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Delineation of heavy metal uptake pathways (seawater and food) in the variegated scallop *Chlamys varia*, using radiotracer techniques

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ABSTRACT: The bioaccumulation and depuration kinetics of selected metals (Ag, Co, Hg, Mn and Zn) were determined in the European variegated scallop *Chlamys varia* following exposures via seawater and food, using highly sensitive radiotracer techniques (^{110m}Ag, ^{57}Co, ^{203}Hg, ^{54}Mn and ^{65}Zn). Body distribution of Ag, Co, Mn and Zn was similar for both waterborne and dietary metals. Ag was mainly present in the digestive gland (>80%), Co and Mn were generally localized in similar proportions in the digestive gland and kidneys, and Zn was mainly found in the kidneys (>40%). In contrast, Hg was mainly present in gills during seawater exposure whereas, after exposure through food, it was primarily distributed in the digestive gland. The results from all experiments were integrated into a bioaccumulation model in order to delineate the relative contribution of each metal uptake route in *C. varia*. Computation indicated that food is the main uptake route of Ag, Co, Mn, and Zn in scallop, whereas waterborne and dietary pathways were shown to contribute similarly in the global bioaccumulation of Hg in *C. varia*. Except for Hg, dietary transfer was investigated using 2 different phytoplankton species, *Isochrysis galbana* and *Skeletonema costatum*. For Mn and Zn, the dietary contribution was not influenced by the phytoplankton species used. In contrast, food quality played a major role for Ag and Co intake. For example, when *S. costatum* was used as food, the dietary pathway contributed 97% of the global Ag bioaccumulation, while it contributed only 58% when Ag was ingested with *I. galbana*.

KEY WORDS: Trace elements · Bioaccumulation kinetics · Depuration kinetics · Bioaccumulation mode · Bivalves

INTRODUCTION

Marine invertebrates are exposed to essential and non-essential metals from both the dissolved and the particulate phases. Many previous studies on invertebrates have focussed on the bioaccumulation of dissolved metals and/or their toxic effects. More recently, however, new evidence suggests that food can contribute significantly to metal uptake (e.g. Luoma 1983, 1989, Wang & Fisher 1999).

Among bivalves, the Pectinidae accumulate high levels of metals in their tissues (e.g. Bryan 1973, Bustamante & Miramand 2004, Metian et al. 2008b), even in areas far from anthropogenic sources such as the Southern Ocean (e.g. Mauri et al. 1990, Viarengo et al. 1993). Within this family, the variegated scallop *Chlamys varia* is a common species on the Mediterranean and the European and North African Atlantic rocky shores, where it lives fixed on rocky substrata (Quéro & Vayne 1998). Field studies have shown that this species contains high levels of several trace metals, rare earth elements and radionuclides in its tissues (Bustamante et al. 2002, Bustamante & Miramand 2004, 2005a,b). Since *C. varia* is targeted by commercial fish-

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ery as well as for leisure activities (see e.g. Bustamante & Miramand 2004, 2005a), its high capacity for metal bioaccumulation could potentially play a significant role in contaminant transfer to consumers.

The high capacity of pectinids to bioaccumulate metals stresses the need to better understand metal behaviour and fate in these edible organisms. Field studies have inferred that the dietary pathway could be of importance in the bioaccumulation of some elements in scallops (Palmer & Rand 1977, Uthe & Chou 1987, Bustamante & Miramand 2005a). However, to the best of our knowledge, an actual assessment of the relative contribution of different exposure pathways in pectinids has been carried out in only 2 recent studies, and only for Cd (Metian et al. 2007) and Ag (Metian et al. 2008a).

The aim of the present study was to characterize the bioaccumulation of selected metals (Ag, Co, Hg, Mn and Zn) in the scallop *Chlamys varia* exposed via seawater or its food under controlled laboratory conditions. In addition, the influence of food quality (i.e. phytoplankton species used as food, viz. *Isochrysis galbana* or *Skeletonema costatum*) on the assimilation efficiency of metals was investigated. Whenever possible, a global bioaccumulation model (Landrum et al. 1992) was used to determine the relative contribution of each uptake pathway on the bioaccumulation of the studied metals in *C. varia*.

MATERIALS AND METHODS

Sampling. In spring 2005, 100 variegated scallops *Chlamys varia* were collected from the French Atlantic coast (Pertuis Breton, Charente-Maritime) by SCUBA diving. This location was selected because it shelters the main variegated scallop population along the French coast and is intensively exploited by local fishermen. Exposure to metals is low in this area, as previously reported in Bustamante & Miramand (2005b). Scallops were carefully transported to the International Atomic Energy Agency (IAEA)–Marine Environment Laboratories in Monaco, where they were acclimated to laboratory conditions (constantly aerated open water circuit aquarium; flux: 50 l h^{-1}; salinity: 36 psu; temperature: 17 ± 0.5°C; pH: 8.0 ± 0.1; 12:12 h light:dark cycle) for 8 wk prior to the experiment. Every day during this period, the scallops were fed an algal diet composed of the Bacillariophyceae *Skeletonema costatum* and the Prymnesiophyceae *Isochrysis galbana* (5×10^4 cells ml^{-1}).

Radiotracers and counting. Bioaccumulation and depuration kinetics of ^{110m}Ag, ^{57}Co, ^{203}Hg, ^{54}Mn and ^{65}Zn in scallops were determined using radiotracers of high specific activity purchased from CERCA, France (^{110m}Ag as $AgNO_3$, half-life [$T_{1/2}$] = 249.8 d), from Amersham (^{57}Co as $CoCl_2$, $T_{1/2}$ = 271.8 d), and from Isotope Product Lab (^{203}Hg as $HgCl_2$, $T_{1/2}$ = 46.59 d; ^{54}Mn as $MnCl_2$, $T_{1/2}$ = 312.2 d; ^{65}Zn as $ZnCl_2$; $T_{1/2}$ = 243.9 d).

The radiotracers were counted using a high-resolution γ-spectrometer system composed of 3 Germanium N- or P-type detectors (EGNC 33–195-R, Canberra and Eurysis) connected to a multichannel analyser and a computer equipped with a spectra analysis software (Interwinner 6). The radioactivity of the samples was determined by comparison with standards of known activities and appropriate geometries and was corrected for background and physical decay of the radiotracers. The counting time was adjusted to obtain a propagated counting error of less than 5% (Rodriguez y Baena et al. 2006a).

Seawater exposure. Twenty-five *Chlamys varia* (average wet weight ± SD: 30 ± 7 g) were placed in a 70 l glass aquarium (constantly aerated closed circuit aquarium; same salinity, temperature, pH and light: dark conditions as previously indicated) and exposed for 7 d to ^{110m}Ag (0.4 kBq l^{-1}), ^{57}Co (0.5 kBq l^{-1}), ^{203}Hg (1.4 kBq l^{-1}), ^{54}Mn (0.5 kBq l^{-1}), and ^{65}Zn (0.75 kBq l^{-1}) dissolved in filtered (0.45 µm) seawater, according to the methodology described in Warnau et al. (1996, 1999). In terms of stable metal concentration, these additions corresponded to Ag (15 pmol l^{-1}), Co (0.5 pmol l^{-1}), Hg (12 pmol l^{-1}), Mn (0.4 pmol l^{-1}) and Zn (138 pmol l^{-1}), which were 1 to 3 orders of magnitude lower than the natural concentrations of metals in seawater (Bruland 1983). No change in pH was detectable after the tracer addition. Spikes and seawater were renewed twice a day for the first 2 d and then daily in order to keep radiotracer concentrations in the seawater constant. Activity of the radiotracers in seawater was checked before and after each spike renewal, yielding time-integrated activities in seawater for the 5 radiotracers of 0.25 ± 0.21 kBq ^{110m}Ag l^{-1}, 0.31 ± 0.11 kBq ^{57}Co l^{-1}, 1.39 ± 0.72 kBq ^{203}Hg l^{-1}, 0.34 ± 0.23 kBq ^{54}Mn l^{-1} and 0.73 ± 0.14 kBq ^{65}Zn l^{-1} (Rodriguez y Baena et al. 2006b). Every day during renewal of seawater and spike, the scallops were briefly (30 min) fed *Isochrysis galbana* and *Skeletonema costatum* (5×10^4 cells ml^{-1}) in clean seawater.

Nine tag-identified scallops were collected daily from Days 1 to 4 and then again on Day 7 and were whole-body radioanalyzed alive (same individuals each time). At the end of the 7 d exposure period, 5 individuals (not belonging to the tag-identified batch) were dissected. Shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues were separated and radioanalyzed in order to assess the distribution of radiotracers in the body.

The remaining scallops were placed under non-contaminating conditions (constantly aerated open circuit;

flux: 50 l h^{-1}; same salinity, temperature, pH and light: dark conditions as previously indicated) for 36 d (91 d for ^{203}Hg), and the 9 tag-identified individuals were radioanalyzed at regular intervals in order to follow the depuration kinetics of the radiotracers from the scallops. During the whole depuration period, scallops were fed daily with *Isochrysis galbana* and *Skeletonema costatum* (5×10^4 cells ml^{-1}). Four individuals were collected at the end of the depuration period and dissected as previously described.

Food exposure. *Isochrysis galbana* and *Skeletonema costatum* were used separately to study the influence of food quality (phytoplankton species) on the dietary transfer of ^{110m}Ag, ^{57}Co, ^{54}Mn, and ^{65}Zn in *Chlamys varia*. Indeed, the contrasting nature of the 2 phytoplankton species — *I. galbana* is a naked flagellate, whereas *S. costatum* is a Si-walled diatom — allows for a representation of the complexity of the phytoplankton diet of scallops (Mikulich & Tsikhon-Lukamina 1981, Shumway et al. 1987). Transfer of ^{203}Hg in *C. varia* was investigated with *I. galbana* only.

One culture of each phytoplankton species was exposed to radiotracers (Table 1) during their growing phase (7 d for *Isochrysis galbana* and 10 d for *Skeletonema costatum*) and then separated from the spiked seawater (1 µm filtration on Osmonic filters) following the method described in Metian et al. (2007). The radiolabelled phytoplankton species were γ-counted before and after the filtration in order to determine the distribution coefficient (K_{df}) of each radiotracer for each species. Each phytoplankton species was resuspended in a 70 l closed-circuit aquarium to reach a cell density of 5×10^4 cells ml^{-1}. This cell density was selected in order to avoid the production of pseudofaeces (Metian et al. 2007). Twelve individuals (6 in each of the two 70 l aquaria; average whole-body wet weight ± SD: 18 ± 4 g) were acclimated for 1 wk prior to the feeding experiments. Scallops were allowed to feed on radiolabelled phytoplankton for 2 h (pulse-chase feeding method; see e.g. Warnau et al. 1996, Metian et al. 2007). After the feeding period, all scallops were whole-body γ-counted and then placed in non-contaminating, flowing seawater conditions (50 l h^{-1}), with daily feeding on *I. galbana* and *S. costatum* (5×10^4 cells ml^{-1}).

All individuals were then whole-body γ-counted at different time intervals to follow the depuration kinetics of ingested ^{110m}Ag, ^{57}Co, ^{203}Hg, ^{54}Mn and ^{65}Zn. In the *Isochrysis galbana* experiment, all individuals were collected after 30 d of depuration, dissected and radioanalyzed to determine the distribution of the radiotracers in the body (shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues). For the *Skeletonema costatum* experiment, the depuration period lasted 18 d and specimens were not dissected.

Preliminary feeding experiments were also carried out in order to assess the ingestion rate (IR, in g g^{-1} d^{-1}) of both phytoplankton species by *Chlamys varia* fed 5×10^4 cells ml^{-1} following the method described in Metian et al. (2008a).

Data analyses. Bioaccumulation of radiotracers from seawater was expressed as concentration factors (CF) which is the ratio between the radioactivity in scallops (Bq g^{-1} wet wt) and the time-integrated activity in the seawater (Bq g^{-1}) over time. Bioaccumulation kinetics were fitted using a simple exponential kinetic model (Eq. 1) or a linear model (Eq. 2):

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

$$CF_t = k_u t \quad (2)$$

where CF_t and CF_{ss} ($CF_{ss} = k_u / k_e$) are the concentration factors at time t (d) and at steady state, respectively; k_u and k_e are the uptake and depuration rate constants (d^{-1}), respectively (Whicker & Schultz 1982, Warnau et al. 1996).

Depuration of radiotracers (seawater and food experiments) was expressed in percent of remaining radioactivity (radioactivity at time t divided by the initial radioactivity measured in scallops at the beginning of the decontamination period, multiplied by 100). The percentage of remaining activity was plotted against time and depuration kinetics were described by a single- (Eq. 3) or a double-component exponential model (Eq. 4):

$$A_t = A_0 e^{-k_e t} \quad (3)$$

$$A_t = A_{0s} e^{-k_e st} + A_{0l} e^{-k_e lt} \quad (4)$$

Table 1. *Isochrysis galbana* and *Skeletonema costatum*. Activity (kBq l^{-1}) of radiotracers added to the phytoplankton culture media, and distribution coefficient (K_{df}) between phytoplankton and seawater determined at the end of the phytoplankton exposure period. nd: not determined

Species	^{110m}Ag		^{57}Co		^{203}Hg		^{54}Mn		^{65}Zn	
	Activity (kBq l^{-1})	K_{df}	Activity (kBq l^{-1})	K_{df}	Activity (kBq l^{-1})	K_{df}	Activity (kBq l^{-1})	K_{df}	Activity (kBq l^{-1})	K_{df}
I. galbana	4	4.43×10^4	3	1.35×10^3	4.5	1.74×10^4	3.5	1.61×10^5	3.5	1.12×10^5
S. costatum	5	6.86×10^5	5	8.47×10^5	nd	nd	5	4.42×10^6	5	6.62×10^6

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}), and the subscripts s and l annotate the 'short-lived' and 'long-lived' components, respectively. For each exponential component (s and l), a biological half-life can be calculated ($T_{b1/2s}$ and $T_{b1/2l}$) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b1/2} = \ln 2/k_e$. With regard to the feeding experiments, the 'long-lived' exponential term describes the proportion of the radiotracer ingested with food that is actually absorbed by the organism and slowly eliminated, while the corresponding A_{0l} represents the assimilation efficiency (AE) of the radiotracer (Warnau et al. 1996).

Model constants and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation, respectively, using the nonlinear curve-fitting routines in the Statistica 6 software. Best-fitting models were selected according to the highest determination coefficient and the examination of residuals. The level of significance for statistical analyses was always set at $\alpha < 0.05$ %.

Bioaccumulation model. The relative contribution of each exposure pathway was determined using the bioaccumulation model originally proposed by Thomann (1981) and Landrum et al. (1992), and further used, revised and developed by other authors (e.g. Thomann et al. 1995, Wang et al. 1996, Reinfelder et al. 1998, Metian et al. 2008a). The term A_{0l} in this model allows for the consideration of only the fraction of the radiotracer taken up by the organisms from seawater that was actually absorbed (Metian et al. 2008a). In addition, the growth rate was considered to be negligible over the duration of the experiment in the original model (e.g. Reinfelder et al. 1998).

The total activity concentration of the radiotracer in the organism (C_t, Bq g^{-1}) was defined as the sum of each concentration resulting from the uptake through the dissolved and food pathways:

$$C_t = C_{w,ss} + C_{f,ss} \quad (5)$$

where $C_{w,ss}$ is the radiotracer concentration in scallops (Bq g^{-1}) at steady state taken up from the dissolved phase (Eq. 6), and $C_{f,ss}$ is the radiotracer concentration in scallops (Bq g^{-1}) at steady state obtained from food (Eq. 7):

$$C_{w,ss} = (A_{0l,w}\, k_{u,w}\, C_w) / k_{e,w} \quad (6)$$

$$C_{f,ss} = (AE\ IR\ C_f) / k_{e,f} \quad (7)$$

where A_{0l} is the proportion (%) of radiotracer taken up from seawater that was lost according to the long-lived compartment, k_u and k_e are the uptake and depuration rate constants (d^{-1}), C_w is the activity concentration of the radiotracer in seawater used in the experiment (Bq g^{-1}), AE is the assimilation efficiency (%) of the radiotracer ingested with the food, IR is the rate of phytoplakton ingestion by the scallop (g g^{-1} d^{-1}), C_f is the activity concentration (Bq g^{-1}) of the radiotracer measured in the food used in the experiment, and 'w' and 'f' subscripts denote the water and food exposure pathways, respectively. The relative contribution of each exposure pathway is then assessed from the relations:

$$\%\ \text{Seawater} = C_{w,ss} / (C_{f,ss} + C_{w,ss}) \quad (8)$$

$$\%\ \text{Food} = C_{f,ss} / (C_{f,ss} + C_{w,ss}) \quad (9)$$

RESULTS

Seawater pathway

The bioconcentration of ^{110m}Ag, ^{57}Co, ^{54}Mn and ^{65}Zn in whole-body *Chlamys varia* was best described by a saturation exponential model ($r^2 \geq 0.81$), whereas ^{203}Hg bioconcentration was best fitted by a linear model ($r^2 = 0.82$) (Fig. 1, Table 2). After 7 d of exposure, the estimated uptake rate constant (k_u) allowed ranking of the radiotracers according to the following order of bioavailability: ^{110m}Ag > ^{203}Hg > ^{54}Mn > ^{65}Zn > ^{57}Co.

Table 3 shows the concentration factors reached by the whole organisms (CF_{7d} in toto) and their body compartments at the end of the exposure period. Based on the measured CF_{7d} in toto, radiotracer bioavailability can be ranked similarly to when it is based on k_u: ^{110m}Ag (2586 ± 1186) > ^{203}Hg (652 ± 179) > ^{54}Mn (138 ± 36) = ^{65}Zn (155 ± 31) > ^{57}Co (92 ± 36).

Among the tissues and organs, the digestive gland, the kidneys, and the gills displayed the highest bioconcentration capacities. ^{110m}Ag was hyperconcentatred by all scallop tissues (CF_{7d} > 2000) and especially by the digestive gland (CF_{7d} = 132 346 ± 38 091). ^{203}Hg was highly concentrated in gills (CF_{7d} = 13 377 ± 3280) whereas essential metals (^{57}Co, ^{54}Mn and ^{65}Zn) were mainly concentrated in the kidneys (CF_{7d} = 107 ± 57 for ^{57}Co, 172 ± 78 for ^{54}Mn and 8749 ± 5259 for ^{65}Zn).

Regarding the distribution of radiotracers in the body, ^{110m}Ag and ^{203}Hg were mainly present in the digestive gland (87 ± 2 %) and the gills (61 ± 1 %), respectively, whereas ^{65}Zn was mainly found in the kidneys (40 ± 12 %), and ^{57}Co was more present in the digestive gland (Fig. 2). Finally, ^{54}Mn was distributed quite homogeneously among the different tissues and organs.

After the exposure period, non-contaminating conditions were restored and depuration kinetics of radiotracers were followed for 36 d for all radiotracers except for ^{203}Hg (91 d). The whole-body depuration of ^{110m}Ag was best fitted by a single-component exponen-

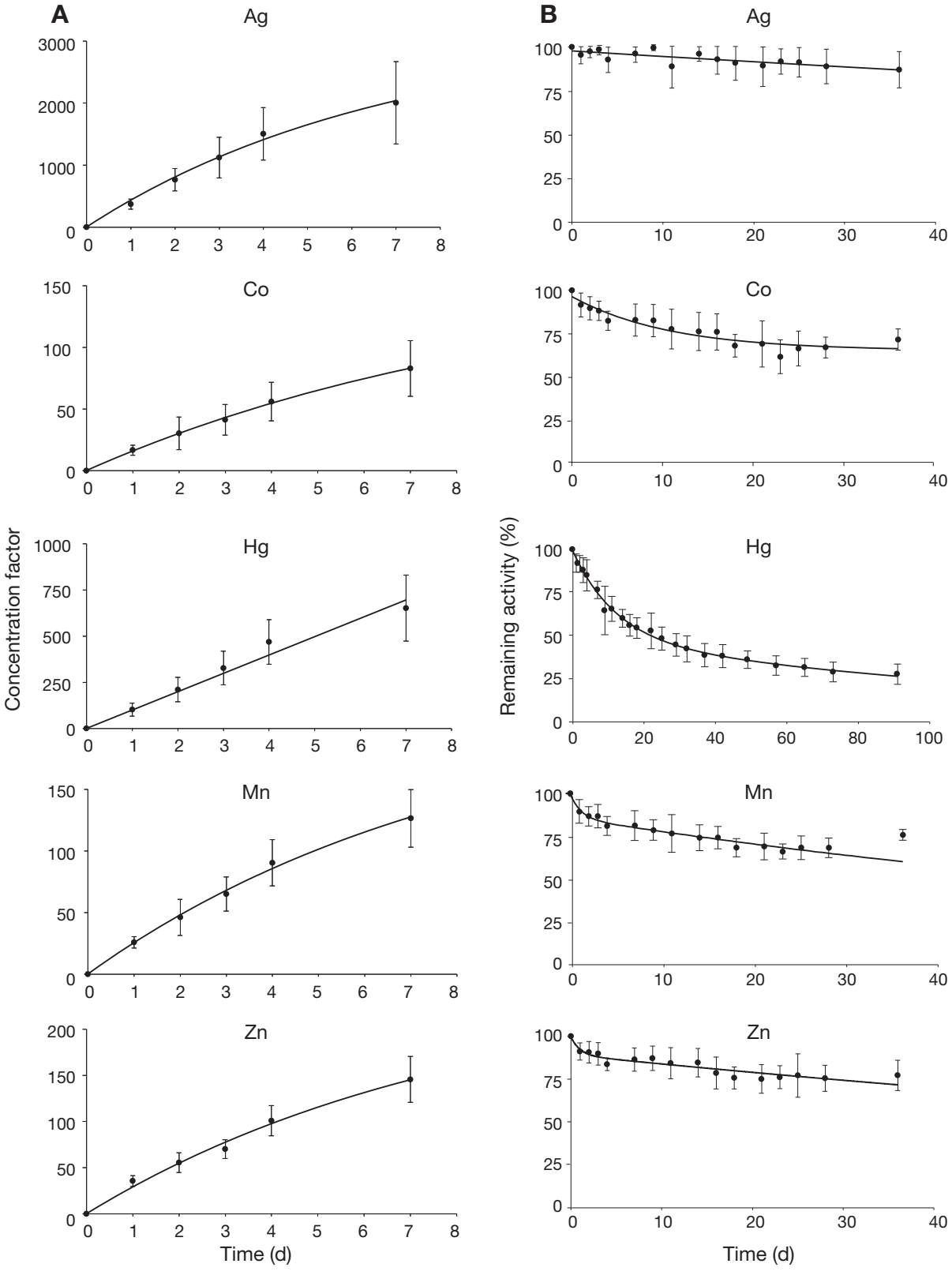

Fig. 1. *Chlamys varia*. (A) Bioaccumulation kinetics (mean concentration factor ± SD, n = 9) and (B) depuration kinetics (mean % remaining activity ± SD, n = 9) of selected radiotracers. Scallops were exposed for 7 d in seawater (A) then maintained for 36 d (91 d for ^{203}Hg) in non-contaminating conditions

Table 2. *Chlamys varia*. Parameters of the bioaccumulation and depuration kinetics of selected radiotracers in scallops (n = 9) after 7 d exposure to the radiotracers in seawater followed by 36 d (91 d for Hg) depuration under non-contaminating conditions. CF_{ss}: concentration factor at steady state; k_u, k_{el}: uptake and depuration rate constant (d^{-1}), respectively; A_{0l}: activity (%) lost according to the long-lived exponential component; $T_{b1/2}$: biological half-life (d); ASE: asymptotic standard error; R^2: determination coefficient of the kinetics; nd: not determined. Probability of the model adjustment: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001

Metal	Uptake phase			Depuration phase			
	CF_{ss} ± ASE	k_u ± ASE	R^2	A_{0l} ± ASE	k_{el} ± ASE	$T_{b1/2l}$ ± ASE	R^2
^{110m}Ag	3169 ± 978**	466 ± 59****	0.81	97.8 ± 1.7****	0.003 ± 0.001****	211 ± 46****	0.14
^{57}Co	161 ± 120**	16.7 ± 2.1****	0.82	86.0 ± 4.4****	0.009 ± 0.003***	73 ± 20***	0.56
^{203}Hg	nd	99.3 ± 3.9****	0.82	50.4 ± 7.9****	0.007 ± 0.003*	95 ± 34*	0.93
^{54}Mn	223 ± 70	27.0 ± 2.4****	0.91	85.4 ± 2.2****	0.010 ± 0.002****	69 ± 10****	0.66
^{65}Zn	253 ± 69***	30.9 ± 2.3****	0.93	89.1 ± 1.9****	0.006 ± 0.001****	115 ± 23****	0.45

Table 3. *Chlamys varia*. Concentration factors (CF, mean ± SD, n = 5) in different body compartments of the scallop

Tissues	^{110m}Ag	^{57}Co	^{203}Hg	^{54}Mn	^{65}Zn
Digestive gland	132 346 ± 38 091	49 ± 20	9545 ± 1412	44 ± 24	355 ± 127
Gills	5200 ± 1446	18 ± 6	13 377 ± 3280	23 ± 6	282 ± 88
Kidneys	15 437 ± 10 849	107 ± 57	3923 ± 3074	172 ± 78	8749 ± 5259
Intestine	2501 ± 1796	8 ± 8	1381 ± 357	19 ± 16	189 ± 166
Gonad	9972 ± 8601	7 ± 5	1822 ± 1293	15 ± 13	266 ± 341
Foot	9488 ± 5549	4 ± 3	875 ± 148	30 ± 16	239 ± 63
Mantle	2092 ± 1852	8 ± 4	1608 ± 229	13 ± 6	145 ± 24
Adductor muscle	2008 ± 793	4 ± 1	300 ± 114	8 ± 2	243 ± 96
Remaining tissues	8471 ± 6327	18 ± 12	2576 ± 914	34 ± 19	222 ± 131
Whole body	2586 ± 1186	92 ± 36	652 ± 179	138 ± 36	155 ± 31

tial model, whereas depuration kinetics of ^{57}Co, ^{203}Hg, ^{54}Mn and ^{65}Zn were best described by a double-component exponential model (Fig. 1). All radiotracers were efficiently absorbed (A_{0l} > 50%) and retained ($T_{b1/2l} \geq 10$ wk) by *Chlamys varia* (Table 2).

After 36 d of depuration, the dissections highlighted the storage role of the digestive gland for ^{110m}Ag, of the gills for ^{203}Hg and of the kidneys for ^{57}Co, ^{54}Mn and ^{65}Zn (Fig. 2). Interestingly, the proportion of ^{110m}Ag (86 ± 2%) in the digestive gland and of ^{65}Zn (59 ± 20%) in the kidneys was similar at the end of the bioaccumulation and depuration periods, whereas the proportion of ^{57}Co, ^{203}Hg and ^{54}Mn in kidneys and those of ^{203}Hg in the digestive gland increased with time during the depuration phase (Fig. 2).

Dietary pathway

The preliminary assessment of the IR of the 2 phytoplankton species indicated that *Chlamys varia* ingested both *Isochrysis galbana* and *Skeletonema costatum* at similar rates, i.e. IR = 0.087 ± 0.028 g g^{-1} d^{-1} (p = 0.01).

In order to evaluate whether different phytoplankton species influence the assimilation and retention of ^{110m}Ag, ^{57}Co, ^{54}Mn, and ^{65}Zn by *Chlamys varia*, depuration kinetics were followed after 2 h pulse-chase feeding experiments using either of the 2 radiolabelled phytoplankton species (*Isochrysis galbana* and *Skeletonema costatum*).

Whole-body depuration kinetics of all radiotracers in *Chlamys varia* fed radiolabelled *Isochrysis galbana* or *Skeletonema costatum* were best fitted by a double-component exponential equation (Fig. 3). AE of radiotracers ingested with *S. costatum* was higher than when ingested with *I. galbana* (Table 4), whereas the biological half-lives of radiotracers ingested with either of the species did not show a significant difference, except for ^{65}Zn, which was more strongly retained when ingested with *S. costatum*. Comparison of the retention capacities of the scallops when exposed through seawater or food indicated that the $T_{b1/2l}$ of all dietary metals were shorter than that of waterborne elements.

Dietary transfer of ^{203}Hg in *Chlamys varia* was only investigated with *Isochrysis galbana* as the food source. This element was assimilated with an efficiency of 32% and retained with a $T_{b1/2l}$ of 10 ± 4 d.

At the end of the depuration period of the *Isochrysis galbana* feeding experiment, the distribution of ^{110m}Ag and ^{65}Zn (Fig. 2) was similar to that deter-

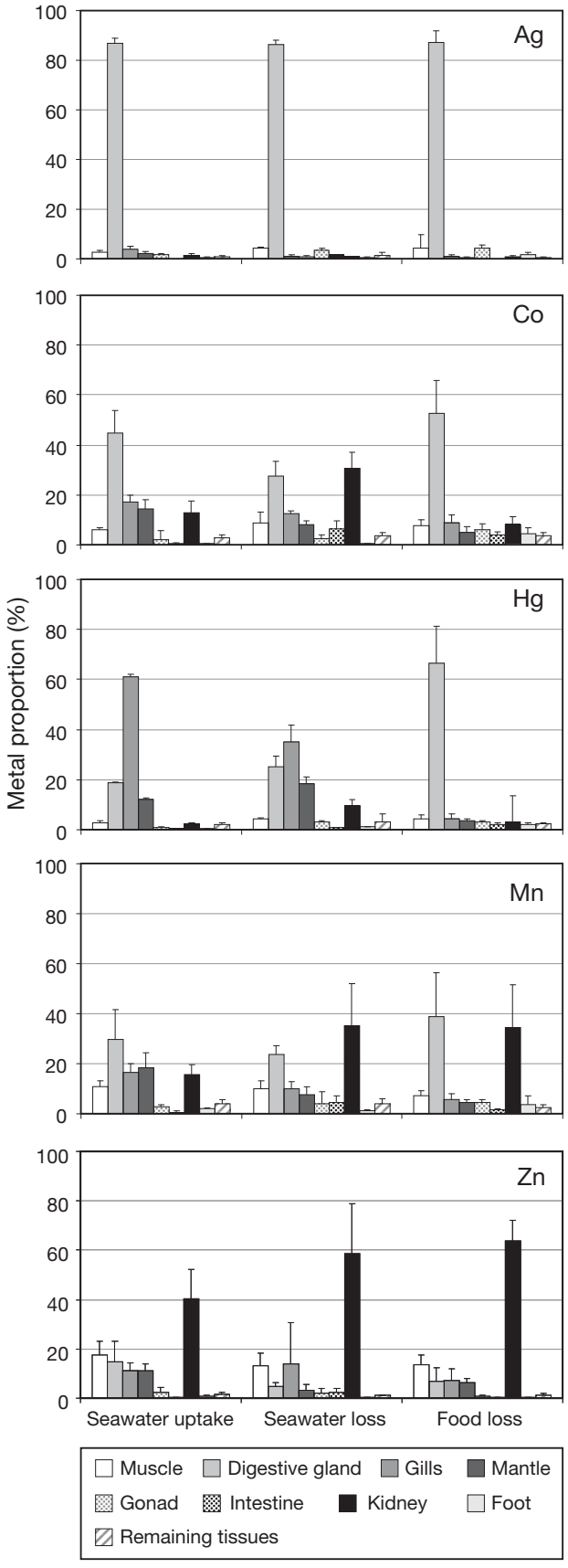

Fig. 2. *Chlamys varia*. Body distribution (%, mean ± SD) of selected radiotracers after 7 d exposure to the radiotracers via seawater (seawater uptake, n = 5), at the end of the 36 d (91 d for Hg) depuration period following the seawater exposure (seawater loss, n = 4), and after a 2 h feeding on radiolabelled *Isochrysis galbana* followed by 30 d under non-contaminating conditions (food loss, n = 6)

mined after seawater exposure. When assimilated via the food, ^{110m}Ag was mainly present in the digestive gland (87 ± 5%) and ^{65}Zn in the kidneys (64 ± 9%). For the other radiotracers, the digestive gland contained a higher proportion than observed in the seawater experiment. Indeed, most of ^{203}Hg (67 ± 15%) and ^{57}Co (52 ± 13%) was present in the digestive gland, whereas ^{54}Mn occurred in similar proportions in the digestive gland (39 ± 17%) and the kidneys (34 ± 17%).

Bioaccumulation model

The relative contribution of each exposure pathway to the global bioaccumulation (see 'Material and methods') was calculated using the parameters determined in the different experiments and given in Tables 2 & 4 and in the text (IR = 0.087 g g^{-1} d^{-1}). The relative contribution of each exposure pathway for all metals is shown in Fig. 4. The dietary pathway appears to play a major role in metal bioaccumulation in *Chlamys varia*.

Comparison of the model runs using the parameters obtained from the 2 feeding experiments (viz. using *Isochrysis galbana* or *Skeletonema costatum* as food source) indicated that the influence of food quality on the dietary contribution to global bioaccumulation is metal-dependent. Indeed, when the scallops were fed ^{110m}Ag-labelled *S. costatum*, food was the main uptake pathway (contributing 96% of the global ^{110m}Ag bioaccumulation). In contrast, when fed ^{110m}Ag-labelled *I. galbana*, food and seawater pathways contributed similarly to global bioaccumulation of ^{110m}Ag in *Chlamys varia* (53 and 47%, respectively). A more pronounced trend has been observed for ^{57}Co: the main ^{57}Co uptake route was food (100% contribution) when scallops were fed *S. costatum*, whereas a dramatic shift to seawater as the predominant uptake pathway (contributing 82% to global bioaccumulation) occurred when scallops were fed *I. galbana*. In contrast, ^{54}Mn and ^{65}Zn were always mainly accumulated via the food (≥91%) for both phytoplankton species. For ^{203}Hg, the food tested (*I. galbana*) and seawater contributed equally to the global bioaccumulation of this metal in *C. varia*.

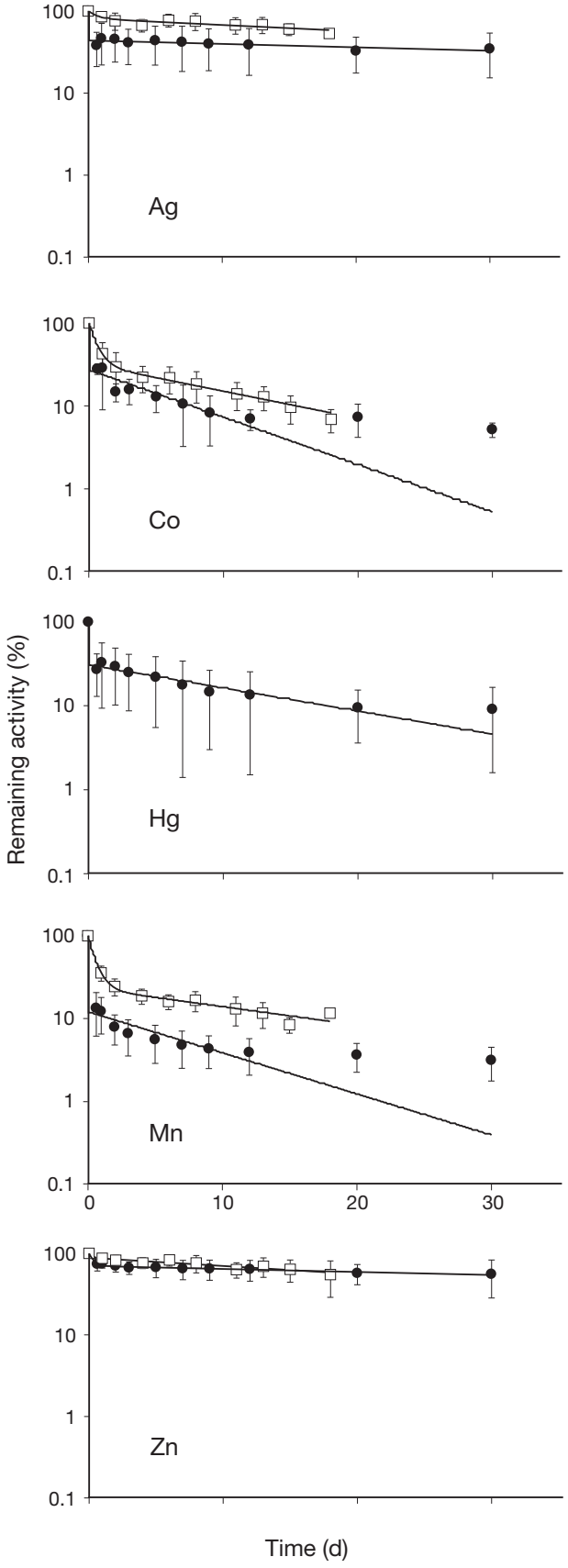

Table 4. *Chlamys varia*. Parameters of the depuration kinetics of selected radiotracers after 2 h feeding on radiolabelled *Isochrysis galbana* (n = 6) and *Skeletonema costatum* (n = 6), followed by 30 and 18 d under non-contaminating conditions, respectively. A_{0l}: activity (%) lost according to the long-lived exponential component (= assimilation efficiency, AE); k_{el}: depuration rate constant (d^{-1}); $T_{b1/2l}$: biological half-life (d); ASE: asymptotic standard error; R^2: determination coefficient of the kinetics. Probability of the model adjustment: $^{ns}p > 0.05$; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$

Metal	A_{0l} ± ASE	k_{el} ± ASE	$T_{b1/2l}$ ± ASE	R^2
I. galbana				
^{110m}Ag	44.4 ± 3.9****	0.011 ± 0.008^{ns}	64 ± 49^{ns}	0.49
^{57}Co	17.7 ± 3.6****	0.061 ± 0.029*	11 ± 5*	0.93
^{203}Hg	31.6 ± 4.1****	0.067 ± 0.024**	10 ± 4**	0.77
^{54}Mn	8.6 ± 1.3****	0.058 ± 0.023*	12 ± 5*	0.99
^{65}Zn	70.0 ± 4.1****	0.008 ± 0.005^{ns}	83 ± 45^{ns}	0.39
S. costatum				
^{110m}Ag	79.0 ± 5.5****	0.014 ± 0.007**	48 ± 25**	0.42
^{57}Co	31.2 ± 5.3****	0.071 ± 0.022***	10 ± 3***	0.92
^{54}Mn	23.7 ± 2.5****	0.054 ± 0.013***	13 ± 3***	0.98
^{65}Zn	87.8 ± 5.1****	0.022 ± 0.007**	31 ± 10**	0.41

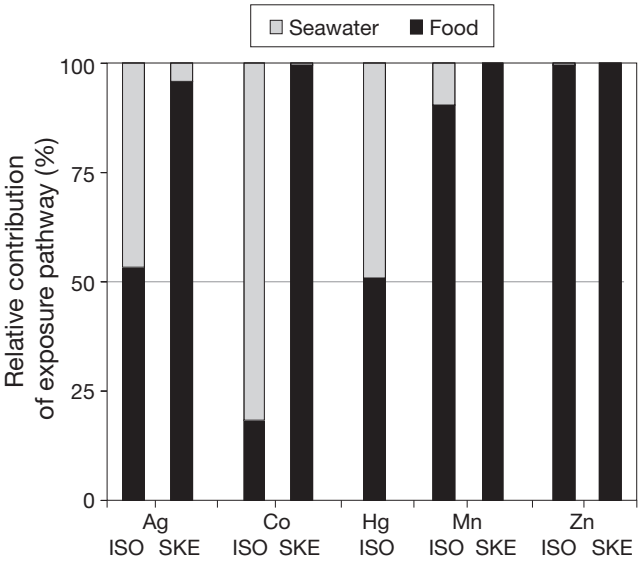

Fig. 4. *Chlamys varia*. Contribution (%) of dissolved (grey) and dietary (black) exposure pathways to global bioaccumulation of the selected elements. Dietary exposure tested with 2 phytoplankton species, *Isochrysis galbana* (ISO) and *Skeletonema costatum* (SKE)

Fig. 3. *Chlamys varia*. Depuration kinetics (mean % remaining activity ± SD, n = 6) of selected radiotracers after a 2 h feeding on radiolabelled *Isochrysis galbana* (●) followed by 30 d under non-contaminating conditions, and radiolabelled *Skeletonema costatum* (□) followed by 18 d under non-contaminating conditions

DISCUSSION

In the available literature on metal bioaccumulation in the Pectinidae, the importance of dietary transfer to the global metal bioaccumulation has been suggested (e.g. Palmer & Rand 1977, Uthe & Chou 1987, Bustamante & Miramand 2005b), but it has only been experimentally demonstrated for Cd and Ag (Metian et al. 2007, 2008c). For example, according to Bustamante & Miramand (2005b), the very high metal concentrations typically recorded in the digestive gland and kidneys of *Chlamys varia* and the relatively low levels in the gills suggest that uptake via food is a major pathway of metal bioaccumulation in this species. However, great caution should be exercised in the interpretation of organotropism data from the field as an indicator of the metal uptake pathway. Indeed, the results presented here showed that, for example, Ag was efficiently bioaccumulated and mainly stored in the digestive gland regardless of whether the scallops were exposed via their food or via seawater. Moreover, food was found to not always be the dominant contributing pathway; for example, seawater and diet contributed similarly when scallops were fed *Isochrysis galbana*.

To the best of our knowledge the present study, using radiotracer techniques and modelling, is the first to delineate the contributions of the uptake pathways of the 5 metals in *Chlamys varia*. Results of the bioaccumulation model indicate that the dietary pathway plays an important role in the global metal bioaccumulation in *C. varia*. When scallops were fed *Skeletonema costatum*, the food pathway was always the main route of bioaccumulation for all studied metals (food contributing ≥ 96%). When *C. varia* were fed *Isochrysis galbana*, either the dietary pathway was the main route of bioaccumulation (Mn and Zn, food contribution ≥ 91%), or seawater and food contributed equally (Ag and Hg, the latter element not having been tested with *S. costatum*). In a previous study on the blue mussel *Mytilus edulis*, Wang et al. (1996) showed that the dietary pathway was the main route of bioaccumulation for Se, but not for Ag, Am, Cd, Co and Zn, for which dissolved and dietary pathways contributed equally. The trend observed in the present study for the uptake of Zn by *C. varia* (dietary pathway contributing ≥ 99%) was the opposite of that reported for *M. edulis*. Interestingly, the data on the bioaccumulation and depuration kinetics of Mn and Zn in *C. varia* indicate that these elements are not efficiently bioconcentrated from seawater ($k_u \approx 30$ d^{-1}) compared to Ag or Hg (k_u = 466 and 100 d^{-1}, respectively). Moreover, even though dietary Mn displayed limited assimilation and retention in *C. varia*, food was the main uptake pathway for this metal.

Several studies have investigated the mechanisms influencing dietary metal bioavailability in filter-feeders (e.g. Borchardt 1983, Wang & Fisher 1996, Ng et al. 2005). Factors such as the cytosolic proportion of the metal in phytoplankton cells (Reinfelder et al. 1997) or the nature of the metal/phytoplankton binding (Ng & Wang 2005) have been shown to influence the bioavailability of a given metal ingested with food. Consequently, our results show that metal bioaccumulation characteristics of phytoplankton influence the bioavailability of the metals ingested by *Chlamys varia*. In this study, all metals displayed a higher AE in *C. varia* when ingested with *Skeletonema costatum* than when ingested with *Isochrysis galbana*. In the case of Ag and Co in particular the phytoplankton species determined the relative contribution of the dietary pathway to global metal bioaccumulation. Indeed, depending on whether *I. galbana* or *S. costatum* was ingested, the main route of Co bioaccumulation in the scallops switched between food and seawater. Since the retention capacity of Co did not differ depending on the phytoplankton species used, the observed difference was most probably due to the difference in AE ($AE_{I.\ galbana}$ = 18%, $AE_{S.\ costatum}$ = 31%) and/or to the difference in the K_{df} of the 2 phytoplankton species ($K_{df\ I.\ galbana} = 10^3$, $K_{df\ S.\ costatum} = 8 \times 10^5$). An overview of our results suggests that the affinity of the metal for the phytoplankton species (viz. the value of K_{df} measured during the food experiments; see Table 1) is a key factor in determining the relative contribution of the dietary pathway. Indeed, the K_{df} of Mn and Zn for both phytoplankton species was always higher than 10^5, whereas the K_{df} of Ag and Co had a lower value for *I. galbana* ($K_{df} = 4 \times 10^4$ for Ag and 10^3 for Co) than for *S. costatum* (K_{df} = 1 and 3 orders of magnitude higher, respectively).

At the end of the seawater exposure and the depuration phase following the seawater and food exposures of *Chlamys varia*, body targeting by the different metals was investigated. The results were consistent with previous studies carried out on different scallop species (e.g. Bryan 1973, Mauri et al. 1990, Bustamante & Miramand 2005a). The storage capacity of an organ is generally thought to be related to its role in the organism's metabolism and to detoxification processes occurring in this organ. For example, metal storage in pectinid tissues is typically attributed to metals binding to cytosolic proteins in the digestive gland and gills, to co-precipitation with sulfur in the digestive connective tissue, and to their precipitation on mineral granules in kidneys (e.g. George et al. 1980, Stone et al. 1986, Fowler & Gould 1988, Martoja et al. 1989, Mauri et al. 1990). The body distribution of the tested metals was generally relatively similar for seawater and food exposure, except for Co and espe-

cially for Hg. Indeed, Hg was mainly distributed in gills when scallops were exposed via seawater, whereas it was mainly accumulated in the digestive gland after dietary exposure. Comparison with other pectinids is difficult due to the scarcity of published data related to Hg. To the best of our knowledge, only Bargagli et al. (1998) reported a higher Hg concentration in the gills than in the digestive gland of the Antarctic scallop *Adamussium colbecki* (0.86 ± 0.22 vs. 0.35 ± 0.08 µg g^{-1} dry weight). Nevertheless, given that (1) gills and digestive gland of *C. varia* bioaccumulate dissolved and dietary Hg most efficiently, and that (2) both seawater and food pathways contribute similarly to the global bioaccumulation of this metal, our results suggest that the gills and the digestive gland of *C. varia* could be used as biomonitoring organs for Hg, since they would provide information on both the environmental contamination level and on the source of the contamination.

It is known that methylation of Hg and its accumulation at the base of the food chain can determine its transfer to higher organisms (see e.g. Mason et al. 1996). While relatively low AEs have previously been reported for inorganic Hg in several marine organisms (e.g. Fowler et al. 1978, Riisgård & Hansen 1990, Metian et al. 2008c), methyl-Hg is more readily bioaccumulated in bivalves than inorganic Hg (e.g. Fowler et al. 1978, Mason et al. 1996). In addition, retention of methyl-Hg in bivalve tissues is also higher than retention of the inorganic form ($T_{b1/2}$ is 2.7 to 4.7 times longer). However, the present study did not focus on methyl-Hg and additional research should be carried out to further assess possible differences in the availability and retention of inorganic vs. methyl-Hg in scallops.

The behaviour and fate of Ag in *Chlamys varia* tissues has been well studied (e.g. Martoja et al. 1989, Metayer et al. 1990, Bustamante & Miramand 2005a). The exposure to elevated dietary and/or dissolved concentrations of Ag decreased the ability of *C. varia* to produce byssus threads, preventing adequate fixation on the substratum (Metayer et al. 1990). Under these conditions, the foot was shown to contain both high concentrations and proportions of Ag. In the present study, which considered realistic environmental exposure concentrations, Ag was mainly bioconcentrated and distributed in the digestive gland of *C. varia* regardless of the exposure mode (food and seawater). This is consistent with previous observations made *in situ* (Bustamante & Miramand 2005a). Both experimental and field work support the central role of scallop digestive glands in the detoxification and storage of Ag, which is likely due to its precipitation with sulphur within the cells (Ballan-Dufrançais et al. 1985, Martoja et al. 1989).

CONCLUSION

The present study determined the relative contribution of the 2 main exposure pathways, food and seawater, to the bioaccumulation of metals in the variegated scallop *Chlamys varia* and confirmed the suspected importance of the dietary pathway. The dietary contribution was shown to depend on the metal and/or the phytoplankton species used as food. The results presented here will be useful for the interpretation of field data on metal concentrations in *C. varia* organs and tissues. In addition, as the dietary pathway was generally most important, metal concentrations in scallop food need to be carefully considered during field studies. This is particularly true when metal toxicity is being assessed, since most available toxicity tests currently used are focusing on the assessment of dissolved metal toxicity.

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