The major proportion of ^{241}Am was always associated with the egg capsule membrane during the course of the experiment. In contrast, the distribution of ^{134}Cs varied greatly among the egg compartments over time. A much lower percentage of ^{134}Cs was found in the eggshell (always < 38%), whereas the percentages in the peri-embryonic liquid and the embryo differed significantly ($P_{G\text{-}test} < 0.05$) from one sampling time to another.

DISCUSSION

Only very limited information concerning trace element metabolism in the eggs of cephalopods is available in the literature (Suzuki *et al.* 1978, Guary & Fowler 1982, Ueda *et al.* 1985, Bustamante *et al.* 2002, 2004). Among the cephalopods, cuttlefish undertake coastal to open sea migrations as they evolve from juveniles to adults, and then return to coastal waters for reproduction. Contaminant-enrichment of coastal waters due to anthropogenic activities may have adverse effects on the embryos, potentially influencing the recruitment and eventual dynamics of cuttlefish populations. The strong toxicity of various trace elements with respect to the larvae of marine invertebrates is well known (e.g. Calabrese *et al.* 1973, Martin *et al.* 1981, Warnau *et al.*

1996a). However, similar information for cephalopods is limited to only a few metals (viz. Ag, Cd, Co and Zn; Bustamante *et al.* 2002, 2004), and to the best of our knowledge nothing concerning radionuclides has been reported to date.

When spawned, cuttlefish eggs are surrounded by an eggshell the thickness of which is approximately 1.5 mm (Lemaire 1971). This shell, composed of albumin and other proteins, hardens when coming into contact with seawater. It also becomes thinner during embryonic development, and is almost transparent at the moment of hatching (Wolf et al, 1985). In general, the concentrations of toxic metals within the eggs remain very low until the end of the embryogenesis, and they are in the same concentration range as those in the yolk of early spawned eggs (Miramand *et al.* in press). Moreover, previous experiments with radiotracers have shown that Cd and Co were accumulated in the eggshell which constituted an effective protective barrier preventing the incorporation of waterborne metals in the embryo during its development (Bustamante *et al.* 2002, 2004). In contrast, Ag and to a lesser extent Zn were incorporated after a few days of exposure, suggesting a limited capacity of retention for Ag in eggshell and a facilitated transport across the eggshell of the biologically-essential element Zn. It is therefore likely that the physical-chemical properties of the different elements determine their capability to pass through the eggshell. It is evident that more detailed tracer studies are needed in order to provide a clearer picture of the specific processes involved in the selectivity of the eggshell towards certain trace elements.

A major objective of our experiments with radionuclides was to compare the behaviour of a particle-reactive element which has no known biological function, viz. ^{241}Am, with a biologically-reactive element such as^{134}Cs which acts as an analogue element of potassium (K). During embryonic development, ^{241}Am was readily taken up from seawater reaching a CF of 20 ± 3 after 11 d of exposure (Fig. 1). Approximately 98% of the incorporated ^{241}Am remained associated with the capsule membrane of the eggs during the entire experiment (Table 1). Similar to other transuranium nuclides, ^{241}Am is well known for its specific affinity for the calcareous structures of echinoderms (Grillo *et al.* 1981, Guary *et al.* 1982, Fowler & Carvalho 1985, Warnau *et al.* 1996b) and bivalves (Guary 1980, Bjerregaard *et al.* 1985, Fisher *et al.* 1996), and therefore it might be expected to accumulate in the cuttlebone of the embryo. In fact, our results clearly demonstrate that the eggshell acts as a very effective shield against the incorporation of transuranium nuclides.

In contrast to ^{241}Am, the ^{134}Cs CF remained very low with bioaccumulation reaching a steady state after only 6 d of exposure (Fig. 1). In fact, ^{134}Cs was readily transferred across the eggshell and was not specifically trapped on the membrane, which at any time only contained between 13 and 38% of the total radionuclide

activity (Table 1). However, the percentage of ^{134}Cs in the peri-embryonic fluid and the embryo varied significantly throughout the experiment ($p_{G\text{-}test} < 0.05$), suggesting that this element is not specifically sequestered within the egg. It is therefore likely that rapid exchange between the internal compartments (peri-embryonic fluid and embryo) and the external medium (seawater) occurred. In general, ^{134}Cs displayed a quite different behaviour compared to other elements, especially ^{241}Am, ^{109}Cd, and ^{57}Co, which are mainly bound to the eggshell (Bustamante *et al.* 2002, 2004, this study). In contrast, ^{134}Cs is readily transferred across the membrane much like K which is a major element occurring in high concentrations in muscular tissues. However, the low concentrations of ^{134}Cs in the embryo indicate a poor incorporation of this element in muscle during ontogenesis. Any reduction of Cs incorporation in the muscular tissues of cephalopods is likely a reflection of the very low stable Cs concentration relative to K in these organisms, as for example the Cs (7.5 ng g^{-1} wwt) and K (3,100 µg g^{-1} wwt) levels measured in the mantle of the purpleback flying squid *Sthenoteuthis oualaniensis* (Ichihashi *et al.* 2001). Therefore, a comparative examination of the results of the few investigations examining metal incorporation during cuttlefish embryogenesis (Bustamante *et al.* 2002, 2004, Miramand *et al.* in press, this study) allows defining 3 distinct categories of elements: (1) those efficiently trapped on the eggshell which results in protecting embryos against metal toxicity (i.e. ^{241}Am, ^{109}Cd, and ^{57}Co), (2) those which are firstly trapped on/in the membrane and which subsequently cross the eggshell, probably when both specific and non-specific sites for binding are occupied (i.e. ^{110m}Ag and ^{65}Zn), and (3) those which are readily transferred across the eggshell in both directions (i.e. ^{134}Cs). However the specific mechanisms involved remain poorly understood, and further detailed investigations which specifically address the physiological mechanisms controlling eggshell selectivity in metal accumulation and transfer should be considered in the future.

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Table I. *Sepia officinalis*. Distribution (%; mean values ± SD, n = 3) of ^{241}Am and ^{134}Cs among the cuttlefish egg compartments at different times during their exposure via seawater.

Fig. 1. *Sepia officinalis*. Whole-body uptake kinetics of ^{241}Am (A) and ^{134}Cs (B) in cuttlefish eggs exposed for 11 d to radionuclides in seawater (concentration factor, CF; mean ± SD, n = 3).

Table I.

Egg compartments	**1 d**		**3 d**		**6 d**		**11 d**	
	^{241}Am	^{134}Cs	^{241}Am	^{134}Cs	^{241}Am	^{134}Cs	^{241}Am	^{134}Cs
Eggshell	99 ± 1	20 ± 10	99 ± 1	38 ± 16	98 ± 2	13 ± 4	98 ± 2	28 ± 8
Peri-embryonic fluid	< 1	63 ± 12	1 ± 1	20 ± 5	2 ± 1	57 ± 4	2 ± 1	23 ± 2
Embryo	< 1	17 ± 4	< 1	42 ± 13	< 1	30 ± 1	< 1	49 ± 6

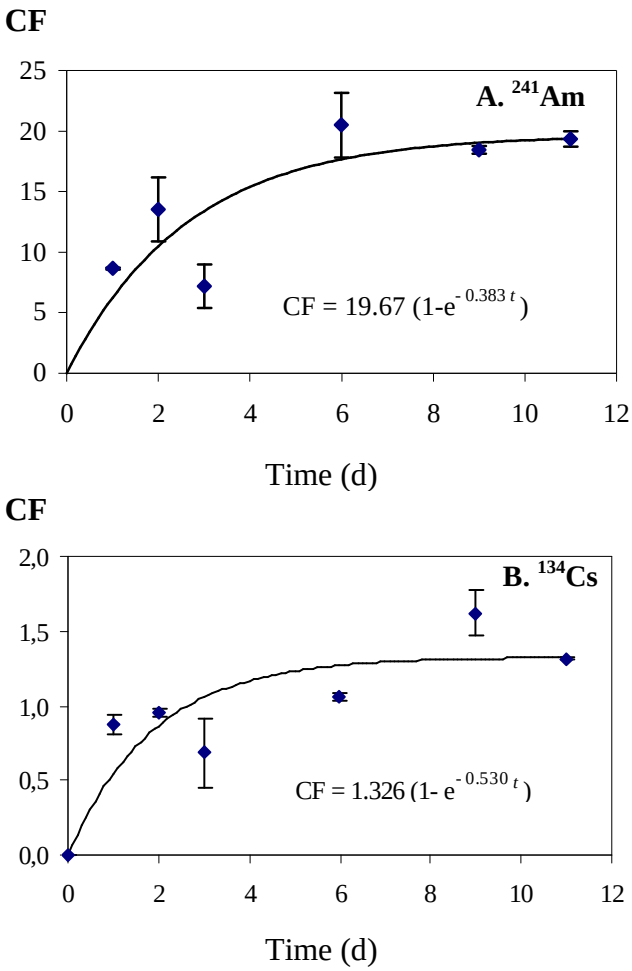
CF
A. 241Am
CF = 19.67 (1-e- 0.383 t)
Time (d)
CF
B. 134Cs
CF = 1.326 (1- e- 0.530 t)
Time (d)

Fig. 1.