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ABSTRACT: Uptake and depuration kinetics of inorganic mercury (Hg) was investigated in the juvenile common cuttlefish *Sepia officinalis* following exposures via seawater and food using sensitive radiotracer technique (^{203}Hg). The cuttlefish readily concentrated ^{203}Hg when exposed via seawater, with whole body CF > 260 after only 10d of exposure. The total Hg accumulated from seawater was depurated relatively fast with a $Tb_{½}$ of 17d. During both exposure and depuration periods, accumulated Hg was mainly (> 70%) associated with the muscular parts of the cuttlefish. However, the proportion of the whole body Hg content associated with the digestive gland increased during exposure and depuration phases, suggesting that the metal was transferred from the muscles towards this organ for detoxification. When fed with radiolabelled food, cuttlefish displayed high assimilation efficiency (AE > 90%) and the metal was found to be mainly located in the digestive gland (60% of the whole Hg content). Nevertheless, high depuration rates resulted in short $Tb_{½}$ (i.e., 4 d), suggesting that this organ has a major role in Hg detoxification and depuration. Whatever the exposure pathway was, low proportion of Hg (< 2%) was found in the cuttlebone. Assessment of the relative contribution of the dietary and dissolved exposure pathways to inorganic Hg bioaccumulation in juvenile cuttlefish revealed that Hg was mainly accumulated from food that contributed for 77 ± 16% of the global metal bioaccumulation.

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

INTRODUCTION

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

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

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

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

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

MATERIALS AND METHODS

Organisms and radiotracer

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

The radiotracer, ^{203}Hg [as $^{203}HgNO_3$; $t_{½}$ = 46.59 d], was purchased from Isotope Product Laboratory, USA. Stock solutions were prepared in 1 N nitric acid to obtain final radioactivity allowing the use of spikes of only a few microliters (typically 5 µl).

Experimental procedure

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

spiked with ^{203}Hg (0.6 kBq l^{-1}). In terms of stable metal, this concentration corresponded to 20 ng l^{-1}. To facilitate the recurrent counting of each individual during the experiment, the juveniles were held individually in separate circular plastics boxes (10 cm diameter, 5 cm height) covered up and down with a meshed plastic net to allow for free water circulation. Radiotracer and seawater were renewed every second day to maintain water quality and radiotracer activity constant. Radiotracer activities in seawater were checked before and after each water renewal in order to determine the time-integrated radiotracer activities (Rodriguez y Baena et al. 2006a, Warnau et al. 1996). Juveniles were separated in two groups: the first group contained 16 tag-identified individuals and the second was composed of unidentified animals for the body distribution analyses. At different time intervals, radiotracer activities were counted in the same tag-identified juveniles (n = 16) all along the experiment. According to their physiological states, the individuals should be removed from the experiment leading the following sampling plan: n=16 from days 0 to 3 and n = 14, 7, 6 at days 6, 9 10, respectively. In addition, after 3 and 9 d of exposure, 3 juveniles of the second group were counted and dissected to determine the radiotracer distribution among the digestive gland, the cuttlebone and the remaining tissues.

After this exposure period, the 6 remaining identified and radiolabelled juveniles (n= 6 from days 0 to 4, 4 at day 6 and 1 at day 11) were held for 11 d in clean flowing water (open circuit with constant aeration; seawater flux 50 l h^{-1}; temperature 17°C; 37 p.s.u.; light/dark cycle 12h/12h). At different time intervals during the depuration period, the same identified juveniles (n = 6) were counted to establish the depuration kinetics of the radiotracer.

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

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

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

Radioanalyses and data treatment

Radioactivities were measured using a high-resolution γ-spectrometry system consisting of four coaxial 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 detectors were calibrated with an appropriate standard for each counting geometry used and measurements were corrected for background and physical decay

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

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

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

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

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

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

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

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively, and k_e is the biological depuration rate constant (d^{-1}). The determination of k_e allows the calculation of the radiotracer biological half-life ($T_{b½}$= ln2 / k_e). In the context of seawater and feeding experiment, A_0 represents the absorption ($A_{0,w}$) and the assimilation (AE) efficiencies, respectively.

Bioaccumulation model

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

(Bq g^{-1}) is equal to the sum of each concentration resulting from the uptake by the different pathways (Eq. 3):

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

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

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

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

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

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

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

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

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

Results

Contamination through seawater

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

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

Contamination through food

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

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

Bioaccumulation model

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

Discussion

Cephalopods are an increasing marine resource for world fisheries (Boyle & Rodhouse 2006). In the 1990s alone there was a 40% increase in squid catches worldwide (FAO 2007). Cephalopods are well-known for their capacity to accumulate high levels of non-essential metals, especially Ag and Cd, in their tissues (e.g. Martin & Flegal 1975, Bustamante et al. 1998, 2008). Hence, the intake of contaminants such as metals by human through cephalopod consumption is a matter of concern (e.g. Pierce et al. 2008). Some studies also reported high concentrations of Hg in cephalopods from areas naturally contaminated by cinnabar such the 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 fis 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 nectobenthic cephalopod. In this study, uptake and depuration
257 biokinetics of Hg were determined using inorganic carrier-free ^{203}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.

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

When *Sepia officinalis* was exposed to dissolved Hg, the digestive gland contained 8% and 20% of the total Hg burden after 3 d and 10 d exposure, respectively (Table 2). Therefore, this increasing Hg proportion found in this organ implied that the digestive gland accumulated more efficiently than the others. In a second time, in depuration conditions, ^{203}Hg was release of following a mono-exponential model and whole-body depuration was relatively rapid with a $T_{b½}$ of approx. 17 d. After 11 d of depuration under running seawater, the tissues in direct contact with seawater contained significantly less ^{203}Hg than at the beginning of the depuration period varying from 135 ± 15 Bq g^{-1} and to 95 ± 17 Bq g^{-1} at d 0 and d 11 of the depuration period, respectively, whereas the digestive gland activity remained unchanged

(i.e., from 520 ± 170 Bq g^{-1} at day 0 to 560 ± 30 Bq g^{-1} at day 11; Table 2). These results suggest that 1) the digestive gland shows a stronger retention capacity for Hg than the other compartments, and/or that 2) a metal translocation occurred from the remaining tissues towards the digestive gland. In cephalopods, the digestive gland plays obviously a major role in the digestive processes but also in the detoxification of xenobiotics. Indeed, this organ has already been shown to retain translocated metals from the other tissues (Bustamante et al. 2002a, 2004). The digestive gland is involved in the storage and detoxification of several metals such as Ag, Cd, Cu, or Zn (e.g. Miramand & Bentley 1992, Bustamante et al. 2002b, Dorneles et al. 2007) and persistent organic pollutants such as PCBs (Ueno et al. 2003, Danis et al. 2005, Storelli et al., 2006). However, due to the fact this organ does not store Hg in large amounts as shown in the field study (Bustamante et al. 2006), the digestive gland might be also involved in the depuration of Hg when cuttlefish is exposed to the dissolved metal.

In the case of dietary exposure, the AE of Hg ingested with food was found to be nearly 100% (Table 1). This high degree of Hg assimilation might be due to the very efficient digestive metabolism that characterizes the juvenile cuttlefish (Mangold 1989). Indeed, this early life stage is characterized by a predominant intracellular digestion process (compared to the extracellular digestion which is dominant in adults) (Boucaud-Camou & Roper 1995), which could favour the metal assimilation. Such extreme assimilation efficiency has already been documented in cuttlefish: for instance, 90% and almost 100% AE for Cd and Co, respectively, were reported in juvenile fed brine shrimp (Bustamante et al. 2002a, 2004). Nevertheless, the assimilated Hg was rapidly depurated with a $Tb_{½}$ of 4 d, which suggests that the processes governing Hg elimination are particularly efficient as well. Indeed, the ^{203}Hg activities in the digestive gland dropped from 310 ± 80 Bq g^{-1} to 36 ± 6 Bq g^{-1} during the 22 d depuration period after the feeding (Table 2). These results highlight the efficient excretion capacity of the digestive gland for Hg. Because following dietary exposure the main fraction of the whole

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

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

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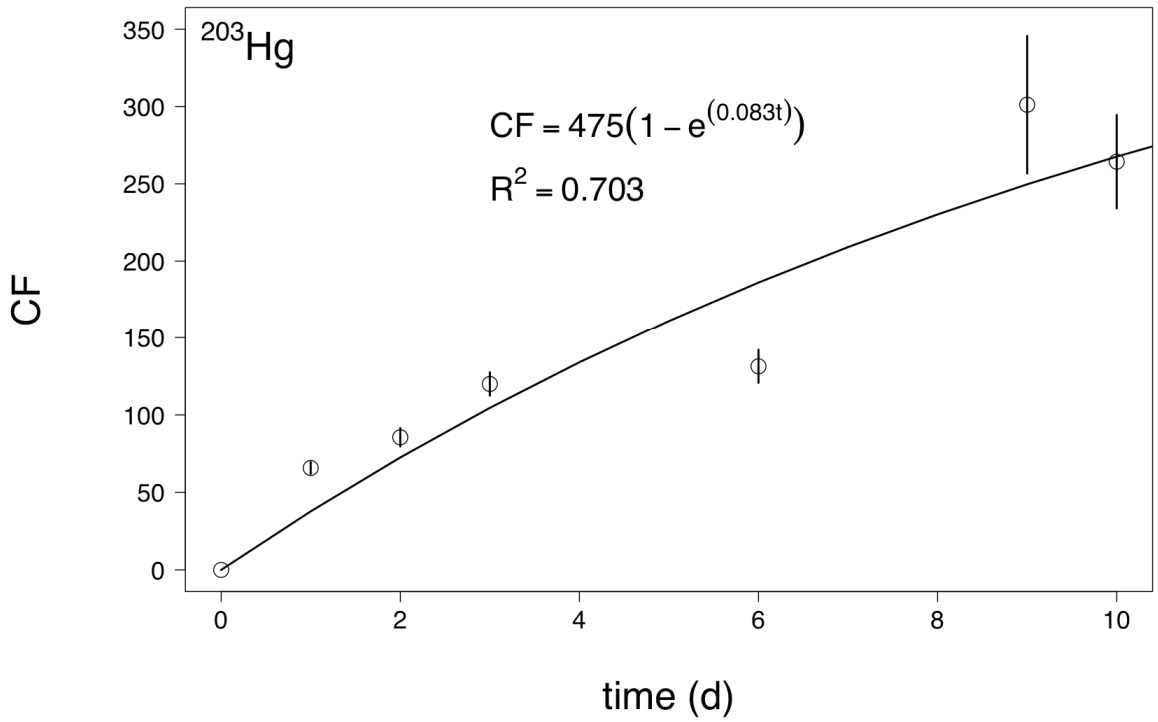

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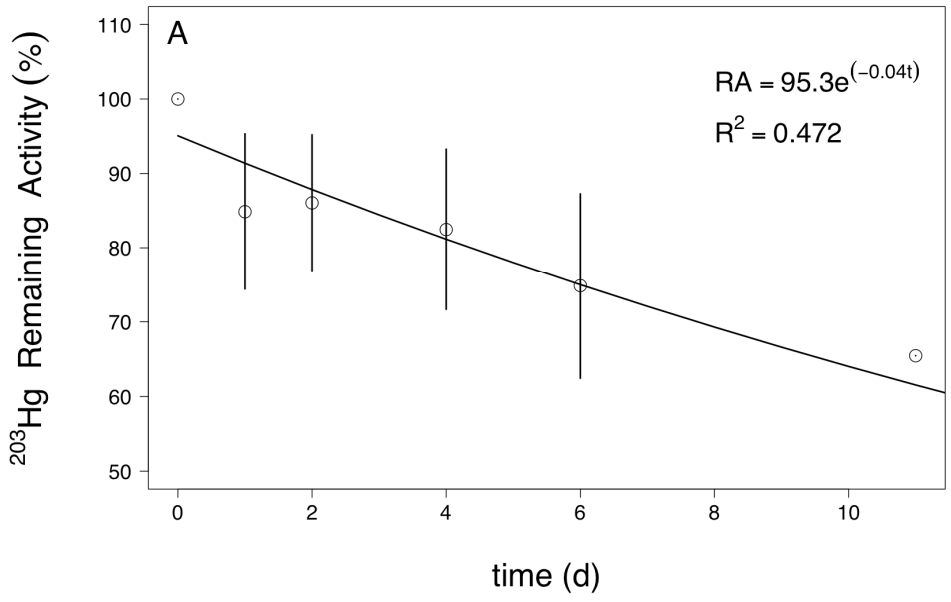
466 Fig. 1: *Sepia officinalis*. Whole-body uptake kinetics of ^{203}Hg in juvenile cuttlefish exposed for 10 days to the
467 radiotracer dissolved in seawater (concentration factors, CF; mean ± SD, n=16 from days 0 to 3 and n = 14, 7, 6
468 at days 6, 9 10, respectively).



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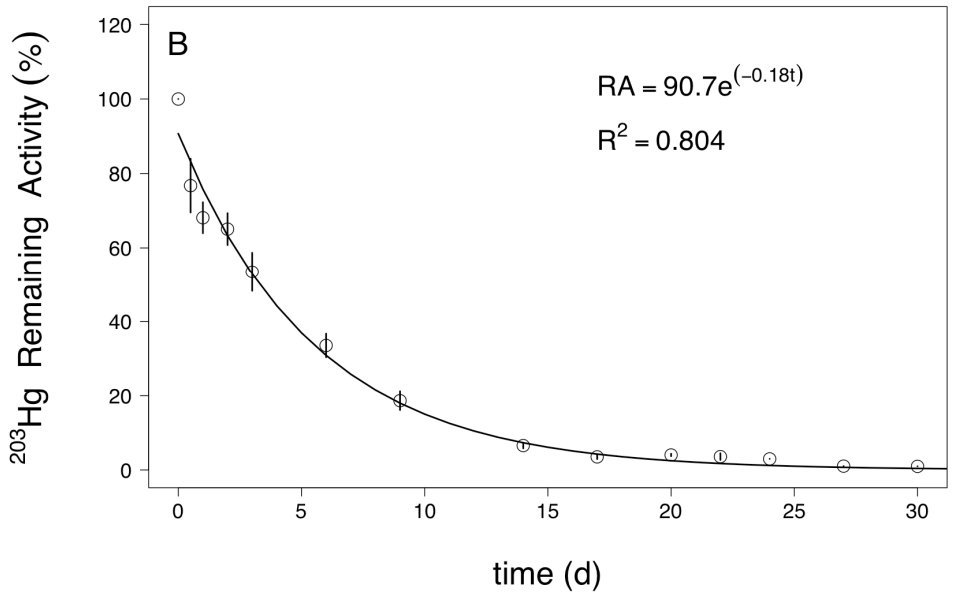

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472 Fig. 2: *Sepia officinalis*. Whole-body loss kinetics of ^{203}Hg (% of remaining activity; mean ± SD) in juvenile
473 cuttlefish A) previously exposed to radiolabelled seawater for 10 days (n= 6 from days 0 to 4, 4 at day 6 and 1 at
474 day 11); 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}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: CF_{ss} concentration and transfer factors at steady state; k_u: uptake rate constant (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 (d^{-1}), $T_{b½}$: 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			
	CF_{ss}	k_e	R^2	A_0 (SE)	k_e	$T_{b½} \pm$ 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

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

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 ± 20	8.3 ± 2.6	1460 ± 480	19.7 ± 3.8	28.2	67.9 ± 10.6	59.9 ± 3.4	63.6 ± 5.8
Cuttlebone	29.7 ± 3.7	2.8± 1.0	47.0 ± 8.8	<1	<1	1.4 ± 0.9	2.4 ± 1.9	9.9 ± 3.2
Remaining tissues	110 ± 10	88.9 ± 3.4	290 ± 80	79.5 ± 3.7	71.8	30.7 ± 9.9	37.7 ± 4.5	26.5 ± 3.3
Whole body	96 ± 15	100	260 ± 70	100	100	100	100	100