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Characterization of ^{241}Am and ^{134}Cs bioaccumulation in the king scallop *Pecten maximus*: investigation via three exposure pathways

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Abstract

In order to understand the bioaccumulation of ^{241}Am and ^{134}Cs in scallops living in sediments, the uptake and depuration kinetics of these two elements were investigated in the king scallop *Pecten maximus* exposed via seawater, food, or sediment under laboratory conditions. Generally, ^{241}Am accumulation was higher and its retention was stronger than ^{134}Cs . This was especially obvious when considering the whole animals exposed through seawater with whole-body concentration factor ($\text{CF}_{7\text{d}}$) of 62 vs. 1, absorption efficiencies (A_{ol}) of 78 vs. 45 for seawater and biological half-lives ($T_{\text{b}/2\text{l}}$) of 892d vs. 22d for ^{241}Am and ^{134}Cs , respectively. In contrast, following a single feeding with radiolabelled phytoplankton assimilation efficiency (AE) and $T_{\text{b}/2\text{l}}$ of ^{134}Cs obtained were higher than those of ^{241}Am (AE: 28% vs. 20%; $T_{\text{b}/2\text{l}}$: 14d vs. 9d). Among scallop tissues, the shells always contained the higher proportion of the total body burden of ^{241}Am whatever the exposure pathway was. In contrast, the whole soft parts presented the major fraction of whole-body burden of ^{134}Cs , which was generally associated with muscular tissues. Our results showed that the two radionuclides have contrasting behaviors in scallops, in relation to their physico-chemical properties.

Keywords: bivalve; scallop; radionuclide; bioaccumulation; uptake; depuration

1. Introduction

During the last sixty years, human activities have resulted in various degrees of contamination of the world seas and oceans with anthropogenic radionuclides (Frielander et al. 2005). Although this contamination tends to decrease (e.g. Toshimichi et al. 2003), it is still a major concern in coastal areas receiving radioactive inputs mainly from industries, nuclear accidents and fallout from nuclear weapon testing and use. Consequently, monitoring programs were established worldwide to monitor the levels of those radionuclides in the marine environment (Nielsen et al. 2007). Generally, surveys are based on the analysis of seawater, sediments and biological samples (e.g., Fegan et al. 2010). Overall, biomonitoring programs present the advantages 1) to reveal the bioavailability of the considered contaminants and 2) to magnify their levels above the analytical detection limits.

The use of marine organisms for monitoring radionuclide contamination is well established (Valette-Silver and Lauenstein 1995, Burger et al. 2007, Thébault et al. 2008). In order to understand field measurements, the characterization of bioaccumulation parameters and/or the relative importance of the different exposure pathways has been carried out for several radionuclides (Ke et al. 2000, Wang et al. 2000, Baines et al. 2005, Borretzen and Salbu 2009). Beside mussels, other bivalve species are used to a lesser extent in biomonitoring programs among which scallops appear of great interest as they highly accumulate trace elements from their environment (Bryan 1973, Bustamante and Miramand 2005, Metian et al. 2008a, 2009a, Pan and Wang 2008). Scallops have also been reported to efficiently concentrate natural and anthropogenic radionuclides such as ^{241}Am , ^{137}Cs , ^{210}Po , ^{238}Pu , ^{239}Pu and ^{90}Sr in their tissues (Miramand et al. 1991; Nonnis Marzano et al. 2000; Bustamante et al. 2002). As for some metals (Brooks and Rumsby 1965, Chouvelon et al. 2009, Bustamante and Miramand 2004), scallops sometimes display higher bioaccumulation capacity for ^{137}Cs than other filter-feeders such as oysters and mussels occurring in the same areas (JCAC

2002). Within the Pectinid family, the current knowledge on concentrations of anthropogenic radionuclides is limited to field measurements (Bustamante et al. 2002, Nonnis Marzano et al. 2000; Miramand and Germain 1986), and to waterborne exposure experiment to Am (Miramand and Germain 1986, Miramand et al. 1991). The importance of other contamination pathways in the bioaccumulation process of Am and Cs in scallops is not known although sediment and food have been considered as possible source of Am and Cs following the analyses carried out on scallops from the field or after laboratory studies (Miramand and Germain 1986, Nonnis Marzano et al. 2000) and by histo-autoradiography approach in laboratory (Miramand et al. 1991). Overall, sediment is considered as a major vector of transuranic elements, among which Am, to biota (Miramand et al. 1982, Bustamante et al. 2006, Ryan 2002). In comparison, Cs transfer from sediments appears relatively limited (Bustamante et al, 2006; Borretzen and Salbu, 2009). Recently, food has been demonstrated as a major pathway for metal bioaccumulation in scallops (Metian et al. 2009ab). Therefore, it appears necessary to determine experimentally the bioaccumulation of radionuclides in scallops via seawater, food and sediments in order to better understand the relative contribution of these three pathways of exposure.

The aim of this study was thus to determine the kinetics of uptake and depuration of ^{241}Am and ^{134}Cs in a typical pectinid from European waters, the king scallop *Pecten maximus*, following its exposure to radiolabelled seawater, food or sediment. The radionuclides were selected for their contrasting characteristics in seawater (particle-reactive Am and soluble Cs). There is a particular interest to study this transuranian and the radiocesium in *P. maximus* since the geographic distribution of this species (European North-Atlantic coasts) coincides with the area subject to direct inputs from the nuclear retreatment plants of Dounreay (Scotland – facility closed in 1996), La Hague (France) and Sellafield (England), which affect the Norway where *Pecten maximus* is cultured (Bergh and Strand, 2001).

2. Materials and Methods

2.1. Sampling

In spring 2004 and 2005, seventy king scallops *P. maximus* were collected on the French Atlantic coast (Pertuis Breton, Charente-Maritime) by SCUBA diving. They were carefully transported to IAEA-MEL premises in Monaco and were acclimated to laboratory conditions for 4 weeks (constantly aerated, open-circuit 800l aquarium; flux: 50 l h⁻¹; salinity: 36 p.s.u.; temperature: 17 ± 0.5 °C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) prior to experimentations. During this period, scallops were fed daily an algal mixed diet (*Isochrysis galbana*, *Skeletonema costatum*).

2.2. Radiotracer and counting

Uptake and depuration kinetics of ²⁴¹Am and ¹³⁴Cs in scallop were determined using high-specific activity radiotracers purchased from Isotope Product Lab (²⁴¹Am nitrate -0.1 N-, T_{1/2} = 433 years; ¹³⁴Cs chloride -0.1 N-, T_{1/2} = 2 years). Tracers were counted using a high-resolution γ-spectrometer system composed of four Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The radioactivity was determined by comparison with standards of known activity and of appropriate geometry. Measurements were corrected for counting efficiency and physical radioactive decay. The counting time was adjusted to obtain a propagated counting error less than 5% (Rodriguez y Baena et al. 2006).

2.3. Seawater exposure

Twenty five *P. maximus* (average weight ± SD: 208 ± 46 g) were placed in a 70-l glass aquarium (constantly aerated, closed-circuit aquarium; salinity: 36 p.s.u.; temperature: 17 ± 0.5 °C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) and simultaneously exposed for 7d to ²⁴¹Am and to ¹³⁴Cs dissolved in 0.45 µm filtrated seawater (0.3 and 1.4 kBq l⁻¹, respectively). No change in pH was detectable after the tracer addition. Spiked seawater was renewed twice a

day during the first two days and then daily in order to keep radioactivity in seawater constant. Activity of the ^{241}Am and ^{134}Cs in seawater was checked before and after each spike renewal, yielding time-integrated activities of $0.13 \pm 0.09 \text{ kBq l}^{-1}$ and $1.23 \pm 0.03 \text{ kBq l}^{-1}$ (Nine tag-identified scallops were collected at different time intervals and were whole-body radioanalyzed alive (same identified individual each time). At the end of the 7d exposure period, 5 scallops among the 25 were sacrificed and dissected. Shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues were separated and radioanalyzed in order to assess the radionuclide body distribution. The remaining scallops were then placed in non contaminating conditions (constantly aerated, open-circuit aquarium; flux: 50 l h^{-1} ; salinity: 36 p.s.u.; temperature: $17 \pm 0.5 \text{ }^{\circ}\text{C}$; pH: 8.0 ± 0.1 ; light/dark cycle: 12 h/12 h) for 36d and the nine tag-identified individuals were regularly radioanalyzed alive in order to follow the depuration of ^{241}Am and ^{134}Cs from the scallops. Three non-exposed individuals were introduced in the aquarium in order to control possible tracer recycling from the contaminated scallops. During the 36d depuration period, scallops were fed daily with *Skeletonema costatum* and *Isochrysis galbana* ($5.10^4 \text{ cells ml}^{-1}$). At the end of the depuration period, four contaminated scallops were collected and dissected into several body compartments as previously described.

2.4. Food exposure

The Bacillariophyceae *Skeletonema costatum* was used to study the transfer of ^{241}Am and ^{134}Cs to scallops through their diet. Phytoplankton cells were exposed to $4.5 \text{ kBq } ^{241}\text{Am l}^{-1}$ and $7 \text{ kBq } ^{134}\text{Cs l}^{-1}$ during their exponential growing phase (10d). After that period, phytoplankton medium was filtrated (1 μm -mesh size; Osmonic filters), and the phytoplankton cells resuspended in a 70-l aquarium (constantly aerated, closed-circuit aquarium; salinity: 36 p.s.u.; temperature: $17 \pm 0.5 \text{ }^{\circ}\text{C}$; pH: 8.0 ± 0.1 ; light/dark cycle: 12 h/12 h) at a cell concentration of $5 \cdot 10^4 \text{ cell ml}^{-1}$ to avoid pseudofeces production by the scallops.

Nine *P. maximus* (average weight $\pm$ SD: 199 ± 32 g) had been placed in the aquarium for one week before the feeding experiment. Scallops were then allowed to feed on radiolabelled *S. costatum* for 2h. After the feeding period, all scallops were γ -counted and flowing seawater conditions (50 l h^{-1}) were restored in the aquarium. Individuals were then whole-body γ -counted alive at different time intervals to follow the depuration kinetics of both elements. Three non-exposed individuals were introduced in the aquarium in order to control possible tracer recycling from the contaminated scallops. During the 21d depuration period, scallops were fed daily with *Skeletonema costatum* and *Isochrysis galbana* (5.10^4 cells ml^{-1}). Four contaminated individuals were randomly collected after 21d and dissected to determine the radionuclide distribution among the different body compartments (shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues). Radiolabelled *S. costatum* did not allow contaminating significantly the scallops with ^{134}Cs . Therefore, another phytoplankton species was used to study the trophic transfer of this radionuclide. To this end, the Haptophyceae *Isochrysis galbana* was used following the same method as previously described for *S. costatum* except that phytoplankton cells were exposed to ^{134}Cs during 7d (growing phase of *I. galbana*). Six *P. maximus* (average weight $\pm$ SD: 127 ± 14 g) were exposed and whole-body γ -counted alive at different time of the depuration experiment (16d). Four individuals were dissected at the end of the depuration period to determine the ^{134}Cs distribution among the different body compartments (same as described above).

2.5. Sediment exposure

Since *P. maximus* lives buried into the sediment, ^{241}Am and ^{134}Cs exposure through sediment was assayed. Sediment was collected in Wimereux (North-Atlantic coast of France). Sediment grain size distribution was measured on a Mastersizer micro and the evaluation of the dry/wet weight ratio was calculated after freeze drying in a LABCONCO Freezone18.

Aerated sediment (9 kg) was placed in a plastic container, spiked with ^{241}Am (8 kBq) and ^{134}Cs (13 kBq) for 6d with constant agitation, then used to form a homogeneous sediment layer of 4 cm height in a 20-l aquarium. Weakly bound radioisotopes were allowed to leach overnight under flowing seawater (50 l h^{-1}). Ten *P. maximus* (average weight $\pm$ SD: 118 ± 5 g) were then placed for 13d in the aquarium (open circuit; parameters as previously described), and six tag-identified individuals were regularly whole-body radioanalyzed alive. Sediment samples were also regularly collected and γ -counted to verify that the radiotracer activities in sediment remained constant. Activity of ^{241}Am and ^{134}Cs in sediment was constant all along the exposure period (8.2 ± 0.8 and $13.1 \pm 3.0 \text{ Bq g}^{-1}$ wet wt, respectively). At the end of the exposure period, four scallops were collected, dissected (shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues), weighed and radioanalyzed to determine the radionuclide body distribution. The remaining six scallops were placed in non-contaminating depuration conditions for 31d (new 20l glass aquarium with clean sediment under flowing seawater, 50 l h^{-1} , daily feeding on *Skeletonema costatum* and *Isochrysis galbana* at $5 \times 10^4 \text{ cells ml}^{-1}$), and regularly γ -counted. The radioactivity in sediment was regularly checked in order to ensure that no tracer recycling occurred in the sediment. Although no radioactivity was detected, the whole sediment was renewed after 1 week. After 31d of depuration, four scallops were collected and dissected as described above to determine body distribution of ^{241}Am and ^{134}Cs .

2.6. Data analysis

Uptake of the radioisotope was expressed in term of concentration factors (CF: ratio between the radioisotope activity in scallops -Bq g^{-1} wet wt- and time integrated activity in the seawater -Bq g^{-1} -) over time for the seawater exposure and in term of transfer factors (TF: ratio between the radioisotopes activity in scallops -Bq g^{-1} wet wt- and time-integrated activity in the sediment -Bq g^{-1} wet wt-) over time for the sediment exposure of *P. maximus*.

Uptake kinetics of ^{241}Am and ^{134}Cs in whole-body scallops were fitted (Statistica® 6) using a simple exponential kinetic model (Eq. (1)) or using a linear model (Eq. (2)):

$$CF_t = CF_{ss} (1 - e^{-k_e t}) \text{ (Eq. 1)}$$

$$CF_t = k_u t \text{ (Eq. 2)}$$

where CF_t and CF_{ss} ($CF_{ss} = k_u/k_e$) are the concentration factors at time t (d) and at steady state, respectively; k_u and k_e are the uptake and depuration rate constants (d^{-1}), respectively (Whicker and Schultz, 1982).

Depuration of ^{241}Am and ^{134}Cs (seawater, food and sediment experiments) was expressed in terms of percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in scallops at the beginning of the decontamination period*100). The percentages of remaining activity were plotted against time and depuration kinetics were described by a double-component exponential model (Eq. (3)):

$$A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t} \text{ (Eq. 3)}$$

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}); ‘s’ and ‘l’ are the subscripts for the short-lived’ and ‘long-lived’ components. For each exponential component (s and l), a biological half-life can be calculated ($T_{b/2s}$ and $T_{b/2l}$) from the corresponding depuration rate constant (k_{es} and k_{el} , respectively) according to the relation $T_{b/2} = \ln 2/k_e$ (Warnau et al. 1996). Regarding feeding experiments, the ‘long-lived’ exponential term describes the fraction of the radiotracer ingested with food that is actually absorbed by the organism (Warnau et al. 1996). The corresponding A_{0l} represents the assimilation efficiency (AE) of the considered radiotracer. The best fitting regression models were selected according to highest determination coefficient and examination of residuals. The level of significance for statistical analysis was always set at $\alpha = 0.05$.

3. Results

No mortality of scallops was recorded neither during acclimation period nor during the different experiments.

3.1. Seawater exposure

Uptake of ^{241}Am in whole-body *P. maximus* displayed linear kinetics ($R^2 = 0.80$; Fig. 1A, 1B and Table 1) whereas the uptake of ^{134}Cs displayed exponential kinetics reaching a steady state ($R^2 = 0.79$). The values estimated for the kinetic parameters and their associated statistics are shown in Table 1. The concentration factors measured at the end of the exposure period ($\text{CF}_{7\text{d}}$) of ^{241}Am and ^{134}Cs were 63 ± 18 and 1.0 ± 0.2 in whole body scallops, respectively. In the case of ^{134}Cs , the estimated steady-state CF calculated by the model (CF_{SS}) reached 0.94 ± 0.05 (Fig. 1B and Table 1).

Calculated $\text{CF}_{7\text{d}}$ for the different compartments and organs are shown in Table 2. ^{241}Am is systematically more concentrated than ^{134}Cs when considering the same compartment of the scallops (up to 2 orders of magnitude). The shells of the scallops displayed higher capacities of ^{241}Am bioconcentration than their whole soft parts (CFs: 130 vs. 30) whereas the opposite was observed for ^{134}Cs (CFs: 1 vs. 3). Among the soft tissues, the digestive gland and the kidneys presented the highest CF of ^{241}Am and ^{134}Cs , respectively (Table 2).

At the end of the uptake experiment, the highest ^{241}Am load was in the shell (more than 90% of the total body load) and that of ^{134}Cs was in whole soft parts (more than 70% of the total load; Table 2). Among soft tissues, ^{241}Am was mainly contained in the mantle, digestive gland and gills (34, 23 and 18% of total body load, respectively; Table 2) whereas ^{134}Cs was mainly present in the adductor muscle and the mantle (41 and 24% of total body load, respectively; Table 2).

When non-contaminating conditions were restored, the whole-body depuration kinetics of both ^{241}Am and ^{134}Cs were best described by a two-component exponential model (Fig. 1C

and Table 1). The major part of the bioaccumulated ^{241}Am was efficiently absorbed (A_{01} : 78%) whereas only 45% of the bioaccumulated ^{134}Cs was absorbed in *P. maximus*. The estimated depuration rate constant of the long-lived components (k_{el}) for ^{134}Cs was $0.031 \pm 0.004 \text{ d}^{-1}$ and, consequently, the derived biological half-life reached $22 \pm 3 \text{ d}$ (Table 1). In the case of ^{241}Am , the depuration rate constant was not significantly different from 0 ($p < 0.05$), thus the corresponding $T_{b/2l}$ may thus be considered as infinite. However, an estimation of $T_{b/2l}$ based on the mean value of k_{el} is shown in Table 1 (892d).

After 36d of depuration, the distribution of both radionuclides between the shell and the whole soft parts remained similar to the one observed at the end of the exposure period: ^{241}Am was mainly found in the shell and ^{134}Cs in the soft tissues (Table 2). Within soft tissues, radionuclide distribution displayed a different pattern than the one observed at the end of the exposure period (Table 2). Indeed, the digestive gland contained the major part of total ^{241}Am load (43%) while the adductor muscle was the main storing organ for ^{134}Cs (76%). Nevertheless, the activities of both radionuclides in all the compartments of *P. maximus* decreased along the depuration phase (data not shown).

3.2. Dietary exposure

The depuration kinetics of the radionuclides ingested with food in whole body *P. maximus* were best fitted by a double exponential model (Fig. 2A and Table 1). ^{241}Am and ^{134}Cs displayed similar assimilation efficiencies ($20\% < AE < 30\%$) and close depuration rate constants, k_{el} , respectively 0.08 ± 0.02 and 0.05 ± 0.02 , which give close $T_{b/2l}$ ($9 \pm 2 \text{ d}$ and $14 \pm 7 \text{ d}$, respectively). At the end of the depuration period, ^{241}Am load was essentially in the shell (88% of the total body load) whereas most of the Cs was associated to the soft tissues; Table 2). Among the soft tissues, the digestive gland contained the main part of ^{241}Am (46%) whereas ^{134}Cs was mainly distributed between the digestive gland, the kidney, the mantle, the

adductor muscle and the remaining tissues, with the digestive gland presenting the higher average load, i.e., 27% (Table 2).

3.3. Sediment exposure

Bioaccumulation of sediment-bound radionuclides was measured in *P. maximus*. However, their whole-body uptake kinetics could not be fitted by a model having a biological meaning (Fig. 2B and Table 3). At the end of the exposure period, the highest transfer factor (TF) measured in for whole scallops was 0.011 ± 0.003 for ^{241}Am and 0.005 ± 0.002 for ^{134}Cs . Overall, the different body compartments displayed low $\text{TF}_{13\text{d}}$ with elevated standard deviations (Table 3). Consequently, none of the tissues could be identified as the main bioaccumulation organ using TF. However, in terms of body distribution, the shell displayed the main part of both radionuclides ($> 75\%$). Among soft tissues, the digestive gland and the adductor muscle contained the major part of ^{241}Am ($47 \pm 17\%$) and ^{134}Cs ($41 \pm 13\%$), respectively (Table 3).

The whole-body depuration kinetics of both ^{241}Am and ^{134}Cs after exposure to spiked sediment were best described by a two-compartment exponential equation (Fig. 2C and Table 1). The results indicated that 78% of ^{241}Am and 68% of ^{134}Cs previously bioaccumulated were efficiently retained, with biological half-lives of $79 \pm 42\text{d}$ and $74 \pm 49\text{d}$, respectively. At the end of the 31d depuration period, both radionuclides were mainly associated to the shell (Table 3). The distribution of ^{241}Am showed that in soft tissues, the major part of the radionuclide was retained in the digestive gland with $49 \pm 7\%$ of the total ^{241}Am load.

4. Discussion

Biomonitoring programs using scallops (Class Bivalvia, family Pectinidae) already exist (Fegan et al. 2010, JCAC 2002). Interestingly, scallops show a high accumulation capacity for radionuclides (Miramand et al. 1991, Nonnis Marzano et al. 2000, Bustamante et al. 2002)

that can be accumulated to higher degrees than in other bivalve species such as mussels (JCAC 2002). However, little is known about the behavior of radionuclides in scallops and their mode of uptake in comparison to other bivalve families such as Ostreidae or Mytilidae (Ryan 2002). To the best of our knowledge, experimental investigations were limited to the description of the uptake of waterborne ^{241}Am in *P. maximus* and to the localization of this transuranic element in the digestive gland cells (Miramand and Germain 1986, Miramand et al. 1991). Therefore, there is a lack of information on the uptake and retention of Am by scallops following other natural exposure pathways (i.e., food and sediment) although food and/or sediment were shown to constitute the main pathway of accumulation for Ag, Cd, Co, Pb, Zn (Metian et al. 2007, 2008b, 2009abc). Concerning Cs, no information on its bioaccumulation by scallops is currently available in the literature.

Using realistic activities of dissolved ^{241}Am (i.e., within the range of environmental levels), our study confirmed that ^{241}Am was efficiently accumulated in hard and soft tissues of the scallops, reaching a whole-body CF of 63 after 7d of exposure. This CF value is relatively high for bivalves. For example, CF were 10-30 after 5d of exposure in *Mytilus edulis* (Bjeregaard et al. 1985), 230 after 28d of exposure in *Cerastoderma edule* and 140 after 31d of exposure in *Scrobicularia plana* (Miramand et al. 1987). This CF was higher than in other marine invertebrates such as the sea urchin *Paracentrotus lividus* for comparable exposure time (i.e., approx. 30; Warnau et al. 1996). For *P. maximus*, Miramand and Germain (1986) showed a CF of 80 but after a longer exposure period to radiolabelled seawater (i.e., 38d). Based on the linear model describing the uptake kinetics, the scallops from the present experiment should accumulate ^{241}Am up to a CF of 360 after 38d. This difference could be due to the size/weight difference of the studied organisms since the scallops of Miramand and Germain (1986) were half-lighter than our organisms. Indeed, previous works have shown

314 that this factor affects metal bioaccumulation (Boyden 1974, 1977, Warnau et al. 1995,
315 Hédouin et al. 2007).

316 Following waterborne exposure, ^{241}Am was more efficiently concentrated than ^{134}Cs in
317 *Pecten maximus* (~20 times higher, Table 1). This difference is often found when marine
318 organisms are exposed to these two radionuclides through seawater (Warnau et al. 1996;
319 Bustamante et al. 2006). It could be related to physico-chemicals properties of each
320 radionuclide: as a transuranic radionuclide, americium (III) is strongly particulate reactive
321 (Ryan 2002) while Cs is soluble and not reacting with particles. Such reactive properties of
322 Am would lead to direct adsorption onto shells. It is therefore not surprising that ca. 95% of
323 the accumulated ^{241}Am was found on the shell of the scallops (Table 2). After they have been
324 accumulated from seawater, the radionuclides were depurated with very different rates with a
325 much shorter biological half-life for Cs (22d) than for Am (892d; Table 1). Such very long
326 biological half-life for Am might result from its retention on the shell. ^{134}Cs was mainly
327 present in the soft tissues, with 76% in the adductor muscle and the mantle (Table 2). This
328 specific accumulation pattern in muscular tissues is related to the analogous behavior of Cs^+
329 for K^+ (Ke et al. 2000, Smith et al. 2002, Lacoue-Labarthe et al. 2010). The predominant
330 distribution of ^{241}Am in the calcitic skeleton/endoskeleton have been shown by several
331 authors (Grillo et al. 1981, Guary et al. 1982, Fowler and Carvalho 1985). Fowler and
332 Carvalho (1985) demonstrated a positive correlation between the ^{241}Am CF in different
333 echinoderm species and the proportion of calcitic endoskeleton in the body wall of those
334 species. Recently, Zuykov et al. (2009) showed a preferential accumulation of ^{241}Am in the
335 organic periostracum of bivalve's shell. In *P. maximus*, it is noteworthy that the shell
336 contained most of ^{241}Am (up to 95%) whatever the exposure pathway was (seawater, food or
337 sediment, Tables 2 and 3). According to our results of dietary exposure, it clearly appears
338 that, beside a direct adsorption of dissolved ^{241}Am on the shell, the radionuclide is also

translocated from soft tissues (e.g. the digestive gland) to the shell. It is important to note that the relative affinity of ^{241}Am with the shell of *P. maximus* has been previously observed in the field (Miramand and Germain 1986). A good perspective for the temporal record of ^{241}Am in shell would be the use of ICP-MS coupled to laser ablation that has been already used in scallop for chronological survey of other elements (Thébault et al. 2009).

When scallops were fed radiolabelled phytoplankton, dietary ^{241}Am and ^{134}Cs were relatively poorly assimilated (AE: 28 and 20%, respectively; Table 1). Furthermore, both radionuclides were rapidly depurated, resulting in relatively short biological half-lives (between 1 and 2 weeks). These AEs are much lower than in echinoderms and cephalopods for which they vary from 33 to 90% and from 30 to 60%, respectively (Ryan 2002, Bustamante et al. 2006). However, our results for ^{241}Am are consistent with data reported for other bivalves, which displayed low ^{241}Am assimilation. For example, AE of ^{241}Am ranged between 2 and 13% in mussels of the genus *Mytilus* (Baines et al. 2005). Such low AE by mussels could be related to the radionuclide association with mineral fractions of phytoplankton (e.g. diatom shells) or on algal cell surfaces (e.g., Chlorophyceae such as *Dunaliella tertiolecta*) due to the particle-reactive properties of ^{241}Am (Fisher et al. 1983, Fisher and Teyssié 1986).

The scarcity of data on ^{134}Cs assimilation in marine invertebrates is conspicuous, especially compared to ^{241}Am data on the subject. Nevertheless, ^{134}Cs is not well bioaccumulated by phytoplankton. Heldal et al. (2001) working on ^{134}Cs uptake of five species (three prymnesiophytes and two diatoms) have shown that phytoplankton is unlikely to influence the Cs build-up in marine food webs and Cs flux to deep waters. Bivalves generally showed low AE for Cs with values ranging between 0.4 and 10% in the green mussel *Perna viridis* (Wang et al. 2000). In predators, reported AEs for Cs are higher, ranging between 44 and 58% in the gastropod *Babylonia formosae habei* and between 23 and 29% in the cuttlefish *Sepia officinalis* (Wang et al. 2000, Bustamante et al. 2006).

364

365 Regarding their way of living and their nutrition, scallops are in direct contact with bottom
366 sediments and predispose scallop to filter and ingest contaminated particles. For a species
367 filtering large quantities of sediment particles, the TF obtained at the end of the exposure were
368 quite low (TF_{13d} of ^{241}Am and $^{134}Cs < 0.011$ with a maximum $TF < 0.05$ over the whole
369 exposure period) but consistent with other sediment exposure studies (Miramand et al. 1982,
370 Bustamante et al. 2006, Borretzen and Salbu 2009).

371 In scallops from the field, radionuclide activity provides an integrated value of the
372 bioaccumulation process. However, experimental approaches are compulsory to quantify the
373 different physiological parameters of element bioaccumulation and to determine the relative
374 importance of the different exposure pathways (Warnau and Bustamante 2007). According to
375 the bioaccumulation kinetics of the radionuclides, Am seems to be mainly accumulated from
376 the dissolved phase since exposure to particule-associated Am (food and sediment) resulted in
377 quite poor absorption and retention, compared to ^{241}Am -dissolved bioaccumulation (efficient
378 uptake and strong retention). In the case of the Cs, the kinetic analyses did not clearly
379 revealed a trend on the major uptake pathway, even though the low amount of Cs
380 bioaccumulated through sediment exposure (whole body $TF < 0.01$) was highly absorbed (A_{01}
381 of $68 \pm 7\%$) and strongly retained ($T_{b/2} = 74d$). The use of a bioaccumulation model would
382 allow to further explore the importance of each exposure pathways *sensu* Thomann et al.,
383 (1995) and their use have been already developed and applied on scallops in previous studies
384 (Metian et al. 2007, 2008b, 2009b). However, variability of the kinetic parameters obtained
385 during the sediment experiment was high because the exposure was relatively short. Thus the
386 bioaccumulation model with 3 exposure pathways could not be run in the present study.
387 Experiments with longer exposure periods will be necessary to better specify these
388 parameters.

389

390 **5. Conclusion**

391 The present study provided new information about the different bioaccumulation pathways of
392 ^{241}Am and ^{134}Cs that scallops are facing in the field. In this context, our data showed that the
393 shell and the adductor muscle appeared to be the best scallop compartments for monitoring
394 respectively ^{241}Am and ^{134}Cs in the marine environment.

395

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404

405 **6. References**

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Table 1. Whole-body uptake and depuration kinetic parameters of ^{241}Am and ^{134}Cs in *Pecten maximus* following different exposure experiments:

1) exposed for 7d to waterborne radionuclides (n = 9) followed by 36d of depuration (n = 9);

2) after a 2h feeding on radiolabelled *Skeletonema costatum* for ^{241}Am followed by 21d of depuration (n = 9) and *Isochrysis galbana* for ^{134}Cs followed by 16d of depuration (n = 6)

3) exposed for 13d via the radiolabelled sediments (n = 6) and then maintained for 31d in clean sediment and running seawater (n = 6)

Experiment	Radionuclide	Uptake			Depuration				
		$\text{CF}_{\text{ss}} \pm \text{ASE}$	$k_u \pm \text{ASE}$	R^2	$A_{0s} \pm \text{ASE}$	$T_{b/2s} \pm \text{ASE}$	$A_{0l} \pm \text{ASE}$	$T_{b/2l} \pm \text{ASE}$	R^2
1) Seawater	^{241}Am	-	9.53 ± 0.35^d	0.80	22.76 ± 4.34^d	1.0 ± 0.4^a	77.64 ± 2.95^d	892	0.35
	^{134}Cs	0.94 ± 0.05^d	0.55 ± 0.07^d	0.79	54.87 ± 3.88^d	0.5 ± 0.1^d	45.02 ± 2.92^d	22 ± 3^d	0.89
2) Feeding	^{241}Am	-	-	-	79.72 ± 4.46^d	0.4 ± 0.1^d	20.29 ± 3.80^d	9 ± 2^c	0.93
	^{134}Cs	-	-	-	71.93 ± 6.30^d	0.1	28.07 ± 4.37^d	14 ± 7^a	0.88
3) Sediment	^{241}Am		n.a.		21.89 ± 8.74^a	0.7	78.22 ± 6.24^d	79 ± 42	0.35
	^{134}Cs		n.a.		32.01 ± 11.63^b	0.2	67.99 ± 6.54^d	74 ± 49	0.24

Uptake parameters: CF_{ss} : concentration factors at steady state; k_u : uptake rate constant (d^{-1}).

Depuration parameters: A_{0s} and A_{0l} : remaining activity (%) according to the short-and the long-lived exponential component, respectively; $T_{b/2}$: biological half-life (d). ASE: asymptotic standard error; r^2 : determination coefficient of the uptake or depuration kinetics.

Probability of the model adjustment: ^a $p < 0.05$. ^b $p < 0.01$. ^c $p < 0.001$. ^d $p < 0.0001$

n.a.: information not available.

Table 2. Concentration Factors (mean CF $\pm$ SD) and body distribution (mean % $\pm$ SD) of ^{241}Am and ^{134}Cs in *Pecten maximus* during seawater (after 7d of exposure and after 36d of depuration) and feeding experiments (21d after feeding with *Skeletonema costatum* for ^{241}Am and 16d after feeding with *Isochrysis galbana* for ^{134}Cs).

Radionuclides	Seawater contamination			Food contamination
	Uptake (7d, n = 5)		Loss (36d, n = 4)	Loss (21 or 16d, n = 4)
	Concentration Factor	Distribution (%)	Distribution (%)	Distribution (%)
^{241}Am				
Digestive gland	140 $\pm$ 51	23 $\pm$ 3	43 $\pm$ 6	46 $\pm$ 11
Gills	53 $\pm$ 30	18 $\pm$ 3	9 $\pm$ 1	7 $\pm$ 2
Kidneys	40 $\pm$ 14	2 $\pm$ 0	3 $\pm$ 1	3 $\pm$ 1
Intestine	109 $\pm$ 75	2 $\pm$ 1	< 1	5 $\pm$ 4
Gonad	23 $\pm$ 12	5 $\pm$ 2	9 $\pm$ 2	4 $\pm$ 2
Foot	44 $\pm$ 23	2 $\pm$ 1	< 1	2 $\pm$ 2
Mantle	30 $\pm$ 10	34 $\pm$ 2	19 $\pm$ 2	27 $\pm$ 7
Adductor muscle	8 $\pm$ 3	10 $\pm$ 2	7 $\pm$ 3	1 $\pm$ 1
Remaining tissues	71 $\pm$ 14	6 $\pm$ 4	8 $\pm$ 4	2 $\pm$ 2
Whole soft part	30 $\pm$ 8	7 $\pm$ 3	5 $\pm$ 0	12 $\pm$ 7
Shell	130 $\pm$ 21	93 $\pm$ 3	95 $\pm$ 0	88 $\pm$ 7
^{134}Cs				
Digestive gland	6 $\pm$ 2	10 $\pm$ 5	14 $\pm$ 2	27 $\pm$ 7
Gills	3 $\pm$ 1	11 $\pm$ 7	1 $\pm$ 1	4 $\pm$ 2
Kidneys	8 $\pm$ 1	3 $\pm$ 1	2 $\pm$ 0	16 $\pm$ 10
Intestine	2 $\pm$ 0	< 1	< 1	1 $\pm$ 1
Gonad	3 $\pm$ 0	6 $\pm$ 3	1 $\pm$ 0	5 $\pm$ 1
Foot	4 $\pm$ 1	2 $\pm$ 0	< 1	3 $\pm$ 1
Mantle	2 $\pm$ 0	24 $\pm$ 5	5 $\pm$ 0	16 $\pm$ 16
Adductor muscle	3 $\pm$ 1	41 $\pm$ 13	76 $\pm$ 3	14 $\pm$ 10
Remaining tissues	4 $\pm$ 0	2 $\pm$ 1	< 1	11 $\pm$ 11
Whole soft part	3 $\pm$ 1	76 $\pm$ 6	92 $\pm$ 2	n.a.
Shell	< 1	24 $\pm$ 6	8 $\pm$ 2	n.a.

n.a.: information not available.

Table 3. Transfer Factors (mean TF $\pm$ SD) of ^{241}Am and ^{134}Cs in *Pecten maximus* after 13d of exposure via sediment and body distribution (mean % $\pm$ SD) of ^{241}Am and ^{134}Cs at the end of the 13d exposure (n = 4) and 31d depuration periods (n = 4)

Radionuclides	Sediment contamination		
Compartments	Uptake (13d, n = 4)		Loss (31d, n = 4)
	Transfer Factor	Distribution (%)	Distribution (%)
²⁴¹Am			
Digestive gland	0.18 ± 0.07	47 ± 17	49 ± 7
Gills	0.02 ± 0.02	10 ± 10	8 ± 1
Kidneys	0.04 ± 0.04	3 ± 3	3 ± 1
Intestine	0.08 ± 0.07	2 ± 2	9 ± 9
Gonad	0.31 ± 0.31	14 ± 14	4 ± 2
Foot	0.03 ± 0.01	2 ± 1	6 ± 2
Mantle	< 0.01	14 ± 9	3 ± 1
Adductor muscle	< 0.01	3 ± 3	16 ± 2
Remaining tissues	0.04 ± 0.02	4 ± 4	1 ± 1
Whole soft part	0.02 ± 0.02	19 ± 11	8 ± 2
Shell	0.03 ± 0.01	81 ± 11	92 ± 2
¹³⁴Cs			
Digestive gland	0.02 ± 0.02	16 ± 11	19 ± 13
Gills	< 0.01	11 ± 5	16 ± 18
Kidneys	0.02 ± 0.01	3 ± 1	31 ± 40
Intestine	0.06 ± 0.06	4 ± 4	3 ± 3
Gonad	0.04 ± 0.04	12 ± 6	12 ± 13
Foot	0.08 ± 0.08	10 ± 10	3 ± 1
Mantle	< 0.01	18 ± 8	3 ± 2
Adductor muscle	< 0.01	41 ± 13	11 ± 11
Remaining tissues	0.02 ± 0.02	5 ± 3	2 ± 1
Whole soft part	0.005 ± 0.003	25 ± 1	24 ± 16
Shell	0.006 ± 0.002	75 ± 1	76 ± 16

Figures captions:

Fig. 1. Uptake kinetics of (A) ^{241}Am and (B) ^{134}Cs in *Pecten maximus* exposed for 7d to dissolved radiotracers (n=9) and their following 36d of depuration kinetics (C) (n=9).

All values are mean $\pm$ SD.

Fig. 2. Kinetics of ^{241}Am and ^{134}Cs in *Pecten maximus* (A) Depuration after a 2h feeding on radiolabelled *Skeletonema costatum* for ^{241}Am followed by 21d of depuration (n = 9) and *Isochrysis galbana* for ^{134}Cs followed by 16d of depuration (n = 6); Uptake and depuration kinetics of ^{241}Am and ^{134}Cs in *Pecten maximus* (B) exposed for 13d to the radiolabelled sediments (n = 6) and (C) then maintained for 31d in clean sediment and seawater (n = 6).

All values are mean $\pm$ SD.

Figure 1

Figure 2