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## **Bioaccumulation of inorganic Hg by the juvenile cuttlefish *Sepia officinalis* exposed to <sup>203</sup>Hg radiolabelled seawater and food**

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1 **Bioaccumulation of inorganic Hg by the juvenile cuttlefish *Sepia officinalis***  
2 **exposed to <sup>203</sup>Hg radiolabelled seawater and food**

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27 **ABSTRACT:** Uptake and depuration kinetics of inorganic mercury (Hg) was investigated in  
28 the juvenile common cuttlefish *Sepia officinalis* following exposures via seawater and food  
29 using sensitive radiotracer technique ( $^{203}\text{Hg}$ ). The cuttlefish readily concentrated  $^{203}\text{Hg}$  when  
30 exposed via seawater, with whole body CF > 260 after only 10d of exposure. The total Hg  
31 accumulated from seawater was depurated relatively fast with a  $T_{b_{1/2}}$  of 17d. During both  
32 exposure and depuration periods, accumulated Hg was mainly (> 70%) associated with the  
33 muscular parts of the cuttlefish. However, the proportion of the whole body Hg content  
34 associated with the digestive gland increased during exposure and depuration phases,  
35 suggesting that the metal was transferred from the muscles towards this organ for  
36 detoxification. When fed with radiolabelled food, cuttlefish displayed high assimilation  
37 efficiency (AE > 90%) and the metal was found to be mainly located in the digestive gland  
38 (60% of the whole Hg content). Nevertheless, high depuration rates resulted in short  $T_{b_{1/2}}$  (i.e.,  
39 4 d), suggesting that this organ has a major role in Hg detoxification and depuration.  
40 Whatever the exposure pathway was, low proportion of Hg (< 2%) was found in the  
41 cuttlebone. Assessment of the relative contribution of the dietary and dissolved exposure  
42 pathways to inorganic Hg bioaccumulation in juvenile cuttlefish revealed that Hg was mainly  
43 accumulated from food that contributed for  $77 \pm 16\%$  of the global metal bioaccumulation.

44

45

46 **Keywords:** mercury; bioaccumulation; kinetics; body distribution; cephalopod; relative  
47 contribution.

48

## 49 INTRODUCTION

50 Mercury (Hg) is one of the metals of highest concern in the marine environment as it is  
51 readily methylated by micro-organisms, bioaccumulates in marine biota and consistently  
52 biomagnifies along the food chain (Cossa 1990). Among marine organisms, most of the  
53 available information on Hg is related to fish, mainly because of their importance as a food  
54 source for human. In fish, most of the Hg (i.e. > 95%) is methylated and is therefore  
55 bioavailable for upper trophic levels (Bloom 1992). Hence, fish consumption is an important  
56 source of Hg for human (Svensson et al. 1992) and is of particular health concern (Clarkson  
57 1990).

58 In contrast to fish, information on Hg in cephalopods tissues is scarce despite the fact that  
59 these molluscs represent an increasing component of the world fisheries (Boyle & Rodhouse,  
60 2006, FAO 2007). In addition, information on Hg in cephalopod is essentially limited to the  
61 main species targeted by fisheries and mainly reports metals levels in edible tissues, i.e.  
62 mantle muscle, arms and fins (e.g. Buzina et al. 1989, Sapunar et al. 1989, Plessi et al. 2001).  
63 Recently, a study on a large range of cephalopod species from the North Eastern Atlantic  
64 waters reported that Hg concentrations varied on two orders of magnitude among cephalopod  
65 species and that the metal was mainly stored under organic form in the muscular tissues  
66 (Bustamante et al. 2006). Several other studies on cephalopods from the Mediterranean  
67 suggest that these molluscs are able to accumulate high Hg concentrations in their tissues  
68 (Renzoni et al. 1973, Rossi et al. 1993, Storelli & Marcotrigiano 1999). Various factors are  
69 likely to influence Hg concentrations in cephalopods among which the size seems to be of  
70 primary importance (Monteiro et al. 1992, Rossi et al. 1993, Pierce et al. 2008).

71 Although it was suggested that food would be the main source for Hg accumulation in  
72 cephalopod tissues (Bustamante et al., 2006), the relative contribution of dietary and  
73 waterborne pathways has not been assessed in cephalopods. Moreover, as cephalopods are

74 short-lived species, they might be interesting as short-term indicator species of the variation  
75 of Hg concentrations in the environment (Seixas et al. 2005, Pierce et al. 2008).

76 For these reasons, the aim of this work was to investigate the biokinetics of Hg uptake and  
77 depuration in cephalopods in order to better characterize its bioaccumulation, tissue  
78 distribution and retention capacity. The common cuttlefish *Sepia officinalis* was selected as a  
79 model to study Hg transfer in cephalopods from seawater and food.

80

## 81 **MATERIALS AND METHODS**

### 82 **Organisms and radiotracer**

83 Adult cuttlefish were collected by net fishing off Monaco in March and April 2006. They  
84 were acclimated and maintained in open-circuit tanks in the IAEA-MEL premises. After  
85 mating, the eggs laid by a single female were separated to optimise their oxygenation and kept  
86 in a separate aquarium during the whole embryonic development (constantly aerated open  
87 circuit; flux: 50 l h<sup>-1</sup>; salinity: 37 p.s.u; temperature: 17 ± 0.5°C; pH: 8.0 ± 0.1; light/dark  
88 cycle: 12 h/12 h). Hatching occurred approx. 50 d after the spawning. Young cuttlefish were  
89 then maintained in the same aquarium and fed with brine shrimp (*Artemia* sp.) for 5 days  
90 before the experiments.

91 The radiotracer, <sup>203</sup>Hg [as <sup>203</sup>HgNO<sub>3</sub>; t<sub>1/2</sub> = 46.59 d], was purchased from Isotope Product  
92 Laboratory, USA. Stock solutions were prepared in 1 N nitric acid to obtain final radioactivity  
93 allowing the use of spikes of only a few microliters (typically 5 µl).

94

### 95 **Experimental procedure**

96 **Contamination from seawater:** juveniles (n = 23; mean weight ± SD, 0.258 ± 0.009 g) were  
97 placed for 10 d in a 20-l glass aquarium containing 0.45 µm filtered natural seawater  
98 (constantly aerated closed circuit; temperature 17°C; 37 p.s.u.; light/dark cycle 12h/12h)

99 spiked with  $^{203}\text{Hg}$  ( $0.6 \text{ kBq l}^{-1}$ ). In terms of stable metal, this concentration corresponded to 20  
100  $\text{ng l}^{-1}$ . To facilitate the recurrent counting of each individual during the experiment, the  
101 juveniles were held individually in separate circular plastics boxes (10 cm diameter, 5 cm  
102 height) covered up and down with a meshed plastic net to allow for free water circulation.  
103 Radiotracer and seawater were renewed every second day to maintain water quality and  
104 radiotracer activity constant. Radiotracer activities in seawater were checked before and after  
105 each water renewal in order to determine the time-integrated radiotracer activities (Rodriguez  
106 y Baena et al. 2006a, Warnau et al. 1996). Juveniles were separated in two groups: the first  
107 group contained 16 tag-identified individuals and the second was composed of unidentified  
108 animals for the body distribution analyses. At different time intervals, radiotracer activities  
109 were counted in the same tag-identified juveniles ( $n = 16$ ) all along the experiment.  
110 According to their physiological states, the individuals should be removed from the  
111 experiment leading the following sampling plan:  $n=16$  from days 0 to 3 and  $n = 14, 7, 6$  at  
112 days 6, 9 10, respectively. In addition, after 3 and 9 d of exposure, 3 juveniles of the second  
113 group were counted and dissected to determine the radiotracer distribution among the  
114 digestive gland, the cuttlebone and the remaining tissues.  
115 After this exposure period, the 6 remaining identified and radiolabelled juveniles ( $n= 6$  from  
116 days 0 to 4, 4 at day 6 and 1 at day 11) were held for 11 d in clean flowing water (open circuit  
117 with constant aeration; seawater flux  $50 \text{ l h}^{-1}$ ; temperature  $17^\circ\text{C}$ ; 37 p.s.u.; light/dark cycle  
118 12h/12h). At different time intervals during the depuration period, the same identified  
119 juveniles ( $n = 6$ ) were counted to establish the depuration kinetics of the radiotracer.

120

121 ***Contamination through the food:*** brine shrimp (*Artemia* sp.) were exposed for 5 d in a  
122 plastic aquarium containing 4 l of natural seawater spiked with  $^{203}\text{Hg}$  until pools of 25 brine

123 shrimp reached  $3.1 \text{ kBq g}^{-1}$  wet weight. The organisms were subsequently used as food for the  
124 juvenile cuttlefish.

125 As detailed in the seawater experimental procedure, juveniles were separated in two groups of  
126 identified and non-identified animals, which were devoted for the depuration kinetics and the  
127 body distribution studies, respectively. Hence, sixteen newly hatched cuttlefish (mean weight  
128  $\pm$  SD,  $0.297 \pm 0.011 \text{ g}$ ) were placed in individual plastic containers (10 cm diameter, 5 cm  
129 height), and held in a 20-l aquarium under the same conditions as in the previous experiment.  
130 Each juvenile was fed for 1 h with 25 of the previously radiolabelled *Artemia* sp. At the end  
131 of the feeding period, the cuttlefish were immediately counted. From that time on, the  
132 cuttlefish were fed twice a day with uncontaminated *Artemia* sp. for one month and regularly  
133 counted to determine radiotracer depuration kinetics and assimilation efficiency. As  
134 mentioned above, juveniles showing poor health condition were removed leading to a sample  
135 number decrease along the experiment:  $n = 16$  from days 0 to 2, 14 from days 3 to 14, 4 from  
136 days 17 to 22 and 1 from days 24. Throughout the depuration period, faeces were removed  
137 twice a day to reduce possible radiotracer recycling through leaching from the faeces. In  
138 addition, after 3 h, 9 d, and 22 d of exposure, 3 others juveniles were counted and dissected to  
139 determine the radiotracer distribution among the digestive gland, the cuttlebone and the  
140 remaining tissues.

141

## 142 **Radioanalyses and data treatment**

143 Radioactivities were measured using a high-resolution  $\gamma$ -spectrometry system consisting of  
144 four coaxial Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra<sup>®</sup> and Eurysis<sup>®</sup>)  
145 connected to a multi-channel analyzer and a computer equipped with a spectra analysis  
146 software (Interwinner<sup>®</sup> 6). The detectors were calibrated with an appropriate standard for each  
147 counting geometry used and measurements were corrected for background and physical decay

148 of the radiotracer. Counting times were adapted to obtain relative propagated errors less than  
149 5% (Rodriguez y Baena, 2006b). They ranged from 10 min to 30 min for whole juveniles and  
150 from 10 min to 24h for the dissected tissues.

151 Uptake of the  $^{203}\text{Hg}$  from seawater was expressed as change in concentration factors (CF;  
152 ratio between radiotracer content in the juvenile –  $\text{Bq g}^{-1}$  – and time-integrated activity in  
153 seawater –  $\text{Bq g}^{-1}$ ) along time (Warnau et al. 1996). Uptake kinetic was best described by a  
154 saturation equation (Eq.1):

$$155 \quad \text{CF}_t = \text{CF}_{\text{ss}} (1 - e^{-k_e t}) \quad (\text{Eq. 1})$$

156 where  $\text{CF}_t$  and  $\text{CF}_{\text{ss}}$  are the concentration factors at time  $t$  (d) and at steady state, respectively,  
157  $k_e$  is the biological depuration rate constants ( $\text{d}^{-1}$ ) (Whicker & Schultz 1982).

158 Radiotracer depuration kinetics were expressed in terms of change of percentage of remaining  
159 activity (i.e., radioactivity at time  $t$  divided by initial radioactivity measured in the organisms  
160 or in the tissue at the beginning of the depuration period \* 100) along with time.

161 The depuration kinetic was best fitted by a mono-exponential equation (Eq. 2):

$$162 \quad A_t = A_0 e^{-k_e t} \quad (\text{Eq. 2})$$

163 where  $A_t$  and  $A_0$  are the remaining activities (%) at time  $t$  (d) and 0, respectively, and  $k_e$  is the  
164 biological depuration rate constant ( $\text{d}^{-1}$ ). The determination of  $k_e$  allows the calculation of the  
165 radiotracer biological half-life ( $T_{b/2} = \ln 2 / k_e$ ). In the context of seawater and feeding  
166 experiment,  $A_0$  represents the absorption ( $A_{0,w}$ ) and the assimilation (AE) efficiencies,  
167 respectively.

168

### 169 **Bioaccumulation model**

170 The relative contribution of each uptake pathway was determined using the bioaccumulation  
171 model originally proposed by Thomann (1981) and revised by Thomann et al. (1995) and  
172 Metian et al. (2008). In this model, the total concentration of radiotracers in the juveniles,  $C_t$

173 (Bq g<sup>-1</sup>) is equal to the sum of each concentration resulting from the uptake by the different  
174 pathways (Eq. 3):

$$175 \quad C_t = C_{f,ss} + C_{w,ss} \quad (\text{Eq. 3})$$

176 where  $C_{f,ss}$  is the food-derived radiotracer concentration (Bq g<sup>-1</sup>) in juveniles at steady state  
177 (Eq. 4) and  $C_w$  is the water-derived radiotracer concentration (Bq g<sup>-1</sup>) in juveniles at steady  
178 state (Eq. 5).

$$179 \quad C_{f,ss} = (AE \times IR \times C_f) / k_{e,f} \quad (\text{Eq. 4})$$

$$180 \quad C_{w,ss} = (A_{0,w} \times k_{u,w} \times C_w) / k_{e,w} \quad (\text{Eq. 5})$$

181 where  $A_{0,w}$  is the absorption efficiencies (%) of the radiotracer from seawater, AE is the  
182 assimilation efficiency (%) of the radiotracer from food,  $C_f$  and  $C_w$  are the radiotracer  
183 activities in food and seawater (Bq g<sup>-1</sup> and Bq ml<sup>-1</sup>, respectively), respectively, IR is the  
184 ingestion rate (g g<sup>-1</sup> d<sup>-1</sup>),  $k_{u,w}$  is the uptake rate constants (d<sup>-1</sup>) from seawater,  $k_{e,f}$  and  $k_{e,w}$  are  
185 the biological depuration rate constants (d<sup>-1</sup>), for food and water pathways, respectively.

186 The relative contribution (%) of each uptake pathway is then assessed from the relation:

$$187 \quad \% \text{ food} = C_{f,ss} / (C_{f,ss} + C_{w,ss})$$

$$188 \quad \% \text{ seawater} = C_{w,ss} / (C_{f,ss} + C_{w,ss})$$

189

190 Constants (and their statistics) of the best fitting equations (decision based on ANOVA tables  
191 for two fitted model objects) were estimated by iterative adjustment of the models using the  
192 *nls* curve-fitting routine in R freeware. The level of significance for statistical analysis was  
193 always set at  $\alpha = 0.05$ .

194

## 195 **Results**

### 196 **Contamination through seawater**

197 Uptake activity of  $^{203}\text{Hg}$  in whole-body *S. officinalis* was best fitted by a saturation  
198 exponential equation with a calculated  $\text{CF}_{\text{ss}}$  of 480 (Fig. 1; Table 1). The CF actually  
199 measured at the end of the uptake period ( $\text{CF}_{10\text{d}}$ ) of  $^{203}\text{Hg}$  was  $260 \pm 70$  (Table 2). Calculated  
200  $\text{CF}_{10\text{d}}$  for the different organs indicated that  $^{203}\text{Hg}$  was concentrated according to the following  
201 decreasing order: digestive gland ( $1460 \pm 480$ ) > remaining tissues ( $290 \pm 80$ ) > cuttlebone  
202 ( $47 \pm 9$ ). In terms of body distribution,  $^{203}\text{Hg}$  was mainly found in the remaining tissues all  
203 along the exposure period; this compartment accounted for  $89 \pm 3$  and  $80 \pm 4$  % of the whole  
204 body load of  $^{203}\text{Hg}$  after 3 d and 10 d of exposure, respectively (Table 2). The cuttlebone  
205 presented very low Hg activity ( $< 15 \text{ Bq g}^{-1}$ ) and loads ( $< 3\%$ ) whereas the Hg activity in the  
206 digestive gland was increasing with time, varying from  $24 \pm 4 \text{ Bq g}^{-1}$  at day 3 to  $520 \pm 170 \text{ Bq}$   
207  $\text{g}^{-1}$  at day 9 corresponding to 8% and 20% of the total radioactivity loads, respectively.

208

209 After the 10 d exposure period, non-contaminating conditions were restored and depuration  
210 kinetic of  $^{203}\text{Hg}$  was followed for 11 d. The whole-body depuration kinetic of  $^{203}\text{Hg}$  in *S.*  
211 *officinalis* was best described by a mono-exponential model (Fig. 2; Table 1). This result  
212 indicated that 95% of the  $^{203}\text{Hg}$  previously accumulated were depurated with a relatively  
213 biological half-life relatively short (i.e. 17 d, Table 1). After 11 d of depuration conditions,  
214 most of the  $^{203}\text{Hg}$  body load was associated with the remaining tissues (72%) while the  
215 digestive gland proportion increase up to 28% (Table 2).

216

### 217 **Contamination through food**

218 The depuration kinetic of  $^{203}\text{Hg}$  ingested with food in *S. officinalis* was best fitted by a mono-  
219 exponential model, characterized by a biological half-life of 4 d (Fig.2; Table 1) and allowed  
220 an estimated assimilation efficiency of 91%.

221 During the depuration period, the digestive gland displayed highest proportion of the total  
222 body burden of  $^{203}\text{Hg}$ , i.e. 68, 60, 64 % after 3 h, 9 d and 22 d of depuration, respectively  
223 (Table 2). The distribution of  $^{203}\text{Hg}$  remained unchanged all along the loss experiment, with  
224 the cuttlebone always showing the lowest proportion of the radiotracer (Table 2).

225

### 226 **Bioaccumulation model**

227 In order to assess the relative contribution of each uptake pathway to the global Hg  
228 accumulation in *S. officinalis*, the different kinetic parameters obtained for seawater and food  
229 experiments were used to feed the bioaccumulation model, along with other parameters such  
230 as the  $^{203}\text{Hg}$  concentration in seawater and food ( $C_w$ :  $0.364 \text{ Bq ml}^{-1}$  and  $C_f$ :  $3100 \text{ Bq g}^{-1}$ ,  
231 respectively, present study) and the ingestion rate (IR:  $0.07 \text{ g g}^{-1} \text{ d}^{-1}$ , present study value being  
232 congruent with IR value determined by Koueta et al., 1999). Modelling showed that food  
233 represented the main pathway for  $^{203}\text{Hg}$  bioaccumulation in the juvenile of cuttlefish,  
234 contributing to  $77 \pm 16\%$  of the global metal bioaccumulation vs.  $23 \pm 14\%$  for the seawater  
235 pathway.

236

### 237 **Discussion**

238 Cephalopods are an increasing marine resource for world fisheries (Boyle & Rodhouse 2006).  
239 In the 1990s alone there was a 40% increase in squid catches worldwide (FAO 2007).  
240 Cephalopods are well-known for their capacity to accumulate high levels of non-essential  
241 metals, especially Ag and Cd, in their tissues (e.g. Martin & Flegal 1975, Bustamante et al.  
242 1998, 2008). Hence, the intake of contaminants such as metals by human through cephalopod  
243 consumption is a matter of concern (e.g. Pierce et al. 2008). Some studies also reported high  
244 concentrations of Hg in cephalopods from areas naturally contaminated by cinnabar such the  
245 Tyrrhenian and Adriatic Seas (e.g. Renzoni et al. 1973, Rossi et al. 1993, Storelli &

246 Marcotrigiano 1999). However, the dynamic of Hg incorporation and its metabolism in  
247 cephalopods remain poorly understood. In the field, Hg could be methylated that generally  
248 increases its toxicity as a result of its enhanced capacity of penetration across cell membranes  
249 (Boudou et al. 1983). The known methylHg uptake pathways in the biota are the transfer from  
250 the sediment where sediment-associated bacterial floras able to methylate part of the  
251 inorganic Hg (Compeau & Bartha 1985), and the consumption of the prey such as juveniles of  
252 shrimp, crab or fish where Hg is stored under the methylated form in variable proportions.  
253 Considering that sand burying is a transient cryptic behaviour of cuttlefish (Poirier et al.  
254 2004) and the grain size of the sediment selected by these species did not favour the  
255 methylation of Hg, the inorganic form of the metal could also be a significant source of  
256 accumulation for this nektobenthic cephalopod. In this study, uptake and depuration  
257 biokinetics of Hg were determined using inorganic carrier-free  $^{203}\text{Hg}$  in order to measure  
258 metal fluxes in real time at environmentally realistic contaminant concentrations (Warnau et  
259 al. 1996).

260 As a typical cephalopod, the common cuttlefish *Sepia officinalis* has high food intake  
261 requirements to sustain its elevated growth rate. Being active predators, cephalopods have a  
262 high digestion efficiency (Boucher-Rodoni et al. 1987) and food has been shown to constitute  
263 an important source of uptake for various trace elements such as Am, Cd, Co and Zn (e.g.,  
264 Guary & Fowler 1982, Koyama et al. 2000, Bustamante et al. 2002ab, 2004). However,  
265 seawater could be an important bioaccumulation pathway as well, as elements can be taken up  
266 efficiently through the skin and the gills. For instance, it has been shown that seawater is the  
267 main intake pathway for Ag in adult *S. officinalis* (Bustamante et al. 2004). Therefore, there  
268 was a need to provide insights on the bioaccumulation of Hg under controlled experimental  
269 conditions in order to delineate the contribution of its uptake via the dissolved and dietary  
270 pathways.

271 After 10 d of exposure to dissolved  $^{203}\text{Hg}$ , cuttlefish displayed quite elevated whole-body  
272 activities ( $\text{CF} = 260 \pm 70$ ), indicating that Hg was efficiently bioconcentrated from seawater  
273 (Table 2). The estimated steady-state equilibrium ( $\text{CF}_{\text{ss}} = 480 \pm 150$ ) would be reached after 2  
274 weeks of exposure. Among the three considered compartments, the digestive gland displayed  
275 the highest concentration capacity  $^{203}\text{Hg}$  ( $\text{CF} = 1460 \pm 480$ ; Table 2) whereas the cuttlebone  
276 showed the lowest CF (i.e. less than 50; Table 2). This pattern is very similar to that for  
277 experimental data reported for other metals such as Ag, Cd, Co and Zn (Bustamante et al.  
278 2002a, 2004). However, in terms of body burden distribution,  $^{203}\text{Hg}$  was mainly stored in the  
279 remaining tissues (up to 80 % of the total Hg load; Table 2), which are mainly composed of  
280 muscles although they include the skin and the respiratory organs (i.e. gills) corresponding to  
281 the tissues directly exposed to the contaminated seawater. Consistently, Bustamante et al.  
282 (2006) reported that 70-90% of the total Hg body burden was stored in the muscular tissues in  
283 different cephalopod species collected from the North East Atlantic. This could be explained  
284 by the fact that 1) that these tissues represents more than 70% of the total body weight and 2)  
285 that Hg has a stronger affinity for the sulphhydryl groups of muscular proteins rather than of fat  
286 tissue found in fish and/or cephalopod (Bloom 1992, Bustamante et al. 2006).

287 When *Sepia officinalis* was exposed to dissolved Hg, the digestive gland contained 8% and  
288 20% of the total Hg burden after 3 d and 10 d exposure, respectively (Table 2). Therefore, this  
289 increasing Hg proportion found in this organ implied that the digestive gland accumulated  
290 more efficiently than the others. In a second time, in depuration conditions,  $^{203}\text{Hg}$  was release  
291 of following a mono-exponential model and whole-body depuration was relatively rapid with  
292 a  $T_{b/2}$  of approx. 17 d. After 11 d of depuration under running seawater, the tissues in direct  
293 contact with seawater contained significantly less  $^{203}\text{Hg}$  than at the beginning of the  
294 depuration period varying from  $135 \pm 15 \text{ Bq g}^{-1}$  and to  $95 \pm 17 \text{ Bq g}^{-1}$  at d 0 and d 11 of the  
295 depuration period, respectively, whereas the digestive gland activity remained unchanged

296 (i.e., from  $520 \pm 170 \text{ Bq g}^{-1}$  at day 0 to  $560 \pm 30 \text{ Bq g}^{-1}$  at day 11; Table 2). These results  
297 suggest that 1) the digestive gland shows a stronger retention capacity for Hg than the other  
298 compartments, and/or that 2) a metal translocation occurred from the remaining tissues  
299 towards the digestive gland. In cephalopods, the digestive gland plays obviously a major role  
300 in the digestive processes but also in the detoxification of xenobiotics. Indeed, this organ has  
301 already been shown to retain translocated metals from the other tissues (Bustamante et al.  
302 2002a, 2004). The digestive gland is involved in the storage and detoxification of several  
303 metals such as Ag, Cd, Cu, or Zn (e.g. Miramand & Bentley 1992, Bustamante et al. 2002b,  
304 Dorneles et al. 2007) and persistent organic pollutants such as PCBs (Ueno et al. 2003, Danis  
305 et al. 2005, Storelli et al., 2006). However, due to the fact this organ does not store Hg in  
306 large amounts as shown in the field study (Bustamante et al. 2006), the digestive gland might  
307 be also involved in the depuration of Hg when cuttlefish is exposed to the dissolved metal.  
308 In the case of dietary exposure, the AE of Hg ingested with food was found to be nearly 100%  
309 (Table 1). This high degree of Hg assimilation might be due to the very efficient digestive  
310 metabolism that characterizes the juvenile cuttlefish (Mangold 1989). Indeed, this early life  
311 stage is characterized by a predominant intracellular digestion process (compared to the  
312 extracellular digestion which is dominant in adults) (Boucaud-Camou & Roper 1995), which  
313 could favour the metal assimilation. Such extreme assimilation efficiency has already been  
314 documented in cuttlefish: for instance, 90% and almost 100% AE for Cd and Co, respectively,  
315 were reported in juvenile fed brine shrimp (Bustamante et al. 2002a, 2004). Nevertheless, the  
316 assimilated Hg was rapidly depurated with a  $T_{b/2}$  of 4 d, which suggests that the processes  
317 governing Hg elimination are particularly efficient as well. Indeed, the  $^{203}\text{Hg}$  activities in the  
318 digestive gland dropped from  $310 \pm 80 \text{ Bq g}^{-1}$  to  $36 \pm 6 \text{ Bq g}^{-1}$  during the 22 d depuration  
319 period after the feeding (Table 2). These results highlight the efficient excretion capacity of  
320 the digestive gland for Hg. Because following dietary exposure the main fraction of the whole

321 Hg body burden was associated with this organ, it is not surprising that the retention time of  
322 Hg following dietary exposure was 4 times lower than following seawater exposure, i.e. 4 d  
323 vs. 17 d (Table 1).

324 In our experimental conditions, it appeared that the exposure of cuttlefish to contaminated  
325 seawater lead to an accumulation of the Hg in the juvenile remaining tissues (> 80%) mainly  
326 composed of muscular tissues. In the same time, Hg was translocated to the digestive gland  
327 and subsequently eliminated from the organism. Following dietary exposure, inorganic Hg  
328 was assimilated via the digestive gland and then rapidly eliminated. Consequently, the storage  
329 and/or redistribution of the bioaccumulated metal towards the remaining tissues is limited in  
330 cuttlefish. Nonetheless, considering both seawater and dietary exposure, food appears as the  
331 predominant pathway for inorganic Hg bioaccumulation in juvenile cuttlefish. This result is  
332 not surprising considering the relative high ingestion rate and the efficient digestive  
333 metabolism of cephalopods (Lee 1994).

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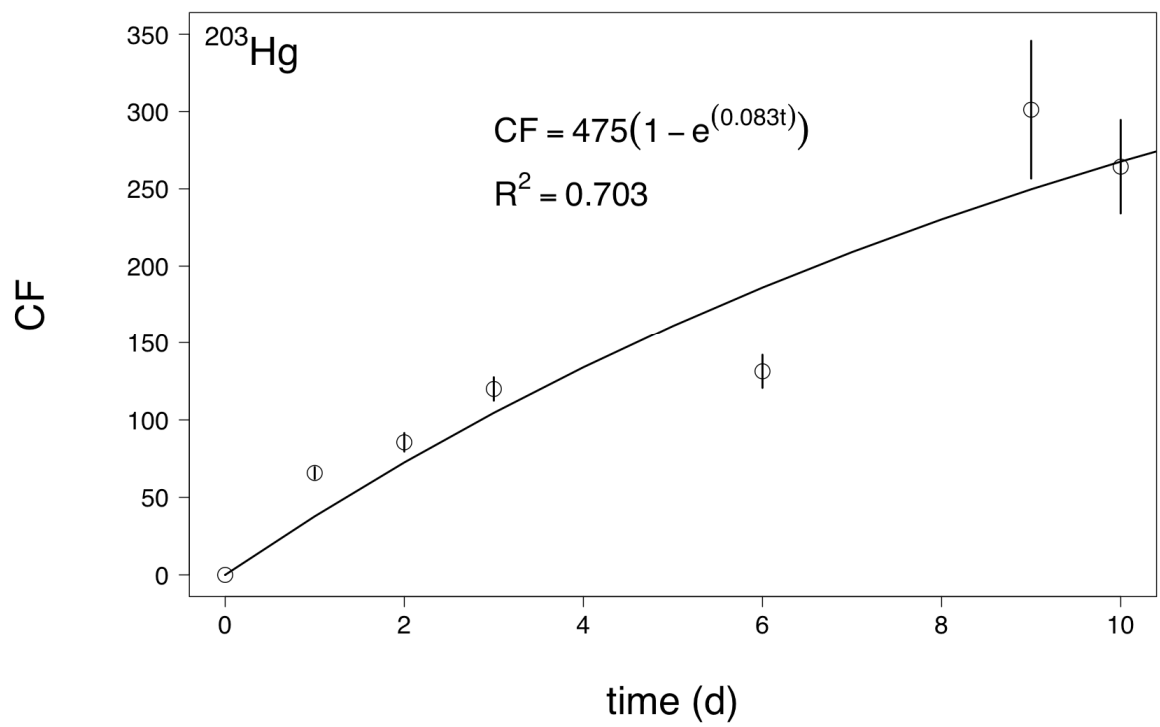
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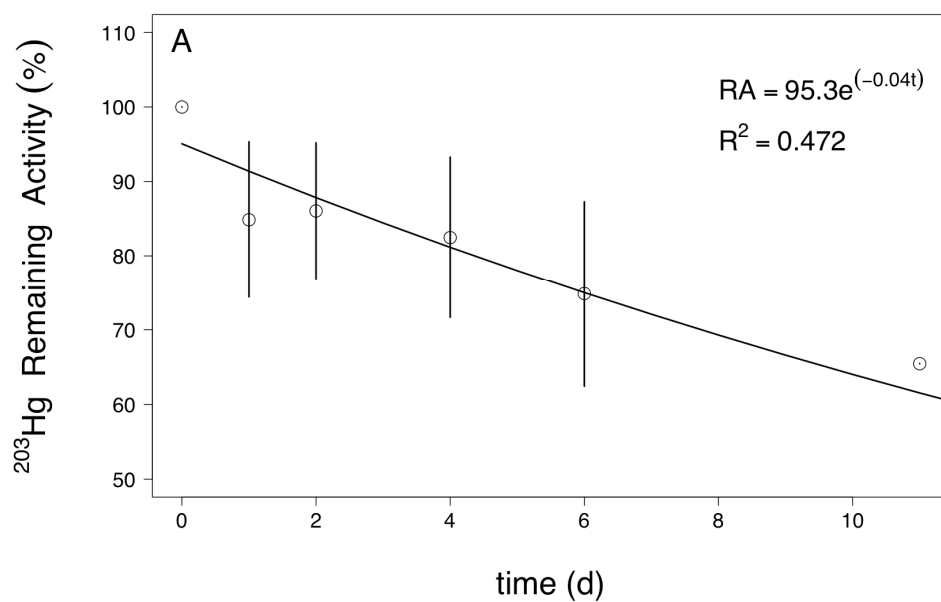
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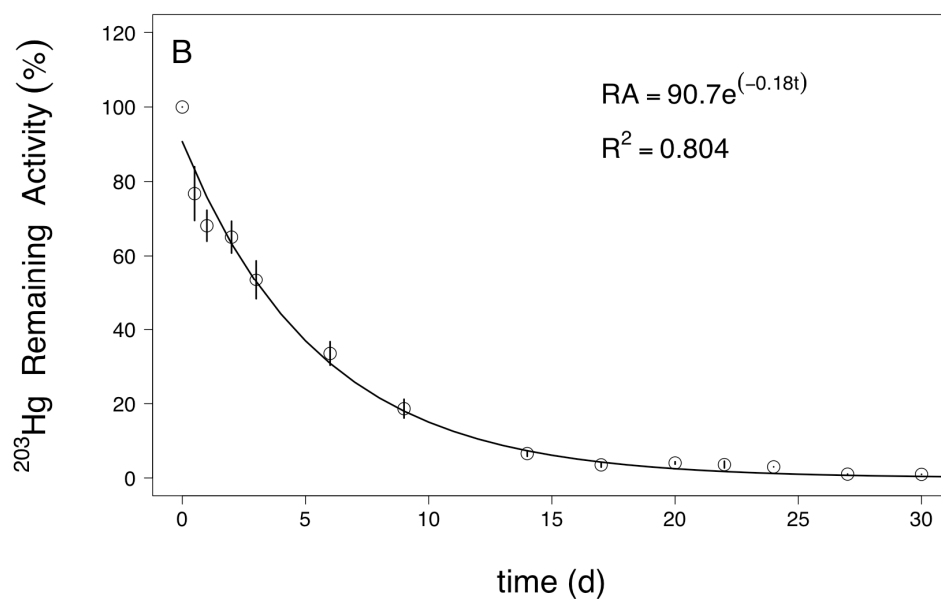


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Fig. 1: *Sepia officinalis*. Whole-body uptake kinetics of  $^{203}\text{Hg}$  in juvenile cuttlefish exposed for 10 days to the radiotracer dissolved in seawater (concentration factors, CF; mean  $\pm$  SD, n=16 from days 0 to 3 and n = 14, 7, 6 at days 6, 9 10, respectively).



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472 Fig. 2: *Sepia officinalis*. Whole-body loss kinetics of  $^{203}\text{Hg}$  (% of remaining activity; mean  $\pm$  SD) in juvenile  
 473 cuttlefish A) previously exposed to radiolabelled seawater for 10 days (n= 6 from days 0 to 4, 4 at day 6 and 1 at  
 474 day 11); B) previously fed with radiolabelled brine shrimp (n = 16 from days 0 to 2, 14 from days 3 to 14, 4 from  
 475 days 17 to 22 and 1 from days 24).

476 Parameters for the best fitting equations are given in Table 1.

Table 1. *Sepia officinalis*. Whole-body uptake and loss kinetic parameters of  $^{203}\text{Hg}$  in whole cuttlefish following different exposure experiments:

1) individuals (n = 16) were exposed 10-d to the radiotracer in seawater then placed 11-d in depuration conditions (n = 6);

2) individuals fed on radiolabelled brine shrimp *Artemia* sp. were placed in depuration conditions for 30 d (n = 16);

Uptake parameters:  $\text{CF}_{\text{ss}}$  concentration and transfer factors at steady state;  $k_u$ : uptake rate constant ( $\text{d}^{-1}$ )

All loss kinetics followed a mono-exponential depuration fit.

Depuration parameters:  $A_0$ : activity (%) lost according to the exponential component; ASE: asymptotic standard error;  $k_e$ : depuration rate constant ( $\text{d}^{-1}$ ),  $T_{b/2}$ : biological half-life (d);  $R^2$ : determination coefficient of the uptake or loss kinetics; p: probability of the model adjustment:

\*\*\* and \*\*: p-values < 0.001 and < 0.01, respectively.

Condition	Uptake			Loss			
	$\text{CF}_{\text{ss}}$	$k_e$	$R^2$	$A_0$ (SE)	$k_e$	$T_{b/2} \pm \text{SE}$ (d)	$R^2$
1) uptake seawater	480 ± 150	0.083 ± 0.036	0.703	-	-	-	-
2) loss seawater	-	-	-	95.3 (2.8) ***	0.041 ***	16.9 ± 3.9	0.472
3) loss food	-	-	-	90.7 (2.6) ***	0.180 ***	3.9 ± 0.3	0.804

1 Table 2. *Sepia officinalis*. Concentration Factors (mean CF  $\pm$  SD) and tissue distribution (mean %  $\pm$  SD) of  $^{203}\text{Hg}$  during seawater and feeding  
 2 experiments.

3

Compartments	Seawater contamination				Food contamination			
	Uptake (3d, n = 3)		Uptake (10d, n=3)		Loss (11d, n=1)	Loss (3h, n=3)	Loss (9d, n=3)	Loss (22d, n=3)
	Concentration Factor	Distribution (%)	Concentration Factor	Distribution (%)	Distribution (%)	Distribution (%)	Distribution (%)	Distribution (%)
Digestive gland	110 $\pm$ 20	8.3 $\pm$ 2.6	1460 $\pm$ 480	19.7 $\pm$ 3.8	28.2	67.9 $\pm$ 10.6	59.9 $\pm$ 3.4	63.6 $\pm$ 5.8
Cuttlebone	29.7 $\pm$ 3.7	2.8 $\pm$ 1.0	47.0 $\pm$ 8.8	<1	<1	1.4 $\pm$ 0.9	2.4 $\pm$ 1.9	9.9 $\pm$ 3.2
Remaining tissues	110 $\pm$ 10	88.9 $\pm$ 3.4	290 $\pm$ 80	79.5 $\pm$ 3.7	71.8	30.7 $\pm$ 9.9	37.7 $\pm$ 4.5	26.5 $\pm$ 3.3
Whole body	96 $\pm$ 15	100	260 $\pm$ 70	100	100	100	100	100