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

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24 **Abstract**

25 In order to understand the bioaccumulation of ^{241}Am and ^{134}Cs in scallops living in sediments,
26 the uptake and depuration kinetics of these two elements were investigated in the king scallop
27 *Pecten maximus* exposed via seawater, food, or sediment under laboratory conditions.
28 Generally, ^{241}Am accumulation was higher and its retention was stronger than ^{134}Cs . This was
29 especially obvious when considering the whole animals exposed through seawater with
30 whole-body concentration factor ($\text{CF}_{7\text{d}}$) of 62 vs. 1, absorption efficiencies (A_{ol}) of 78 vs. 45
31 for seawater and biological half-lives ($T_{\text{b}/2\text{l}}$) of 892d vs. 22d for ^{241}Am and ^{134}Cs , respectively.
32 In contrast, following a single feeding with radiolabelled phytoplankton assimilation
33 efficiency (AE) and $T_{\text{b}/2\text{l}}$ of ^{134}Cs obtained were higher than those of ^{241}Am (AE: 28% vs.
34 20%; $T_{\text{b}/2\text{l}}$: 14d vs. 9d). Among scallop tissues, the shells always contained the higher
35 proportion of the total body burden of ^{241}Am whatever the exposure pathway was. In contrast,
36 the whole soft parts presented the major fraction of whole-body burden of ^{134}Cs , which was
37 generally associated with muscular tissues. Our results showed that the two radionuclides
38 have contrasting behaviors in scallops, in relation to their physico-chemical properties.

39

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

41 **1. Introduction**

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

52 The use of marine organisms for monitoring radionuclide contamination is well established
53 (Valette-Silver and Lauenstein 1995, Burger et al. 2007, Thébault et al. 2008). In order to
54 understand field measurements, the characterization of bioaccumulation parameters and/or the
55 relative importance of the different exposure pathways has been carried out for several
56 radionuclides (Ke et al. 2000, Wang et al. 2000, Baines et al. 2005, Borretzen and Salbu
57 2009). Beside mussels, other bivalve species are used to a lesser extent in biomonitoring
58 programs among which scallops appear of great interest as they highly accumulate trace
59 elements from their environment (Bryan 1973, Bustamante and Miramand 2005, Metian et al.
60 2008a, 2009a, Pan and Wang 2008). Scallops have also been reported to efficiently
61 concentrate natural and anthropogenic radionuclides such as ^{241}Am , ^{137}Cs , ^{210}Po , ^{238}Pu , ^{239}Pu
62 and ^{90}Sr in their tissues (Miramand et al. 1991; Nonnis Marzano et al. 2000; Bustamante et al.
63 2002). As for some metals (Brooks and Rumsby 1965, Chouvelon et al. 2009, Bustamante
64 and Miramand 2004), scallops sometimes display higher bioaccumulation capacity for ^{137}Cs
65 than other filter-feeders such as oysters and mussels occurring in the same areas (JCAC

66 2002). Within the Pectinid family, the current knowledge on concentrations of anthropogenic
67 radionuclides is limited to field measurements (Bustamante et al. 2002, Nonnis Marzano et al.
68 2000; Miramand and Germain 1986), and to waterborne exposure experiment to Am
69 (Miramand and Germain 1986, Miramand et al. 1991). The importance of other
70 contamination pathways in the bioaccumulation process of Am and Cs in scallops is not
71 known although sediment and food have been considered as possible source of Am and Cs
72 following the analyses carried out on scallops from the field or after laboratory studies
73 (Miramand and Germain 1986, Nonnis Marzano et al. 2000) and by histo-autoradiography
74 approach in laboratory (Miramand et al. 1991). Overall, sediment is considered as a major
75 vector of transuranic elements, among which Am, to biota (Miramand et al. 1982, Bustamante
76 et al. 2006, Ryan 2002). In comparison, Cs transfer from sediments appears relatively limited
77 (Bustamante et al, 2006; Borretzen and Salbu, 2009). Recently, food has been demonstrated
78 as a major pathway for metal bioaccumulation in scallops (Metian et al. 2009ab). Therefore, it
79 appears necessary to determine experimentally the bioaccumulation of radionuclides in
80 scallops via seawater, food and sediments in order to better understand the relative
81 contribution of these three pathways of exposure.

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

91 **2. Materials and Methods**

92 **2.1. Sampling**

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

100 **2.2. Radiotracer and counting**

101 Uptake and depuration kinetics of ²⁴¹Am and ¹³⁴Cs in scallop were determined using high-
102 specific activity radiotracers purchased from Isotope Product Lab (²⁴¹Am nitrate -0.1 N-, T_{1/2} =
103 433 years; ¹³⁴Cs chloride -0.1 N-, T_{1/2} = 2 years). Tracers were counted using a high-resolution
104 γ-spectrometer system composed of four Germanium (N- or P-type) detectors (EGNC 33-
105 195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer
106 equipped with a spectra analysis software (Interwinner® 6). The radioactivity was determined
107 by comparison with standards of known activity and of appropriate geometry. Measurements
108 were corrected for counting efficiency and physical radioactive decay. The counting time was
109 adjusted to obtain a propagated counting error less than 5% (Rodriguez y Baena et al. 2006).

110 **2.3. Seawater exposure**

111 Twenty five *P. maximus* (average weight ± SD: 208 ± 46 g) were placed in a 70-l glass
112 aquarium (constantly aerated, closed-circuit aquarium; salinity: 36 p.s.u.; temperature: 17 ±
113 0.5 °C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) and simultaneously exposed for 7d to ²⁴¹Am
114 and to ¹³⁴Cs dissolved in 0.45 μm filtrated seawater (0.3 and 1.4 kBq l⁻¹, respectively). No
115 change in pH was detectable after the tracer addition. Spiked seawater was renewed twice a

116 day during the first two days and then daily in order to keep radioactivity in seawater
117 constant. Activity of the ^{241}Am and ^{134}Cs in seawater was checked before and after each spike
118 renewal, yielding time-integrated activities of $0.13 \pm 0.09 \text{ kBq l}^{-1}$ and $1.23 \pm 0.03 \text{ kBq l}^{-1}$ (
119 Nine tag-identified scallops were collected at different time intervals and were whole-body
120 radioanalyzed alive (same identified individual each time). At the end of the 7d exposure
121 period, 5 scallops among the 25 were sacrificed and dissected. Shell, digestive gland, kidneys,
122 gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues were separated
123 and radioanalyzed in order to assess the radionuclide body distribution. The remaining
124 scallops were then placed in non contaminating conditions (constantly aerated, open-circuit
125 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
126 cycle: 12 h/12 h) for 36d and the nine tag-identified individuals were regularly radioanalyzed
127 alive in order to follow the depuration of ^{241}Am and ^{134}Cs from the scallops. Three non-
128 exposed individuals were introduced in the aquarium in order to control possible tracer
129 recycling from the contaminated scallops. During the 36d depuration period, scallops were
130 fed daily with *Skeletonema costatum* and *Isochrysis galbana* ($5 \cdot 10^4 \text{ cells ml}^{-1}$). At the end of
131 the depuration period, four contaminated scallops were collected and dissected into several
132 body compartments as previously described.

133 **2.4. Food exposure**

134 The Bacillariophyceae *Skeletonema costatum* was used to study the transfer of ^{241}Am and
135 ^{134}Cs to scallops through their diet. Phytoplankton cells were exposed to $4.5 \text{ kBq }^{241}\text{Am l}^{-1}$
136 and $7 \text{ kBq }^{134}\text{Cs l}^{-1}$ during their exponential growing phase (10d). After that period,
137 phytoplankton medium was filtrated (1 μm -mesh size; Osmonic filters), and the
138 phytoplankton cells resuspended in a 70-l aquarium (constantly aerated, closed-circuit
139 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
140 h) at a cell concentration of $5 \cdot 10^4 \text{ cell ml}^{-1}$ to avoid pseudofeces production by the scallops.

141 Nine *P. maximus* (average weight \pm SD: 199 ± 32 g) had been placed in the aquarium for one
142 week before the feeding experiment. Scallops were then allowed to feed on radiolabelled *S.*
143 *costatum* for 2h. After the feeding period, all scallops were γ -counted and flowing seawater
144 conditions (50 l h^{-1}) were restored in the aquarium. Individuals were then whole-body γ -
145 counted alive at different time intervals to follow the depuration kinetics of both elements.
146 Three non-exposed individuals were introduced in the aquarium in order to control possible
147 tracer recycling from the contaminated scallops. During the 21d depuration period, scallops
148 were fed daily with *Skeletonema costatum* and *Isochrysis galbana* (5.10^4 cells ml^{-1}). Four
149 contaminated individuals were randomly collected after 21d and dissected to determine the
150 radionuclide distribution among the different body compartments (shell, digestive gland,
151 kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues).
152 Radiolabelled *S. costatum* did not allow contaminating significantly the scallops with ^{134}Cs .
153 Therefore, another phytoplankton species was used to study the trophic transfer of this
154 radionuclide. To this end, the Haptophyceae *Isochrysis galbana* was used following the same
155 method as previously described for *S. costatum* except that phytoplankton cells were exposed
156 to ^{134}Cs during 7d (growing phase of *I. galbana*). Six *P. maximus* (average weight \pm SD: 127
157 ± 14 g) were exposed and whole-body γ -counted alive at different time of the depuration
158 experiment (16d). Four individuals were dissected at the end of the depuration period to
159 determine the ^{134}Cs distribution among the different body compartments (same as described
160 above).

161 **2.5. Sediment exposure**

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

166 Aerated sediment (9 kg) was placed in a plastic container, spiked with ^{241}Am (8 kBq) and
167 ^{134}Cs (13 kBq) for 6d with constant agitation, then used to form a homogeneous sediment
168 layer of 4 cm height in a 20-l aquarium. Weakly bound radioisotopes were allowed to leach
169 overnight under flowing seawater (50 l h^{-1}). Ten *P. maximus* (average weight \pm SD: 118 ± 5
170 g) were then placed for 13d in the aquarium (open circuit; parameters as previously
171 described), and six tag-identified individuals were regularly whole-body radioanalyzed alive.
172 Sediment samples were also regularly collected and γ -counted to verify that the radiotracer
173 activities in sediment remained constant. Activity of ^{241}Am and ^{134}Cs in sediment was
174 constant all along the exposure period (8.2 ± 0.8 and $13.1 \pm 3.0 \text{ Bq g}^{-1}$ wet wt, respectively).
175 At the end of the exposure period, four scallops were collected, dissected (shell, digestive
176 gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft
177 tissues), weighed and radioanalyzed to determine the radionuclide body distribution. The
178 remaining six scallops were placed in non-contaminating depuration conditions for 31d (new
179 20l glass aquarium with clean sediment under flowing seawater, 50 l h^{-1} , daily feeding on
180 *Skeletonema costatum* and *Isochrysis galbana* at $5 \cdot 10^4 \text{ cells ml}^{-1}$), and regularly γ -counted.
181 The radioactivity in sediment was regularly checked in order to ensure that no tracer recycling
182 occurred in the sediment. Although no radioactivity was detected, the whole sediment was
183 renewed after 1 week. After 31d of depuration, four scallops were collected and dissected as
184 described above to determine body distribution of ^{241}Am and ^{134}Cs .

185 **2.6. Data analysis**

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

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

193
$$\text{CF}_t = \text{CF}_{ss} (1 - e^{-k_e t}) \text{ (Eq. 1)}$$

194
$$\text{CF}_t = k_u t \text{ (Eq. 2)}$$

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

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

203
$$A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t} \text{ (Eq. 3)}$$

204 where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the
205 depuration rate constant (d^{-1}); 's' and 'l' are the subscripts for the short-lived' and 'long-lived'
206 components. For each exponential component (s and l), a biological half-life can be calculated
207 ($T_{b/2s}$ and $T_{b/2l}$) from the corresponding depuration rate constant (k_{es} and k_{el} , respectively)
208 according to the relation $T_{b/2} = \ln 2/k_e$ (Warnau et al. 1996). Regarding feeding experiments,
209 the 'long-lived' exponential term describes the fraction of the radiotracer ingested with food
210 that is actually absorbed by the organism (Warnau et al. 1996). The corresponding A_{0l}
211 represents the assimilation efficiency (AE) of the considered radiotracer. The best fitting
212 regression models were selected according to highest determination coefficient and
213 examination of residuals. The level of significance for statistical analysis was always set at α
214 = 0.05.

215

216 **3. Results**

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

219 **3.1. Seawater exposure**

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

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

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

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

241 and Table 1). The major part of the bioaccumulated ^{241}Am was efficiently absorbed (A_{01} :
242 78%) whereas only 45% of the bioaccumulated ^{134}Cs was absorbed in *P. maximus*. The
243 estimated depuration rate constant of the long-lived components (k_{el}) for ^{134}Cs was $0.031 \pm$
244 0.004 d^{-1} and, consequently, the derived biological half-life reached $22 \pm 3\text{d}$ (Table 1). In the
245 case of ^{241}Am , the depuration rate constant was not significantly different from 0 ($p < 0.05$),
246 thus the corresponding $T_{b/2l}$ may thus be considered as infinite. However, an estimation of $T_{b/2l}$
247 based on the mean value of k_{el} is shown in Table 1 (892d).

248 After 36d of depuration, the distribution of both radionuclides between the shell and the
249 whole soft parts remained similar to the one observed at the end of the exposure period:
250 ^{241}Am was mainly found in the shell and ^{134}Cs in the soft tissues (Table 2). Within soft
251 tissues, radionuclide distribution displayed a different pattern than the one observed at the end
252 of the exposure period (Table 2). Indeed, the digestive gland contained the major part of total
253 ^{241}Am load (43%) while the adductor muscle was the main storing organ for ^{134}Cs (76%).
254 Nevertheless, the activities of both radionuclides in all the compartments of *P. maximus*
255 decreased along the depuration phase (data not shown).

256 **3.2. Dietary exposure**

257 The depuration kinetics of the radionuclides ingested with food in whole body *P. maximus*
258 were best fitted by a double exponential model (Fig. 2A and Table 1). ^{241}Am and ^{134}Cs
259 displayed similar assimilation efficiencies ($20\% < AE < 30\%$) and close depuration rate
260 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 $14 \pm$
261 7d , respectively). At the end of the depuration period, ^{241}Am load was essentially in the shell
262 (88% of the total body load) whereas most of the Cs was associated to the soft tissues; Table
263 2). Among the soft tissues, the digestive gland contained the main part of ^{241}Am (46%)
264 whereas ^{134}Cs was mainly distributed between the digestive gland, the kidney, the mantle, the

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

267 **3.3. Sediment exposure**

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

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

285

286 **4. Discussion**

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

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

301 Using realistic activities of dissolved ^{241}Am (i.e., within the range of environmental levels),
302 our study confirmed that ^{241}Am was efficiently accumulated in hard and soft tissues of the
303 scallops, reaching a whole-body CF of 63 after 7d of exposure. This CF value is relatively
304 high for bivalves. For example, CF were 10-30 after 5d of exposure in *Mytilus edulis*
305 (Bjeregaard et al. 1985), 230 after 28d of exposure in *Cerastoderma edule* and 140 after 31d
306 of exposure in *Scrobicularia plana* (Miramand et al. 1987). This CF was higher than in other
307 marine invertebrates such as the sea urchin *Paracentrotus lividus* for comparable exposure
308 time (i.e., approx. 30; Warnau et al. 1996). For *P. maximus*, Miramand and Germain (1986)
309 showed a CF of 80 but after a longer exposure period to radiolabelled seawater (i.e., 38d).
310 Based on the linear model describing the uptake kinetics, the scallops from the present
311 experiment should accumulate ^{241}Am up to a CF of 360 after 38d. This difference could be
312 due to the size/weight difference of the studied organisms since the scallops of Miramand and
313 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

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

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

355 The scarcity of data on ^{134}Cs assimilation in marine invertebrates is conspicuous, especially
356 compared to ^{241}Am data on the subject. Nevertheless, ^{134}Cs is not well bioaccumulated by
357 phytoplankton. Heldal et al. (2001) working on ^{134}Cs uptake of five species (three
358 prymnesiophytes and two diatoms) have shown that phytoplankton is unlikely to influence the
359 Cs build-up in marine food webs and Cs flux to deep waters. Bivalves generally showed low
360 AE for Cs with values ranging between 0.4 and 10% in the green mussel *Perna viridis* (Wang
361 et al. 2000). In predators, reported AEs for Cs are higher, ranging between 44 and 58% in the
362 gastropod *Babylonia formosae habei* and between 23 and 29% in the cuttlefish *Sepia*
363 *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 ²⁴¹Am and ¹³⁴Cs < 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 ²⁴¹Am-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 (A₀₁
381 of 68 ± 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				
		$\text{CF}_{\text{ss}} \pm \text{ASE}$	$k_{\text{u}} \pm \text{ASE}$	R^2	$A_{0\text{s}} \pm \text{ASE}$	$T_{\text{b}/2\text{s}} \pm \text{ASE}$	$A_{0\text{l}} \pm \text{ASE}$	$T_{\text{b}/2\text{l}} \pm \text{ASE}$	R^2
1) Seawater	^{241}Am	-	$9.53 \pm 0.35^{\text{d}}$	0.80	$22.76 \pm 4.34^{\text{d}}$	$1.0 \pm 0.4^{\text{a}}$	$77.64 \pm 2.95^{\text{d}}$	892	0.35
	^{134}Cs	$0.94 \pm 0.05^{\text{d}}$	$0.55 \pm 0.07^{\text{d}}$	0.79	$54.87 \pm 3.88^{\text{d}}$	$0.5 \pm 0.1^{\text{d}}$	$45.02 \pm 2.92^{\text{d}}$	$22 \pm 3^{\text{d}}$	0.89
2) Feeding	^{241}Am	-	-	-	$79.72 \pm 4.46^{\text{d}}$	$0.4 \pm 0.1^{\text{d}}$	$20.29 \pm 3.80^{\text{d}}$	$9 \pm 2^{\text{c}}$	0.93
	^{134}Cs	-	-	-	$71.93 \pm 6.30^{\text{d}}$	0.1	$28.07 \pm 4.37^{\text{d}}$	$14 \pm 7^{\text{a}}$	0.88
3) Sediment	^{241}Am		n.a.		$21.89 \pm 8.74^{\text{a}}$	0.7	$78.22 \pm 6.24^{\text{d}}$	79 ± 42	0.35
	^{134}Cs		n.a.		$32.01 \pm 11.63^{\text{b}}$	0.2	$67.99 \pm 6.54^{\text{d}}$	74 ± 49	0.24

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

Depuration parameters: $A_{0\text{s}}$ and $A_{0\text{l}}$: remaining activity (%) according to the short- and the long-lived exponential component, respectively; $T_{\text{b}/2}$: 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 Compartments	Seawater contamination		Food contamination	
	Uptake (7d, n = 5)		Loss (36d, 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	Compartments	Sediment contamination		
		Uptake (13d, n = 4)		Loss (31d, n = 4)
		Transfer Factor	Distribution (%)	Distribution (%)
^{241}Am				
	Digestive gland	0.18 \pm 0.07	47 \pm 17	49 \pm 7
	Gills	0.02 \pm 0.02	10 \pm 10	8 \pm 1
	Kidneys	0.04 \pm 0.04	3 \pm 3	3 \pm 1
	Intestine	0.08 \pm 0.07	2 \pm 2	9 \pm 9
	Gonad	0.31 \pm 0.31	14 \pm 14	4 \pm 2
	Foot	0.03 \pm 0.01	2 \pm 1	6 \pm 2
	Mantle	< 0.01	14 \pm 9	3 \pm 1
	Adductor muscle	< 0.01	3 \pm 3	16 \pm 2
	Remaining tissues	0.04 \pm 0.02	4 \pm 4	1 \pm 1
	Whole soft part	0.02 \pm 0.02	19 \pm 11	8 \pm 2
	Shell	0.03 \pm 0.01	81 \pm 11	92 \pm 2
^{134}Cs				
	Digestive gland	0.02 \pm 0.02	16 \pm 11	19 \pm 13
	Gills	< 0.01	11 \pm 5	16 \pm 18
	Kidneys	0.02 \pm 0.01	3 \pm 1	31 \pm 40
	Intestine	0.06 \pm 0.06	4 \pm 4	3 \pm 3
	Gonad	0.04 \pm 0.04	12 \pm 6	12 \pm 13
	Foot	0.08 \pm 0.08	10 \pm 10	3 \pm 1
	Mantle	< 0.01	18 \pm 8	3 \pm 2
	Adductor muscle	< 0.01	41 \pm 13	11 \pm 11
	Remaining tissues	0.02 \pm 0.02	5 \pm 3	2 \pm 1
	Whole soft part	0.005 \pm 0.003	25 \pm 1	24 \pm 16
	Shell	0.006 \pm 0.002	75 \pm 1	76 \pm 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.

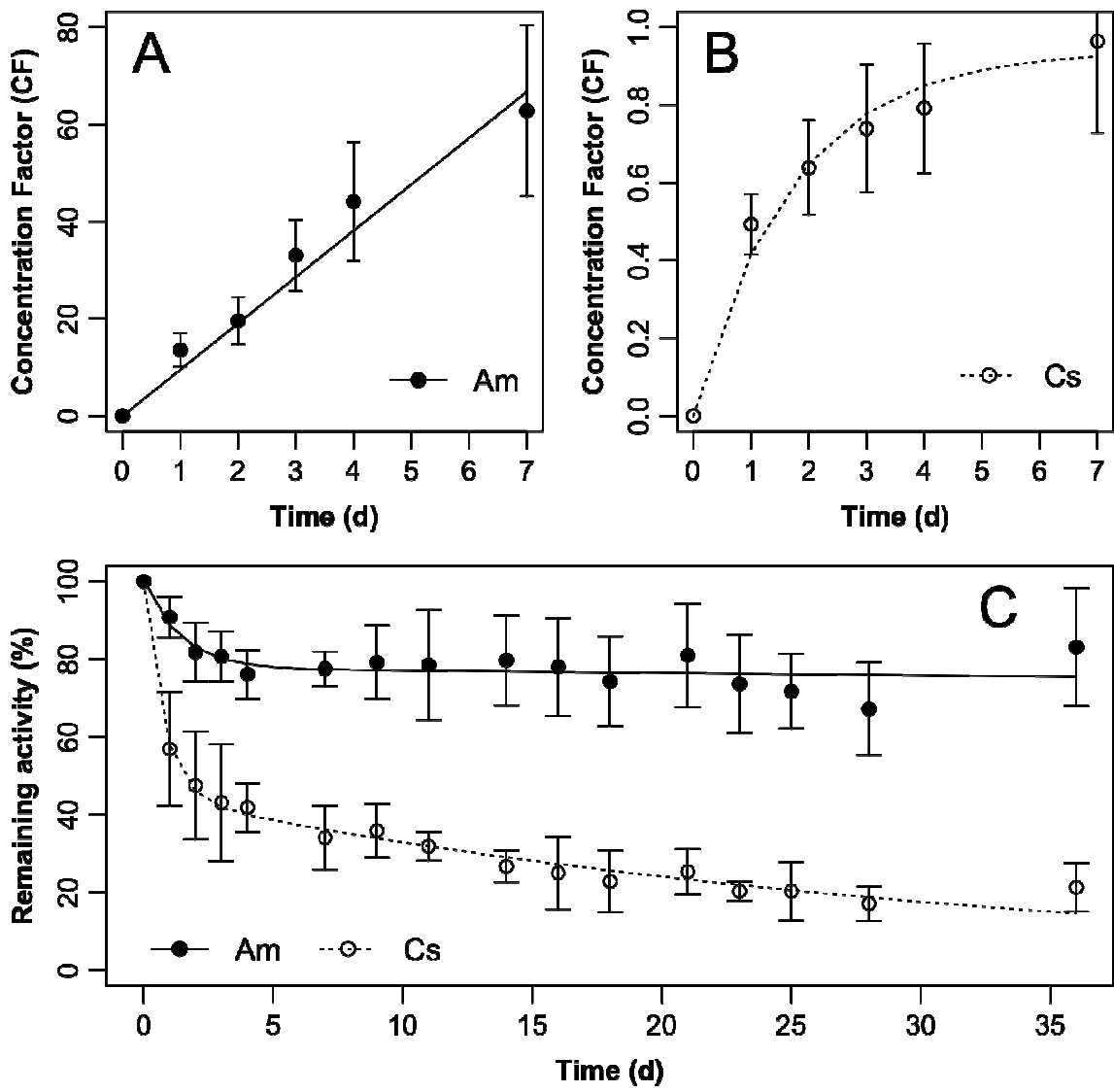


Figure 1

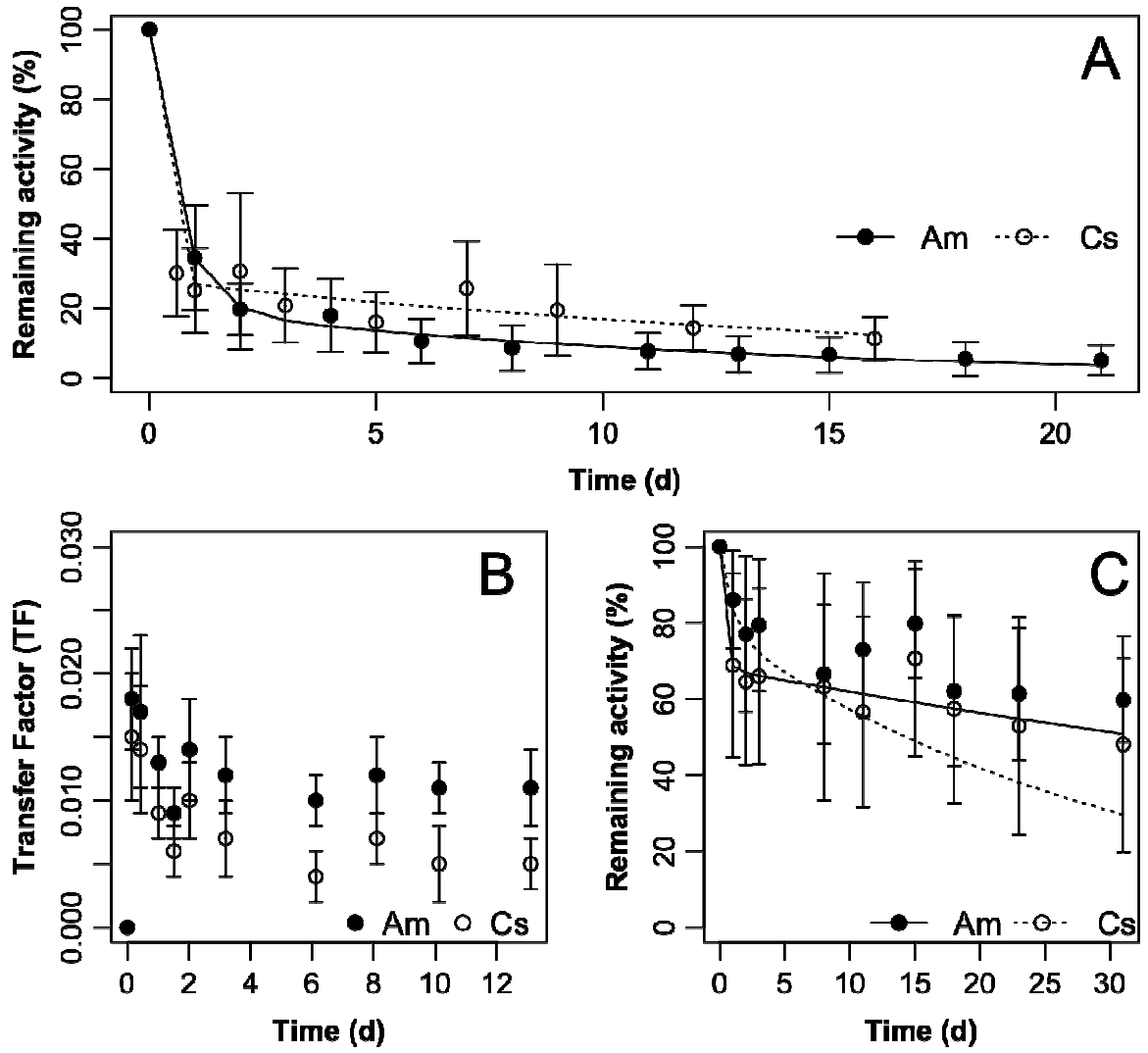


Figure 2