Delineation of 134 Cs uptake pathways (seawater and food) in the variegated scallop Mimachlamys varia

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Abstract: Among bivalves, scallops have been shown to be good bioindicator species for radionuclide monitoring. The present paper looked at the Cs bioaccumulation capacities of the variegated scallop *Mimachlamys varia* exposed separately via seawater and food under laboratory conditions. Results were compared with data previously obtained for the king scallop *Pecten maximus*, the only Pectinid species for which Cs accumulation has been studied in laboratory. Results indicated that *M. varia* has higher uptake capacity (CF: 1.86 ± 0.08) but lower absorption efficiency (A0l: 33 ± 5%) than *P. maximus* when exposed to waterborne Cs (CF of *P. maximus*: 0.94 ± 0.05 and A0l: 45 ± 3%). When scallops were fed radiolabeled phytoplankton, the assimilation efficiency of Cs was similar for the two species (AE: 24 ± 3% for M. varia and 28 ± 4% for *P. maximus*). Interspecific differences in terms of accumulation and retention, can be explained by physiological factors (including size of individuals) and/or difference in storage mecha- nisms. Indeed, organotropism differed between the two scallop species, suggesting the occurrence of specific redistribution mechanisms towards the tissues involved in Cs storage, excretion and detoxification. Finally, the present study examined the relative contribution of the different exposure pathways (seawater and food) to global [134]Cs bioaccumulation for *M. varia*. Results showed that food constitutes the main accumulation pathway, contributing for 77% of the global [134]Cs bioaccumulation.

1. Introduction

Tracking radionuclide contamination using organisms (i.e. biomonitoring) helps understanding the levels as well as the variation (in time and space) of radionuclides that are bioavailable in the environment (Philips and Rainbow, 1993). Since 1950, marine ecosystems were sporadically subjected to radionuclide contamination from industries, nuclear accidents and fallout from nuclear weapon testing and uses. Recently, the accident that occurred in the Daiichi Nuclear Power Plant at Fukushima in Japan, released massive amount of radioactive Cs in the marine environment (Chino et al., 2011; Bailly du Bois et al., 2012).

Among marine organisms, Pectinids have been proposed as good bioindicator candidates of radionuclide contamination (Marzano et al., 2000; Bustamante et al., 2002). This has been confirmed experimentally for the king scallop *Pecten maximus* (Metian et al., 2011). Field surveys (e.g. Marzano et al., 2000) have also documented the capacity of the family of Pectinids to concentrate radionuclides, in particular Cs. However, levels of radionuclides in organisms are influenced by various factors such as geographical origin, season, sexual maturity or species considered (Bryan, 1963; Mauri et al., 1990; Bustamante and Miramand, 2004, 2005). Laboratory characterization of Cs bioaccumulation by Pectinids is currently limited to one species, *P. maximus* (Metian et al., 2011) and since phylogenetically-close species may have different accumulation capacities (as shown for metals in scallops, e.g. Metian et al., 2007 and in oysters, Hedouin et al., 2010a), it is worth comparing Cs bioaccumulation capacities among scallops species.

Therefore, the objective of the present study was to investigate ^{134}Cs bioaccumulation capacities of the variegated scallop *Mimachlamys varia*, exposed via the dissolved and trophic pathways. This species is an interesting model to assess Cs contamination in coastal area given the fact that it settles, with its byssus, on rocky substrates (Shumway and Parsons, 2006) whereas *P.*

maximus lives buried in the sediment. The same protocol as described in Metian et al. (2011) was used in order to allow easy comparison between the two scallop species. In addition, the relative contribution of the different exposure pathways (dissolved vs. dietary) of Cs bioaccumulation in *M. varia* was determined.

2. Materials and methods

2.1. Origin and acclimation of organisms

In spring 2004 and 2005, one hundred variegated scallops *M. varia* were collected on the French Atlantic coast (Pertuis Breton, Charente-Maritime) by SCUBA diving. They were transported to IAEA-EL premises in Monaco and were acclimated to laboratory conditions for 4 weeks (constantly aerated, open-circuit 700 L aquarium; flux: 50 L.h^{-1}; salinity: 36 p.s.u.; temperature: 17 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) prior to experimentation. During this period, scallops were fed daily an algal diet (*Isochrysis galbana*).

2.2. Radiotracer and counting

Uptake and depuration kinetics of ^{134}Cs in scallops were determined using high-specific activity ^{134}Cs purchased from Isotope Product Lab (^{134}CsCl 0.1 N, T½ = 2 years). ^{134}Cs was counted using a high-resolution γ-spectrometer system composed of four Germanium (N- or P-type) detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multi-channel analyzer and a computer equipped with a spectra analysis software (Interwinner® 6). The radioactivity was determined by comparison with standards of known activity and of appropriate geometry. Measurements were corrected for counting efficiency and physical radioactive decay. The counting time was adjusted to obtain a propagated counting error less than 5% (Rodriguez y Baena et al., 2006).

2.3. Seawater exposure

Twenty three *M. varia* (30 ± 7g) were placed in a 70-L aquarium (constantly aerated, closed-circuit aquarium; salinity: 36 p.s.u.; temperature: 17°C ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) and exposed for 7 d to ^{134}Cs dissolved in 0.45 µm filtered seawater (1.4 kBq L^{-1}). Spiked seawater was renewed regularly in order to keep radioactivity constant. The ^{134}Cs in seawater was checked before and after each spike renewal. Nine tag-identified scallops were collected at different time intervals and were whole-body radioanalyzed alive. At the end of the 7 d exposure period, four scallops were collected and dissected into their body compartments (digestive gland, gills, kidneys, intestine, gonad, foot, mantle, adductor muscle and remaining tissues). Samples were radioanalyzed in order to assess the radionuclide body distribution. The remaining scallops were then placed in uncontaminated water in the same conditions for 36 d and the nine identified individuals were regularly radioanalyzed alive in order to follow the depuration kinetics of ^{134}Cs. Three non-exposed individuals were introduced into the aquarium in order to control possible tracer recycling from the contaminated scallops. During the 36 d depuration period, scallops were fed daily with *I. galbana* (5.10^4 cells mL^{-1}). At the end of the depuration period, four contaminated scallops were collected and dissected.

2.4. Dietary exposure

^{134}Cs transfer to scallops through their diet was studied using the Haptophyceae *Isochrysis galbana*. Phytoplankton cells were exposed to 7 kBq ^{134}Cs L^{-1} during their exponential growing phase (7 d). After that period, the phytoplankton medium was filtered (1 µm-mesh size; Osmonic filters), and the phytoplankton cells re-suspended in a 70 L aquarium in the same conditions as previously described for the scallops. Cell density was 5.10^4 cells mL^{-1} to avoid pseudofeces production by the mollusks. Six *M. varia* (17 ± 5g) had been placed in the aquarium for one week before the start of feeding experiment. Scallops were fed with radiolabelled

phytoplankton for 2 h in closed-circuit. After the feeding period, all scallops were γ-counted and flow restored in the aquarium. Depuration was followed during 30 d as described previously (see section 2.3). Three non-exposed individuals were used as controls of tracer recycling. During the 30-d depuration period, scallops were fed daily with *I. galbana* (5.10^4 cells mL^{-1}). Four contaminated individuals were collected after 30 d and dissected to determine the radionuclide body distribution.

2.5. Data analysis

Whole-body uptake and depuration kinetics were fitted using nonlinear regression routines and iterative adjustment (Statistica® 7) and statistical methods described by Warnau et al. (1996a, 1996b) and Metian et al. (2011). The level of significance for statistical analyses was always set at $\alpha = 0.05$. The relative contribution of each pathway (seawater and food) was determined using a global bioaccumulation model described in Hédouin et al (2010a). In addition to the parameters obtained from each biokinetic models, we have used the ingestion rate of *M. varia* (g g^{-1} d^{-1}, wet wt basis) and the Kd$_f$, partition coefficients for phytoplankton calculated experimentally (Metian et al., 2009).

3. Results

3.1. Seawater exposure

Whole-body uptake of ^{134}Cs in *M. varia* followed exponential kinetics, reaching a steady-state equilibrium after approximately 4 days ($R^2 = 0.79$; Fig. 1A and Table 1). The kinetic parameters and their statistics are shown in Table 1. The whole-body concentration factor of ^{134}Cs (i.e. ratio between the radioisotope activity in organisms – Bq g^{-1} wet wt – and time-integrated activity in the seawater – Bq g^{-1}) estimated at steady state (CF$_{ss}$) was 1.86 ± 0.08 (Fig. 1A and Table 1).

Calculated CF_{7d} for the different body compartments are shown in Table 2. Among the tissues and organs, the foot displayed the highest CF closely followed by the kidney and the digestive gland (Table 2). In term of distribution, Cs was mainly located in the adductor muscle, the digestive gland and the mantle with, respectively, 26 ± 3, 24 ± 10 and $23 \pm 5\%$ of the total Cs body burden.

After the exposure period, non-contaminating conditions were restored and depuration of ^{134}Cs was followed for 36 d. The whole-body depuration kinetics of ^{134}Cs was best described by a two-component exponential model (Fig.1B and Table 1). An important part of ^{134}Cs was efficiently absorbed (A_{0l}: $33 \pm 4.6\%$) with an associated long-term biological half-life ($T_{b1/2l}$) of 178 d (not significantly different from infinite; Table 1). After 36 d of the depuration, ^{134}Cs was mainly located in the soft body parts ($72 \pm 7\%$; Table 2). Distribution of ^{134}Cs among the soft tissues displayed a different pattern than that observed at the end of the exposure period (Table 2). Indeed, the digestive gland contained most of ^{134}Cs ($59 \pm 13\%$) and the relative proportion of the total Cs found in the intestine was much higher than the one in the same organ after the exposure phase (10 *vs.* 41%).

3.2. Dietary exposure

The depuration kinetics of the ^{134}Cs ingested with food in *M. varia* was best fitted by a double exponential model (Fig. 1C and Table 1). *M. varia* displayed an assimilation efficiency of $24 \pm 3\%$. The depuration rate constant, k_{el} (0.006 ± 0.006 d^{-1}) was not significantly different from 0 (p>0.05), and therefore the derived $T_{b\frac{1}{2}l}$ is not significantly different from infinite (115 d). At the end of the depuration period, the digestive gland contained the major part of ^{134}Cs ($57 \pm 16\%$, Table 2).

3.3. Bioaccumulation model

The relative contribution of each exposure pathway to the global bioaccumulation of ^{134}Cs was determined using the different kinetic parameters determined in the two experiments, as well as other parameters such as the ^{134}Cs K_{df} in phytoplankton (1.32×10^2 for *I. galbana*; present study) and the ingestion rate of phytoplankton in scallops (IR = 0.0869 g g^{-1}d^{-1}; Metian et al., 2009). Results of the computations indicated that feeding pathway was the major contributor (77%) to the global bioaccumulation of ^{134}Cs in *M. varia* whereas seawater contributed for 23%.

4. Discussion

Pectinids have been used in biomonitoring programmes to assess radionuclide contamination in the marine environment (JCAC, 2002) given their high accumulation capacity shown during field surveys (Marzano et al., 2000; Bustamante et al., 2002). However, the behavior of radionuclides in scallops and their mode of uptake and depuration are less known in comparison to other bivalve families such as Ostreidae or Mytilidae (Cranmore and Harrison, 1975; Ke et al., 2000; Ryan, 2002). To the best of our knowledge, experimental investigations of ^{134}Cs bioaccumulation have been limited to one species *P. maximus* exposed to three pathways. Unlike *P. maximus*, which lives buried in the bottom sediment, *M. varia* settles, with its byssus, on rocky substrates (Shumway and Parsons, 2006); therefore, the exposure of *M. varia* to radionuclides occurs through seawater and food pathways.

Our results show that there are interspecific differences in Pectinids in terms of accumulation and retention of dissolved Cs. Indeed, the concentration factor (CF$_{ss}$) in *M. varia* was two times higher than CF$_{ss}$ of *P. maximus* (Metian et al., 2011). Difference can be attributed to variation of physiological factors (including size of individuals) and/or difference in storage mechanisms (as highlighted by the contrasting body distribution patterns of Cs in both species at the end of exposure; Metian et al., 2011). Similar interspecific differences of CF$_{ss}$ between these two

species have already been highlighted for other radionuclides (^{110m}Ag, ^{109}Cd, ^{54}Mn, ^{210}Pb, ^{65}Zn; Metian et al., 2007, 2008; Metian et al., 2009a, 2009b, 2009c). Interspecific differences also concerned Cs depuration. Indeed, the percentage of long-term retention of ^{134}Cs absorbed by *M. varia* after 36 d of depuration (following a ^{134}Cs exposure through seawater) did not exceed 33 ± 4.6% (Table 1) whereas 45 ± 2.9% of ^{134}Cs was retained by *P. maximus* (Metian et al., 2011). Differences observed in term of retention were radionuclide-dependent: A_{0l} were higher in *P. maximus* for ^{110m}Ag, ^{57}Co, ^{109}Cd and ^{65}Zn than in *M. varia* whereas no differences were observed between the two species for ^{54}Mn and ^{210}Pb (Metian et al., 2007, 2008; Metian et al., 2009a, 2009b, 2009c). For Cs, difference in CF between the two species exposed to the same contamination conditions is related to a higher Cs uptake rate (uptake rate constant: 1.77 d^{-1} *vs.* 0.55 d^{-1}) in *M. varia* compared to *P. maximus* (Metian et al, 2011; Table 1)

Interestingly, interspecific differences can be identified upon a dissolved exposure but not when Pectinids are exposed to ^{134}Cs through food. The present results show that the retention of Cs from food is similar for both scallop's species. Indeed, when *M. varia* were fed with radiolabeled phytoplankton cells (*I. galbana*), ^{134}Cs was similarly assimilated (AE: 24.5 ± 2.9%, Table 1) compared to AE of 28.1 ± 4.4% obtained for *P. maximus* fed in the same conditions (Metian et al., 2011). This similarity in Cs AE between these two Pectinids was not observed for other elements: the AEs of ^{110m}Ag, ^{57}Co, and ^{210}Pb were higher in *M. varia* whereas ^{54}Mn AE was higher for the *P. maximus* (Metian et al., 2007; Metian et al., 2008; Metian et al., 2009a, 2009b, 2009c). To confirm our results for ^{134}Cs, it would be appropriate to test the AE between these two scallop species with other radiolabelled phytoplankton species since Hédouin et al. (2010b) have already demonstrated that the quality of food may affect radionuclide accumulation (^{57}Co, ^{54}Mn and ^{65}Zn) in bivalves.

Globally, our results confirm that Cs is well retained in Pectinids. Figure 2 put these results in perspectives with previous studies on Cs accumulation in bivalves. Whereas ^{134}Cs CF in

Pectinids is similar (or lower in some cases) than other bivalve species, retention is always higher in Pectinids (Figure 2). One has however to keep in mind that some of the observed differences in CF can be partly due to others factor than interspecific bioaccumulation properties, such as difference in ambient salinity. Indeed it is well documented that CF for a given species generally increases with decreasing salinity (e.g. Ke et al., 2000). However, most of the species compared here (see Figure 2) were living in a narrow range of salinity (between 35 and 38 p.s.u.; Nolan and Dahlgaard, 1991 ; Bustamante et al., 2006 ; Qureshi et al., 2007 ; Lacoue-Labarthe et al., 2010 ; Metian et al. 2011).

Percentage of Cs retention from seawater (A_{0l}) and food (AE) is higher in Pectinids than other bivalves. For example, Mytilidae showed low AEs for Cs with values (ranging between 3 and 13% for *Perna viridis*; Table 2, e.g. Wang et al., 2000). However, Figure 2 also shows the reported AEs of Cs in other mollusks (predators) and they can be higher than Pectinids (ranging between 19 and 55% for gastropods and cephalopods; e.g. Wang et al., 2000; Bustamante et al., 2006). These results suggest that food source is key pathway for the transfer of Cs towards molluscs.

In the natural environment, *M. varia* may be exposed to radioactive Cs through seawater and food and each pathway contribute simultaneously to the global bioaccumulation of ^{134}Cs in the organism. Using the kinetic parameters that we obtained experimentally and K_{df} and IR that we measured, the relative contributions of the two exposure pathways were delineated, using the model developed by Landrum et al. (1992) and adapted more recently (Hédouin et al., 2010a). Results indicate that food has a major role in Cs accumulation in *M. varia* in the field (relative contribution of 77% in average). The importance of the trophic pathway in the Cs accumulation has been suggested in taxa such as cephalopods (Bustamante et al., 2006) and fish (Pentreath and Jefferies, 1971; Hewett and Jefferies, 1978) although without direct evidences. In Pectinids, food has been shown to be the major contamination pathway for ^{109}Cd (*M. varia* and *P. maximus*

respectively >99% and 84% of the total contribution; Metian et al., 2007) and for ^{110m}Ag (*P. maximus*: contribution over 98%; Metian et al., 2008) although there is a non-negligible effect of the type of food used in the model.

5. Conclusion

The pectinids, *Mimachlamys varia* included, stand out from bivalves group when one considers their ability to retain^{134}Cs. This characteristic makes them very good candidates to be used in biomonitoring programmes. However, our study highlighted interspecific differences among Pectinids regarding accumulation of waterborne radionuclides: *M. varia* shown a higher capacity to concentrate ^{134}Cs but lower absorption efficiency than *P. maximus*. A global bioaccumulation model indicated that ingestion with food is the major uptake pathway of Cs in *M. varia*. In light with the general recommendation to use multiple species in biomonitoring (e.g. Philips and Rainbow, 1993; Warnau et al., 1996), the present work strongly suggests that the use of *M. varia* in such programmes would be an excellent way to assess the trophically-available Cs and would ideally complement information provided by other species accumulation Cs predominantly from the dissolved phase.

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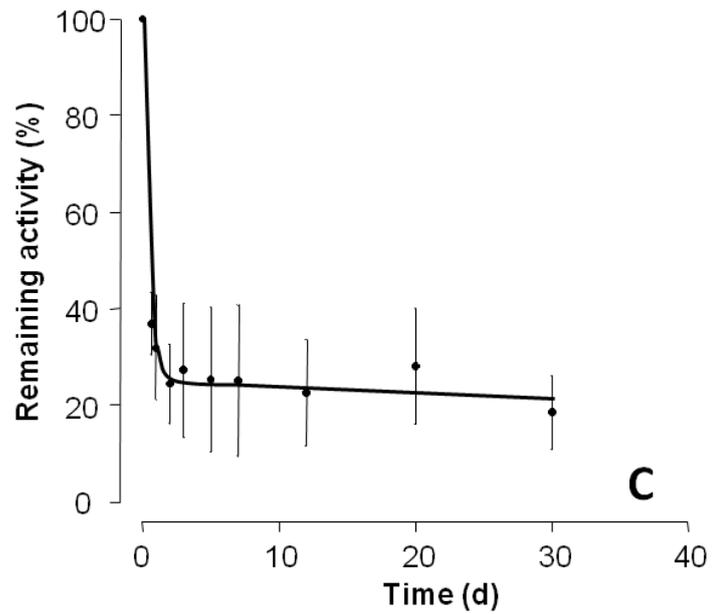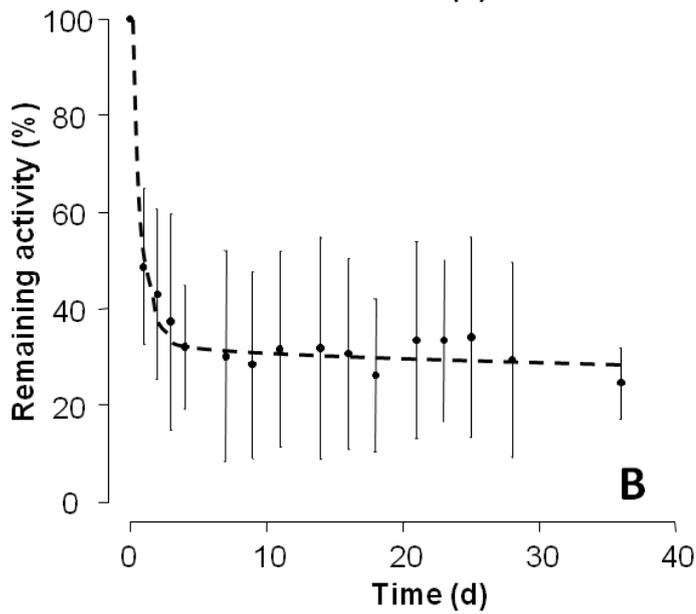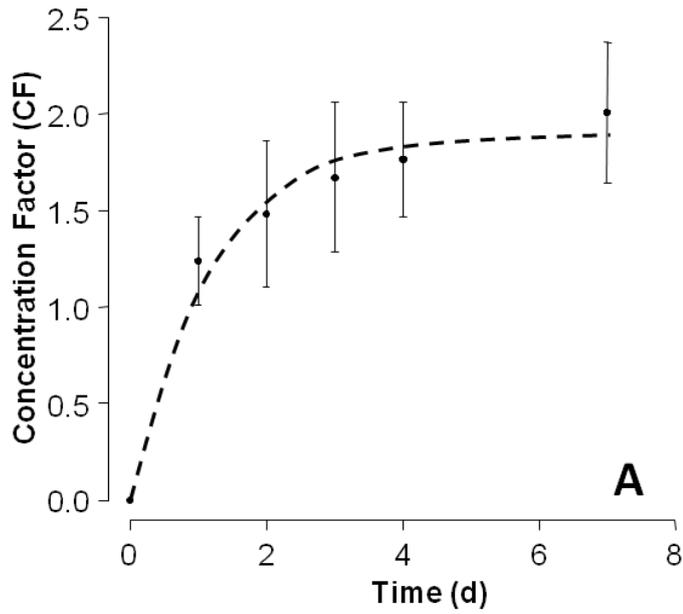
Figure 1. (A) Uptake kinetics of ^{134}Cs in *M. varia* exposed for 7 d to dissolved radiotracers (n=9); (B) subsequent 36-d depuration kinetics (n= 9) and (C) 30-d depuration kinetics after ^{134}Cs-labelled food ingestion (n=6). All values are mean ± SD.
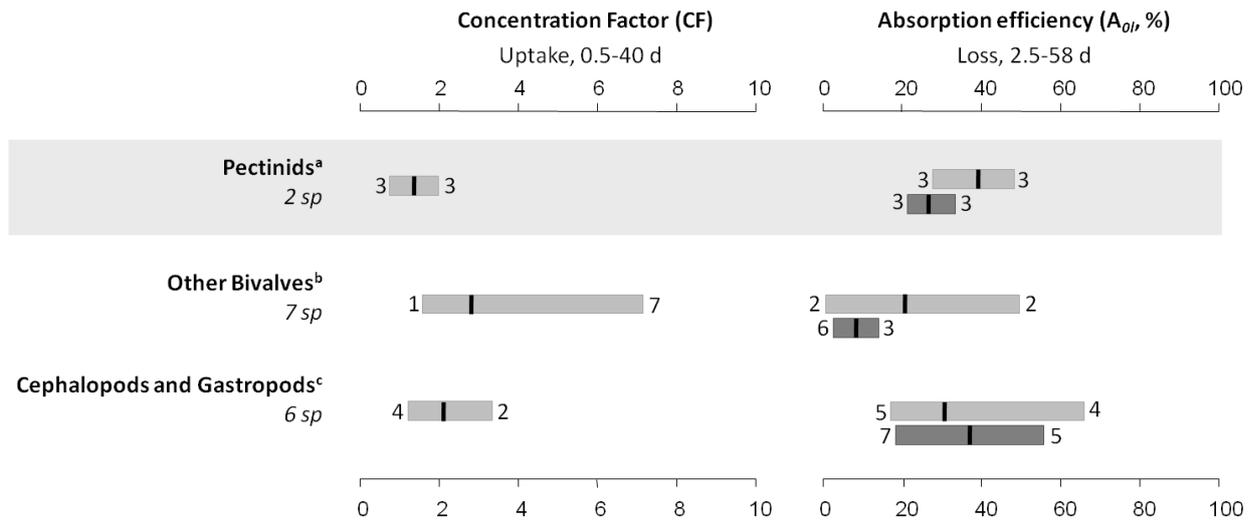
Figure 2. Comparison of concentration factors (CF) and percentages of long-term component absorption efficiency (A_{0I}) for different taxa of mollusks in experimental conditions. Values are mean ± SD. When the values were not directly available, a graphical estimate was performed from uptake and depuration kinetics. Bars are ranges of values. Light grey and dark grey bars represent respectively seawater and food pathways. Black vertical lines indicate means calculated from all the considered values. The numbers next to grey bars represent the number of values below and above the calculated means.

[a]Present study and Metian et al. (2011)

[b](Ueda et al., 1978; Nolan and Dahlgaard, 1991; Hutchins et al., 1998 ; Ke et al., 2000; Wang et al., 2000 ; Güngör et al., 2001; Qureshi et al., 2007; Kalaycı et al., 2013)

[c](Bryan, 1963; Bustamante et al., 2006; Lacoue-Labarthe et al., 2010)

Table 1. Parameters of whole-body uptake and depuration kinetics of ^{134}Cs in *M.varia* 1) exposed for 7 d to waterborne radionuclide (n = 9) and followed by 36 d of depuration (n = 9) and 2) after a 2-h feeding on radiolabelled *I. galbana* followed by 30 d of depuration (n = 6).

Experiment	Uptake			Depuration				
	$CF_{ss} \pm ASE$	$k_u \pm ASE$	R^2	$A_{0s} \pm ASE$	$T_{b\frac{1}{2}s} \pm ASE$	$A_{0l} \pm ASE$	$T_{b\frac{1}{2}l}$	R^2
1) Seawater	1.86 ± 0.08^a	1.77 ± 0.33^a	0.79	66.41 ± 6.92^a	0.6 ± 0.2	33.09 ± 4.63^a	178	0.50
2) Feeding	-	-	-	75.46 ± 5.48^a	0.3 ± 0.1	24.45 ± 2.92^a	115	0.80

Uptake parameters: CF_{ss}: concentration factors at steady state; k_u: uptake rate constant (d^{-1}). Depuration parameters: A_{0s} and A_{0l}: remaining activity (%) according to the short- and the long-lived exponential component, respectively; $T_{b\frac{1}{2}}$: biological half-life (d). ASE: asymptotic standard error; R^2: determination coefficient of the uptake or depuration kinetics.

[a]Probability of the model adjustment: $p < 0.0001$.

Table 2. Concentration Factors (mean CF ± SD) and body distribution (mean % ± SD) of [134]Cs in *M. varia* during seawater (after 7 d of exposure and after 36 d of depuration) and feeding (after 30 d of depuration) experiments.

Compartments	Seawater experiment			Food experiment[a]
	Uptake (7 d, n = 4)		Loss (36d, n = 4)	Loss (30d, n = 4)
	Concentration Factor	Distribution (%)	Distribution (%)	Distribution (%)
Digestive gland	6 ± 4	24 ± 10	59 ± 13	57 ± 16
Gills	4 ± 0.4	15 ± 4	2 ± 1	7 ± 6
Kidneys	6 ± 1	4 ± 2	3 ± 1	6 ± 5
Intestine	2 ± 0.3	<1	10 ± 3	2 ± 1
Gonad	3 ± 1	4 ± 1	4 ± 1	4 ± 2
Foot	7 ± 2	3 ± 1	3 ± 4	7 ± 6
Mantle	3 ± 0.4	23 ± 5	2 ± 1	8 ± 7
Adductor muscle	3 ± 1	26 ± 3	14 ± 9	9 ± 9
Remaining tissues	4 ± 1	3 ± 1	3 ± 1	2 ± 1
Whole soft parts	3 ± 1	41 ± 9	72 ± 7	n.a.
Shell	2 ± 1	59 ± 9	28 ± 7	n.a.

[a] For this experiment, counting errors are comprises between 5-15% due to the low activities measured.