Mar Biol (2009) 156:2063–2075
DOI 10.1007/s00227-009-1237-7

ORIGINAL PAPER

Bioaccumulation of essential metals (Co, Mn and Zn) in the king scallop *Pecten maximus*: seawater, food and sediment exposures

Marc Metian · Michel Warnau · Laetitia Hédouin · Paco Bustamante

Received: 17 October 2008 / Accepted: 10 June 2009 / Published online: 25 June 2009

Abstract In order to understand the bioaccumulation of essential metals in filter-feeding mollusks living in soft sediments, the uptake and depuration kinetics of three elements (Co, Mn and Zn) were investigated in the king scallop *Pecten maximus* exposed via seawater, food, or sediment, using radiotracer techniques. The scallops were collected in April 2005 in the Pertuis Breton, France and acclimated to laboratory conditions for 8 weeks prior to the experimental exposures. Dissolved metals were efficiently bioconcentrated with mean concentration factors (CFs) ranging from 65 (Co) to 94 (Mn) after 7 days of exposure. Feeding experiments using microalgae *Skeletonema costatum* (diatom) or *Isochrysis galbana* (flagellate) showed that metal assimilation efficiency (AE) and retention ($T_{b½}$) were strongly influenced by food source. For Co, AE was higher when ingested with *I. galbana* (29 vs. 4%), whereas Mn and Zn AE was higher for *S. costatum* (82 vs. 44% and 86 vs. 68%, respectively). Transfer factors (TFs) in *P. maximus* exposed to radiolabelled sediment were 3–4 orders of magnitude lower than CFs. Nevertheless, the fraction of sediment-bound metals that was taken up was efficiently absorbed in scallop tissues (>85%). Whatever the exposure pathway, metals were strongly retained in the kidneys of *P. maximus*. Due to poor determination of Mn biokinetics (and related parameters) in scallops exposed through sediment, the relative contribution of the three different pathways could be determined only for Co and Zn using a biodynamic model. The particulate pathway (i.e. food or sediment) appeared to be the main route for bioaccumulation of both metals in this scallop. In addition, even though *P. maximus* displayed different AEs for Co and Zn according to the food, results of the model were only slightly affected, if any, by change in the dietary parameters (AE and depuration rate constant, k_e).

Communicated by J. P. Grassle.

M. Metian · M. Warnau · L. Hédouin
International Atomic Energy Agency-Marine Environment Laboratories (lAEA-MEL), 4 Quai Antoine Ier, 98000 Principality of Monaco, Monaco

M. Metian · M. Warnau (✉) · L. Hédouin · P. Bustamante
Littoral, Environnement et Sociétés (LIENSs), UMR 6250, CNRS-Université de La Rochelle, 2 rue Olympe de Gouges, 17042 La Rochelle Cedex 01, France
e-mail: warnaumichel@yahoo.com

Introduction

Several metals such as Co, Fe, Mn and Zn are essential to the metabolism of organisms. Essential metals are part of the functional groups of various enzymes, play a structural role in respiratory pigments and metalloenzymes or can act as activating co-factors for various enzymes (see e.g. Simkiss 1979; Williams 1981). The development, growth and general health of the organisms are optimal if essential metals are present in sufficient amounts in their tissues. As a consequence, depletion in essential elements can provoke pathological damage and/or physiological alterations in biota (e.g. Förstner and Wittmann 1983). Such a situation is generally reversible when normal concentrations are recovered. Conversely, because of their affinity for biological molecules, essential elements can also provoke toxic effects when their concentrations increase and reach a given threshold value (e.g. Förstner and Wittmann 1983; Rainbow 2002).

Marine organisms accumulate essential metals to satisfy their biological needs but the accumulation can sometimes

greatly exceed the amount required for metabolic processes (e.g. Pecquenat et al. 1969; Coombs 1972; Rainbow 2002). For example, Zn concentrations in barnacles can be very high [up to 100,000 μg Zn g^{-1} dry weight (DW)] while their estimated metabolic requirements are three orders of magnitude lower (Rainbow 2002).

When metabolic threshold concentrations are exceeded, organisms either are able to directly excrete essential metals in excess and/or store them in their tissues, generally as precipitates into mineral granules (Rainbow 2002), hence preventing toxic effects. Such storage mechanisms can lead to very high metal concentrations when these granules remain within the cells or tissues or to transiently elevated concentrations when the granules are eventually excreted (Rainbow 2002).

High concentrations of essential metals such as Mn and Zn have been recorded in pectinid soft tissues and in particular in their kidneys. For example, renal concentrations reported in *Pecten maximus* were 16,000 ± 4,100 μg g^{-1} DW for Mn (Bryan 1973) and from 7,000 to 19,000 μg g^{-1} DW for Zn (Bustamante and Miramand 2004). In this scallop species, both Mn and Zn are stored in renal concretions mainly composed of calcium phosphate (George et al. 1980). Pectinids also store Co in their kidneys up to relatively elevated concentrations (Bustamante and Miramand 2005). For example, in the tropical scallop *Comptopallium radula*, renal Co concentrations (up to 179 μg g^{-1} DW) were more than two orders of magnitude higher than in other soft tissues (Metian et al. 2008b).

The turnover of essential metals in pectinids is thought to be related to the turnover of the fraction trapped in renal granules and hence to their possible excretion through renal tubules (Fowler and Gould 1988). Furthermore, the contribution of the different exposure sources to essential metal bioaccumulation in scallops remains to be determined. The aim of this study was therefore to determine the kinetics of uptake and depuration of three essential metals (Co, Mn and Zn) in a typical pectinid from European waters, the king scallop *P. maximus*, when exposed to these elements via seawater, food or sediment. In order to better understand bioaccumulation processes for the selected essential metals, three levels of biological organization were considered: (1) the whole individual, (2) different organs and tissues and (3) subcellular fractionation in the tissues. Eventually, kinetic parameters obtained from the different experiments were then used in a biodynamic model originally described by Thomann (1981) and reviewed by Landrum et al. (1992) to determine the relative contribution of different exposure pathways to the total bioaccumulation of Co, Mn and Zn in *P. maximus*.

Materials and methods

Sampling

In April 2005, 70 king scallops *P. maximus* were collected on the Atlantic coast of France (Pertuis Breton, Charente-Maritime) by SCUBA diving. They were carefully transported to the IAEA-Marine Environment Laboratories in Monaco and were acclimated to laboratory conditions (constantly aerated 0.45-μm filtered water; open circuit, seawater flux 50 l h^{-1}; salinity 37 p.s.u.; temperature 17 ± 0.5°C; pH 8.0 ± 0.1; 12 h light:12 h dark photoperiod) for 8 weeks prior to experimentation. These laboratory conditions were maintained throughout the experimental period. During the acclimation period, scallops were fed once a day a microalgal diet (initial density: 5×10^4 cells ml^{-1}; Metian et al. 2007) composed of *Skeletonema costatum* (Bacillariophyceae) and *Isochrysis galbana* (Prymnesiophyceae).

Radiotracers and counting

Uptake and depuration kinetics of Co, Mn and Zn in scallops were determined using radiotracers of high specific activity purchased from Amersham, UK (^{57}Co in 0.1 M HCl, $T_{½} = 271.8$ days) and Isotope Product Lab., USA (^{54}Mn in 0.1 M HCl, $T_{½} = 312.2$ days; ^{65}Zn in 0.5 M HCl; $T_{½} = 243.9$ days). The radiotracers were counted using a high-resolution γ-spectrometry system composed of four Germanium—N or P type—detectors (EGNC 33-195-R, Canberra® and Eurysis®) connected to a multichannel analyser and a computer with spectrum 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. 2006a).

Seawater exposure

Twenty-five *P. maximus* (mean wet weight ± SD 208 ± 46 g, mean shell length ± SD 12.3 ± 1.3 cm) were placed in a 70-l glass aquarium (closed circuit; parameters as above) and exposed for 7 days to ^{57}Co, ^{54}Mn and ^{65}Zn (0.4, 0.4 and 0.75 kBq l^{-1}, respectively) dissolved in seawater. No change in pH was detectable after the tracer addition. Spikes and seawater were renewed twice a day for the first 2 days and then daily to keep the radioactivity constant in the seawater. During each renewal of seawater and spike, the scallops were fed briefly (30 min)

Skeletonema costatum and *Isochrysis galbana* (5×10^4 cells ml^{-1}) in clean seawater. According to the procedure of Warnau et al. (1996) and Rodriguez y Baena et al. (2006b), activity of the radiotracers in seawater was checked before and after each spike renewal, yielding time-integrated activities of 0.31 ± 0.12 kBq ^{57}Co l^{-1}, 0.34 ± 0.14 kBq ^{54}Mn l^{-1} and 0.73 ± 0.14 kBq ^{65}Zn l^{-1}.

Nine tag-identified scallops were collected at different time intervals and were whole-body γ-counted alive (same individual each time). At the end of the 7-d exposure period, five scallops (not belonging to the tag-identified batch) were killed and dissected. Shell, digestive gland, gills, kidneys, intestine, gonad, foot, mantle, adductor muscle and the remaining soft tissues were separated and radioanalyzed to assess the body distribution of the radiotracers.

Following the exposure period, the remaining scallops were placed in non-exposure conditions (open circuit, flux 50 l h^{-1}; daily feeding on *S. costatum* and *I. galbana,* 5×10^4 cells ml^{-1}; other parameters as above) for 36 days. The 9 tag-identified individuals were regularly radioanalyzed to follow the depuration kinetics of the radiotracers. Four scallops were collected at days 7, 14, 29 and 36 of the depuration period and dissected as previously described. Different tissues (adductor muscle, gills and digestive gland) were fractionated into soluble and insoluble components (methods below).

Food exposure

Fifteen scallops (mean wet weight ± SD 159 ± 45 g, mean shell length ± SD 11.7 ± 1.2 cm) were exposed to two different radiolabelled foods to approximate the diversity of their phytoplankton diet in the field (Mikulich and Tsikhon-Lukamina 1981; Shumway et al. 1987). One culture of *Skeletonema costatum* (Si-walled diatom) and one of *Isochrysis galbana* (naked flagellate) were previously exposed to ^{57}Co, ^{54}Mn and ^{65}Zn (4 kBq l^{-1} of each tracer) for the duration of their growth phase (10 and 7 days, respectively). At the end of the exposure period, the medium was filtered (1-µm mesh size Osmonic filters; Metian et al. 2007). Phytoplankton cells and spiked medium were collected and radioanalyzed to determine the partition coefficient between microalgae and seawater ($K_{d,f}$) for each radiotracer for each species. Each phytoplankton species was then added to one of the exposure aquaria (final density 5×10^4 cells ml^{-1}).

Phytoplankton resuspension was realized in a 70-l closed-circuit aquarium (parameters as previously described) where scallops ($n = 6$ for *I. galbana* and $n = 9$ for *S. costatum* experiment) had been placed 1 week before for acclimation. After a 2-h feeding on one or the other phytoplankton species (pulse-chase feeding method; Warnau et al. 1996), all scallops were whole-body γ-counted alive and then placed in clean, flowing seawater conditions (parameters as previously described), with daily feeding on non-labelled *S. costatum* and *I. galbana* (5×10^4 cells ml^{-1}). All individuals were then radioanalyzed at different time intervals to follow the whole-body depuration kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn in scallops. Four individuals were collected at the end of the depuration period (viz. after 16 days for scallops fed *I. galbana* and 21 days for those fed *S. costatum*) and dissected to determine (1) the body distribution of the radiotracers among shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues and (2) the subcellular fractionation (see below) in the cells of the digestive gland, gills and adductor muscle.

Sediment exposure

Sediment was collected in Wimereux (North-Atlantic coast of France). Sediment grain size distribution was determined using a Mastersizer Micro v2.12 (Malvern) and the dry/wet weight ratio was calculated after freeze drying using a LABCONCO Freezone18. Aerated sediments (9 kg) were divided between two 5-l plastic bottles, spiked with 292 kBq ^{57}Co, 297 kBq ^{54}Mn and 306 kBq ^{65}Zn (half in each bottle) and then constantly agitated for 6 days according to Danis et al. (2003, 2005) and adapted by Metian et al. (2007). A part of the radiolabelled sediments was then used to form a homogeneous 4-cm sediment layer in a 20-l glass aquarium. Weakly bound radiotracers were allowed to leach from the sediment overnight under flowing seawater (50 l h^{-1}) (Danis et al. 2003, 2005).

Ten *P. maximus* (mean wet weight ± SD 118 ± 5 g, mean shell length ± SD 11.1 ± 0.6 cm) were then placed for 13 days in the aquarium (open circuit; parameters as previously described), during which six tag-identified individuals were regularly radioanalyzed alive. Sediment samples were also regularly collected and γ-counted to verify that the radiotracer activities in sediment remained constant. At the end of the exposure period, four scallops were collected, dissected (shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the remaining soft tissues), weighed and radioanalyzed to determine the radiotracer body distribution. The remaining six scallops were transferred to a new 20-l glass aquarium, placed in depuration conditions for 31 days (clean sediment under flowing seawater, 50 l h^{-1}, daily feeding on *Skeletonema costatum* and *Isochrysis galbana* at 5×10^4 cells ml^{-1}), and regularly γ-counted. The radioactivity in sediment was regularly checked in order to ensure that no tracer recycling occurred in the sediment. Although no radioactivity was detected, the whole sediment was renewed after 1 week. At the end of the loss period

(31 days), four scallops were collected and dissected as described above to determine body distribution of ^{57}Co, ^{54}Mn and ^{65}Zn and their subcellular distribution in the digestive gland, gills and adductor muscle (see below).

Subcellular fractionation

The digestive gland, gills, and adductor muscle were used to assess the partitioning of ^{57}Co, ^{54}Mn and ^{65}Zn between "soluble" and "insoluble" cellular fractions as described by Bustamante and Miramand (2005). Briefly, the organs were homogenized individually with a mortar and pestle on ice with 10 ml of 0.02 M Tris–HCl buffer, 0.25 M sucrose, 1 mM phenylmethylsulfonylfluoride (PMSF, as protease inhibitor), at pH 8.6. The homogenates were centrifuged at 80,000G for 1 h at 5°C in a Sorvall RC28S ultracentrifuge to separate a particle-free supernatant (cytosol, viz. the soluble fraction) from the pellet (insoluble fraction). Homogenate aliquots, cytosols, and pellets were then radioanalyzed.

Data analysis

Uptake kinetics of the radioisotopes were expressed in terms of concentration factors (CFs: ratio between the radiotracer activity in scallops, Bq g^{-1} wet weight, and the time-integrated activity in the seawater, Bq g^{-1}) over time for the seawater exposure and in terms of transfer factors (TF: ratio between the radiotracer activity in scallops, Bq g^{-1} wet weight, and the time-integrated activity in the sediment, Bq g^{-1} wet weight) over time for the sediment exposure. Radiotracer uptake kinetics were best fitted using either an exponential model with saturation (Eq. 1) or a linear model (Eq. 2).

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

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

where CF_t and CF_{ss} are the CFs at time t (days) and at steady state, respectively ($CF_{ss} = k_u/k_e$), and k_u and k_e are the uptake and depuration rate constants (day^{-1}), respectively (Whicker and Schultz 1982).

Radiotracer depuration kinetics were expressed as % of remaining activity (radioactivity at time t divided by initial radioactivity measured in scallops at the beginning of the decontamination period × 100). The % of remaining activity was plotted against time and the depuration kinetics were best described by a simple- (Eq. 3) or a double-component exponential model (Eq. 4).

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

$$A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t} \quad (4)$$

where A_t and A_0 are the remaining activities (%) at time t (days) and 0, respectively; k_e is the depuration rate constant (day^{-1}); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components, respectively. For each exponential component (s and l), a biological half-life can be calculated ($T_{b½\ s}$ and $T_{b½\ l}$) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b\ ½} = \ln 2/k_e$. Regarding feeding experiments, the 'long-lived' exponential term describes the proportion of the radiotracer ingested with food that is actually absorbed by the organism. The corresponding A_{0l} is an estimate of the assimilation efficiency (AE, %) of the considered radiotracer (Temara et al. 1996; Warnau et al. 1996).

The best fitting regression models were selected according to highest determination coefficient and examination of residuals (Lacoue-Labarthe et al. 2008). The level of significance for statistical analyses was always set at $\alpha < 0.05\%$.

Results

Seawater exposure

Pecten maximus efficiently bioconcentrated ^{57}Co, ^{54}Mn and ^{65}Zn from the dissolved phase according to linear uptake kinetics ($R^2 = 0.80$, 0.85 and 0.78, respectively; Fig. 1; Table 1). The uptake rate constants (k_u) increased following the order: ^{57}Co < ^{65}Zn < ^{54}Mn.

The CFs reached at the end of the uptake period ($CF_{7\ d}$) in the whole organism and tissue compartments are presented in Table 2. The in toto $CF_{7\ d}$ were 63 ± 19 for ^{57}Co, 82 ± 24 for ^{54}Mn and 73 ± 21 for ^{65}Zn. The $CF_{7\ d}$ in the shells was higher than in the whole soft tissues for ^{57}Co (121 ± 26 vs. 40 ± 10, respectively) and for ^{54}Mn (122 ± 20 vs. 72 ± 25, respectively). An opposite trend was found for ^{65}Zn (61 ± 21 vs. 170 ± 32). Among soft tissues, the kidneys were the most efficient bioconcentrating organs for the three metals with $CF_{7\ d}$ of 2,374 ± 787 for ^{57}Co, 4,166 ± 1,774 for ^{54}Mn and 5,063 ± 1,897 for ^{65}Zn. $CF_{7\ d}$ values in other soft tissues were at least one order of magnitude lower than those in kidneys. Generally, $CF_{7\ d}$ did not exceed 100 with the exception of the digestive gland and gills for ^{65}Zn (797 ± 134 and 181 ± 9, respectively) and remaining soft tissues for ^{54}Mn and ^{65}Zn (229 ± 319 and 212 ± 75, respectively).

In terms of radiotracer tissue distributions, ^{57}Co, ^{54}Mn and ^{65}Zn were mainly found in kidneys (Fig. 2). These organs contained 68 and 64% of the total body burden of ^{57}Co and ^{54}Mn, respectively, but only 34 ± 4% of the total ^{65}Zn.

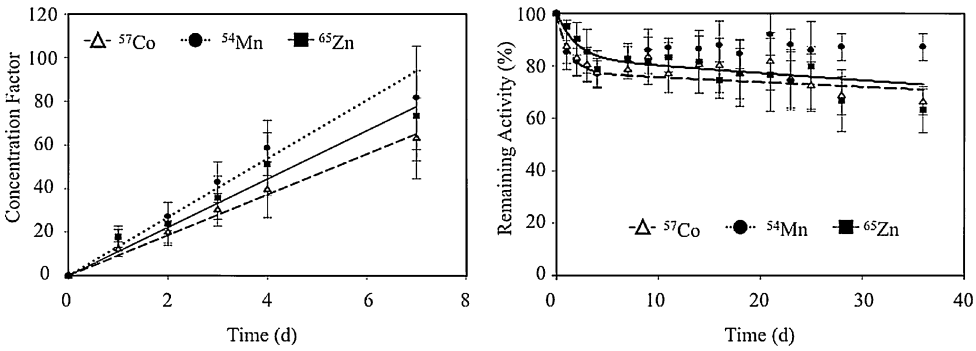

Fig. 1 Uptake and depuration kinetics of ^{57}Co, ^{54}Mn and ^{65}Zn in *Pecten maximus* exposed for 7 days to dissolved radiotracers [concentration factors (CF); mean ± SD; $n = 9$], then maintained for 36 days in clean conditions [remaining activity (%); mean ± SD; $n = 9$]

Table 1 Estimated uptake and depuration kinetic parameters of ^{57}Co, ^{54}Mn and ^{65}Zn in whole scallops *Pecten maximus* (1) exposed for 7 days to dissolved radiotracers ($n = 9$) and then maintained for 36 days in clean seawater ($n = 9$), (2) after a 2-h feeding on radiolabeled *Skeletonema costatum* and *Isochrysis galbana* followed by 21 days ($n = 9$) and 16 days ($n = 6$) in non-exposure conditions, respectively), and (3) exposed for 7 days via the radiolabelled sediments ($n = 6$) and then maintained for 31 days in clean sediment and seawater ($n = 6$)

Experiment	Uptake			Depuration				
Metal	CF_{ss} or TF_{ss} ± SE	k_u ± SE	R^2	A_{0s} ± SE	$T_{b½\ s}$ ± SE	A_{0l} ± SE	$T_{b½\ l}$ ± SE	R^2
Seawater								
^{57}Co	–	9.3 ± 0.3	0.80	25.5 ± 4.1	0.8 ± 0.4	76.6 ± 3.3	280*	0.42
^{54}Mn	–	13.4 ± 0.4	0.85	n.d.	n.d.	n.d.	n.d.	n.d.
^{65}Zn	–	11.1 ± 0.4	0.78	17.6 ± 4.6	1.3 ± 0.6	83.1 ± 4.0	192*	0.55
Food								
^{57}Co : *S. costatum*	–	–	–	96.1 ± 11.5	3.4 ± 1.1	4.19 ± 4.19	12.4	0.36
^{57}Co: *I. galbana*	–	–	–	71.5 ± 14.6	1.0 ± 0.4	28.9 ± 14.3	20	0.36
^{54}Mn : *S. costatum*	–	–	–	27.6 ± 6.7	0.7	82.0 ± 6.8	49 ± 22	0.44
^{54}Mn : *I. galbana*	–	–	–	54.4 ± 5.0	0.2 ± 0.1	43.5 ± 3.7	25 ± 9	0.89
^{65}Zn: *S. costatum*	–	–	–	13.8 ± 3.6	0.03	86.2 ± 1.9	79 ± 18	0.39
^{65}Zn: *I. galbana*	–	–	–	31.5 ± 10.3	0.2	68.4 ± 7.6	193*	0.32
Sediment								
^{57}Co	0.067 ± 0.007	0.014 ± 0.002	0.62	–	–	85.6 ± 3.1	47 ± 9	0.36
^{54}Mn	n.d.	0.014 ± 0.001	0.61	13.4 ± 7.8	0.9	86.6 ± 7.4	68 ± 31	0.45
^{65}Zn	0.028 ± 0.001	0.011 ± 0.001	0.84	12.3 ± 3.9	0.13	87.7 ± 2.0	114 ± 28	0.84

Uptake kinetic parameters: CF_{ss}. and TF_{ss}, concentration and transfer factors at steady state; k_u, uptake rate constant (day^{-1}); depuration kinetic parameters: A_{0s} and A_{0l}, activity (%) lost according to the short- and the long-lived exponential component, respectively; $T_{b½}$, biological half-life (day)

SE asymptotic standard error, R^2 determination coefficient of uptake or depuration kinetics

After the exposure period, unspiked conditions were restored and the radiotracer depuration was followed for 36 days. The whole-body depuration kinetics of ^{57}Co and ^{65}Zn were best described by a double-component exponential model ($R^2 = 0.42$ and 0.55, respectively; Fig. 1; Table 1) whereas it was not possible to determine the depuration kinetic parameters for ^{54}Mn. Nevertheless, the retention of this latter metal was strong as 85 ± 5% of the initial activity was still associated with the scallop tissues at the end of the 36-day depuration period.

The major part of the ^{57}Co and ^{65}Zn taken up was efficiently absorbed in the scallop soft tissues ($A_{0l} > 76\%$). The estimated depuration rate constant of the long-lived component (k_{el}) for both radiotracers was low (<0.004) and not significantly different from 0 ($P \geq 0.3$), indicating that the derived biological half-lives were not significantly different from infinite (Fig. 1; Table 1).

The dissections carried out during the depuration period showed that ^{57}Co, ^{54}Mn and ^{65}Zn were stored in the kidneys of *P. maximus* (Fig. 2): the proportion of ^{57}Co remained constant throughout the depuration period (68–72%; $P_{Tukey} = 0.47$), whereas the proportion of ^{54}Mn and ^{65}Zn increased significantly ($P_{Tukey} < 0.002$ in both cases) from 64 to 88% and from 34 to 69%, respectively (Fig. 2).

Regarding activities (Bq g^{-1} wet weight), renal activity of ^{54}Mn increased linearly throughout the depuration period, whereas ^{65}Zn activity reached a steady state (Fig. 2). For ^{57}Co, the renal activity remained constant throughout

Table 2 Concentration factors (mean CF ± SD, $n = 5$) of ^{57}Co, ^{54}Mn and ^{65}Zn in *Pecten maximus* and its tissues following 7-day exposure to dissolved radiotracers

Compartment	^{57}Co	^{54}Mn	^{65}Zn
Whole body	63 ± 19	82 ± 24	73 ± 21
Shell	120 ± 30	120 ± 20	61 ± 21
Soft tissues	40 ± 10	72 ± 25	170 ± 30
Digestive gland	60 ± 23	76 ± 20	800 ± 130
Gills	19 ± 8	42 ± 14	180 ± 10
Kidneys	2,370 ± 790	4,170 ± 1,770	5,060 ± 1,900
Intestine	14 ± 10	27 ± 17	57 ± 12
Gonad	15 ± 18	29 ± 37	82 ± 47
Foot	8 ± 4	13 ± 1	79 ± 9
Mantle	6 ± 1	9 ± 3	63 ± 15
Adductor muscle	8 ± 2	16 ± 5	54 ± 13
Remainder	49 ± 41	230 ± 320	210 ± 80

the depuration period. The radiotracer depuration kinetics were also followed in the other organs: ^{65}Zn in the digestive gland showed a decrease in both its activities and relative contents. In gills, the radiotracers were depurated following a non-linear regression. Finally, in the adductor muscle, change in radiotracer activity depended on the element considered: ^{57}Co activity decreased steeply, ^{54}Mn significantly increased, and ^{65}Zn remained constant throughout the depuration period (Fig. 2).

From the end of the uptake period to the end of the depuration period, subcellular fractionation was apparent in the digestive gland, gills and adductor muscle (Fig. 3). ^{54}Mn was mainly found in the insoluble fraction, in particular in the adductor muscle (i.e. 70–90%). ^{57}Co was generally higher in the soluble fraction of the studied organs, whereas partitioning of ^{65}Zn was dependent on the organ. This latter element was mainly associated with the soluble fraction in the digestive gland (~60%), was equally distributed between soluble and insoluble fractions in the gills, and mainly in the insoluble fraction in the adductor muscle (~60%).

Food exposure

To evaluate the influence of phytoplankton species on metal assimilation and retention in *P. maximus*, depuration kinetics of the three essentials metals were followed after a pulse-chase feeding, using radiolabelled *Skeletonema costatum* or *Isochrysis galbana*.

Whole-body depuration kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn were best fitted by a double-component exponential model (Fig. 4; Table 1). The assimilation efficiency and retention of the three radiotracers as well as the influence of the food source on these parameters were metal-dependent.

^{57}Co was poorly assimilated with both foods (AE was 29 ± 14% with *I. galbana* and 4 ± 4% with *S. costatum*). However, once incorporated, ^{57}Co was relatively well retained in the scallop tissues ($T_{b½\ 1}$ = 20 and 12 days, respectively). ^{54}Mn ingested with *S. costatum* was much more efficiently assimilated (AE = 82 ± 7%) than when ingested with *I. galbana* (AE = 44 ± 4%), but there was no significant difference in its retention according to the food source ($T_{b½\ 1}$: 49 ± 22 days with *S. costatum* and 25 ± 9 days with *I. galbana*). In the case of ^{65}Zn, assimilation was very high for both phytoplankton strains (AE ≥ 68%). However, when ^{65}Zn was ingested with *S. costatum*, its retention in the scallop tissues was lower ($T_{b½\ 1}$ = 79 ± 18 days) than when ingested with *I. galbana* ($T_{b½\ 1}$ > 193 days).

At the end of the depuration period, the distribution pattern of each radiotracer among the different organs and tissues did not differ according to the phytoplankton species ingested (Tukey test after arcsine data transformation; $P > 0.05$) (Fig. 4). The major part of ^{54}Mn was stored in the kidneys (≥80%), whereas ^{57}Co and ^{65}Zn were mainly found in the digestive gland (22–35%) and kidneys (39–49%). Low tissue activities for ^{57}Co and ^{54}Mn in both feeding experiments generally did not allow an accurate assessment of the subcellular fractionation. The only exception was for ^{54}Mn in the kidneys at the end of the *I. galbana* experiment: 96 ± 2% of ^{54}Mn was associated with the insoluble subcellular fraction. In contrast, the subcellular distribution of ^{65}Zn could be determined accurately (Fig. 5). With *I. galbana* as food, most of the ^{65}Zn was in the insoluble fraction in the kidneys (93 ± 1%). In the digestive gland, ^{65}Zn ingested with *I. galbana* was mainly associated with insoluble components (53 ± 2%) whereas it was mainly found in the soluble fraction when ingested with *S. costatum* (65 ± 7%). Differences between feeding experiments in subcellular fractionation of ^{65}Zn in gills and adductor muscle were not statistically significant.

Sediment exposure

The grain size distribution of sediment used in the experiments was mainly (95.8%) 76–302 μm and its dry/wet weight ratio was 0.80. Radiotracer activities in sediment were quite constant throughout the exposure period, i.e. 17 ± 1 Bq ^{57}Co g^{-1}; 15 ± 2 Bq ^{54}Mn g^{-1}; 36 ± 3 Bq ^{65}Zn g^{-1} wet weight.

Whole-body uptake kinetics of sediment-bound radiotracers were best fitted by a linear model for ^{54}Mn ($R^2 = 0.61$) and by a saturation exponential model in the case of ^{57}Co and ^{65}Zn ($R^2 = 0.62$ and 0.84, respectively) (Fig. 6; Table 1). For both ^{57}Co and ^{65}Zn, steady-state equilibrium was actually reached during the experiment.

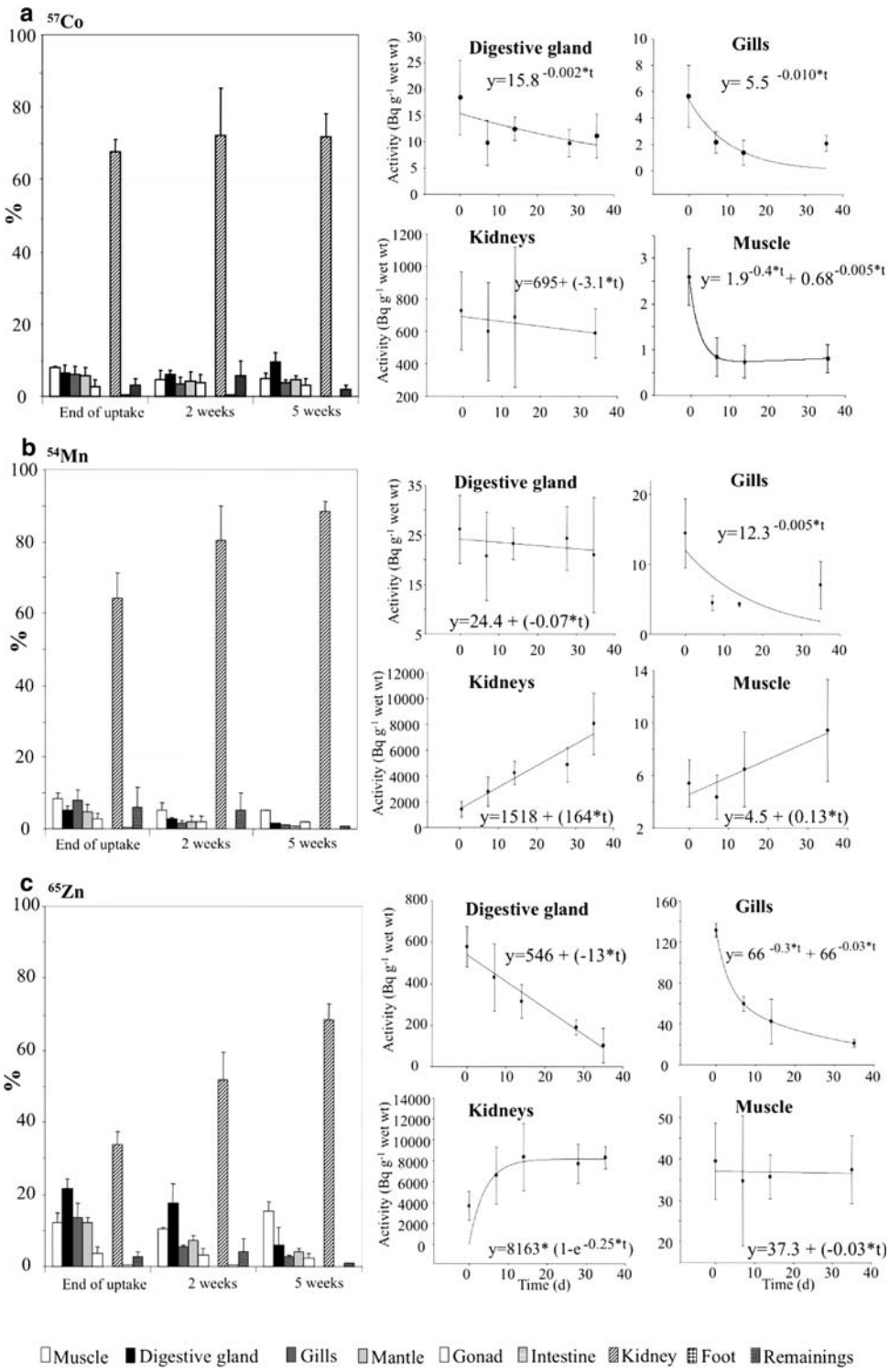

Fig. 2 Distribution of radiotracers among soft tissues of *Pecten maximus* (mean % ± SD; $n = 4$) and kinetics of change in ^{57}Co, ^{54}Mn and ^{65}Zn activities (mean Bq g^{-1} WW ± SD; $n = 4$) in selected organs during the depuration phase after a 7-day exposure to the dissolved radiotracers

After 13 days, the measured $TF_{13\ d}$ in toto reached 0.07 ± 0.04 for ^{57}Co, 0.19 ± 0.12 for ^{54}Mn and 0.05 ± 0.01 for ^{65}Zn (Table 3).

Among the different tissues, the highest $TF_{13\ d}$ were found in the kidneys for all three tracers (Table 3). With the exception of the digestive gland for ^{65}Zn ($TF_{13\ d} = 1.9 \pm 0.6$), the kidneys were the only compartment with a $TF_{13\ d} > 1$.

Among the different tissues, the kidneys also contained the highest fraction (up to 78 ± 9%) of the total body burden of ^{57}Co, ^{54}Mn and ^{65}Zn (Table 4). Although significantly less than kidneys, the digestive gland contained

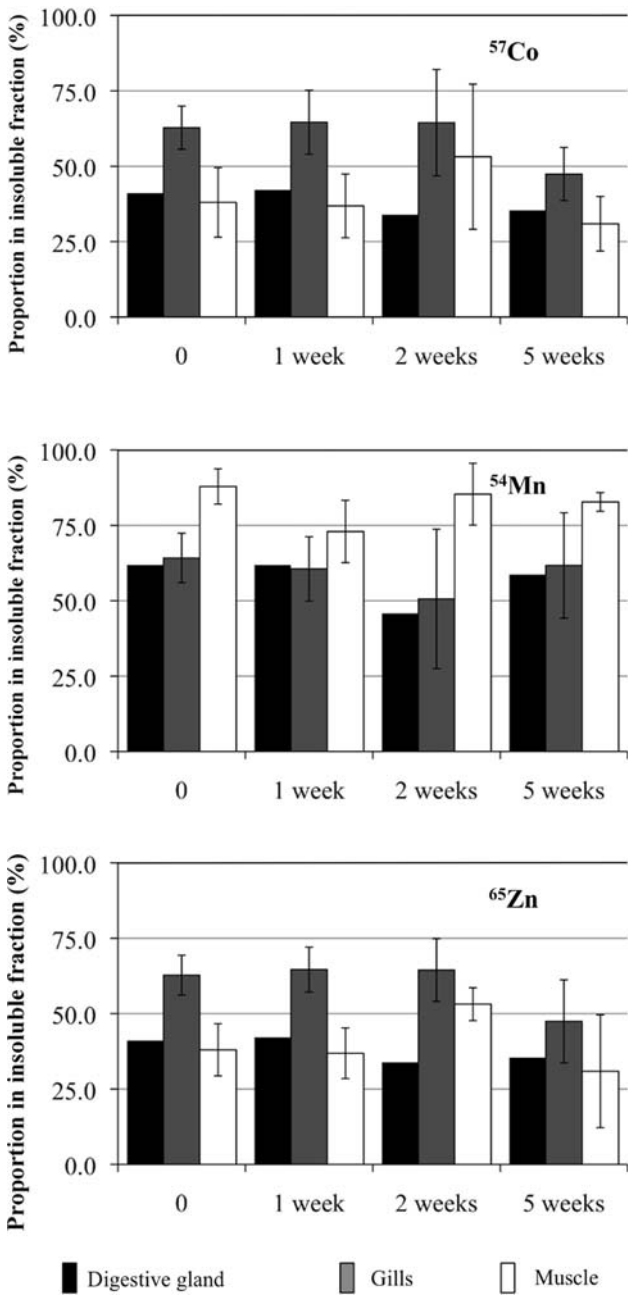

Fig. 3 Radiotracer fraction (%; $n = 4$) in insoluble cellular fraction of selected organs of *Pecten maximus* during 36-day depuration phase following a 7-day exposure to dissolved radiotracers

substantial, but significantly smaller amounts of metals, especially ^{57}Co and ^{65}Zn (i.e. 22 and 24%, respectively).

When non-exposure conditions were restored, whole-body depuration kinetics of ^{57}Co, ^{54}Mn and ^{65}Zn were followed for 31 days. The three metals were depurated according to a two-component exponential model (Table 1). However, for ^{57}Co, the parameters (A_{0s} and k_{es}) of the short-lived component could not be determined accurately. The tissue distribution of the three metals was similar at the beginning and at the end of the depuration phase (Table 4), with the kidneys containing the major part of the total radiotracer contents. In terms of radiotracer activity, there was a decrease in all three radiotracers in the digestive gland during the 31-day depuration period: from 3.9 ± 1.3 to 0.83 ± 0.32 Bq g^{-1} for ^{57}Co, from 0.51 ± 0.24 to 0.18 ± 0.05 Bq g^{-1} for ^{54}Mn, and from 67.9 ± 21.2 to 29.7 ± 11.4 Bq g^{-1} for ^{65}Zn. Similarly, renal activities of ^{57}Co decreased from 40.8 ± 25.8 to 6.5 ± 1.5 Bq g^{-1} but, in contrast, ^{54}Mn and ^{65}Zn activities in kidneys remained constant during that period ($P_{\text{Tukey}} > 0.05$). It is noteworthy that, during the depuration phase, ^{65}Zn activity in the adductor muscle significantly increased from 4.5 ± 0.2 to 5.7 ± 0.7 Bq g^{-1} ($P = 0.033$), leading to a concomitant increase in the radiotracer proportion in this tissue.

During the depuration period, subcellular partitioning was examined in the digestive gland, gills, adductor muscle and kidneys (Fig. 5). Activities in the subcellular fractions could be accurately measured only for ^{65}Zn in all the organs, and for ^{54}Mn in kidneys. ^{54}Mn and ^{65}Zn were always mainly localized in the insoluble subcellular fraction, especially in the kidneys in which the insoluble fraction accounted for 98% of both elements.

Discussion and conclusions

Previous studies of Co, Mn and Zn concentrations in scallop tissues mainly used field samples, and extremely high concentrations of Mn and Zn were reported in pectinid kidneys (i.e. 10–20 mg Mn or Zn g^{-1} DW (Bryan 1973; Mauri et al. 1990; Bustamante and Miramand 2005). Only the Antarctic scallop, *Adamussium colbecki*, had renal Mn and Zn concentrations <200 μg g^{-1} DW (Mauri et al. 1990). Relatively high Co concentrations were also reported in the kidneys of scallops from temperate and tropical regions, i.e. up to 10 μg g^{-1} DW (Bryan 1973; Bustamante and Miramand 2005; Metian et al. 2008b). Field data suggest that the kidneys (and secondarily the digestive gland) play an important role in bioaccumulation of these metals, but virtually no information has been available on the uptake pathways for these elements.

When exposed via the seawater, *P. maximus* efficiently bioconcentrated the three essential metals examined, i.e. on average 65–94 times the metal concentrations present in the surrounding seawater only after 7 days of exposure. In addition, the three essential metals were strongly retained in the scallop tissues. These characteristics, viz. efficient bioconcentration capacity, high absorption and retention efficiencies, suggest that even if low essential metal concentrations occur in the surrounding environment, the

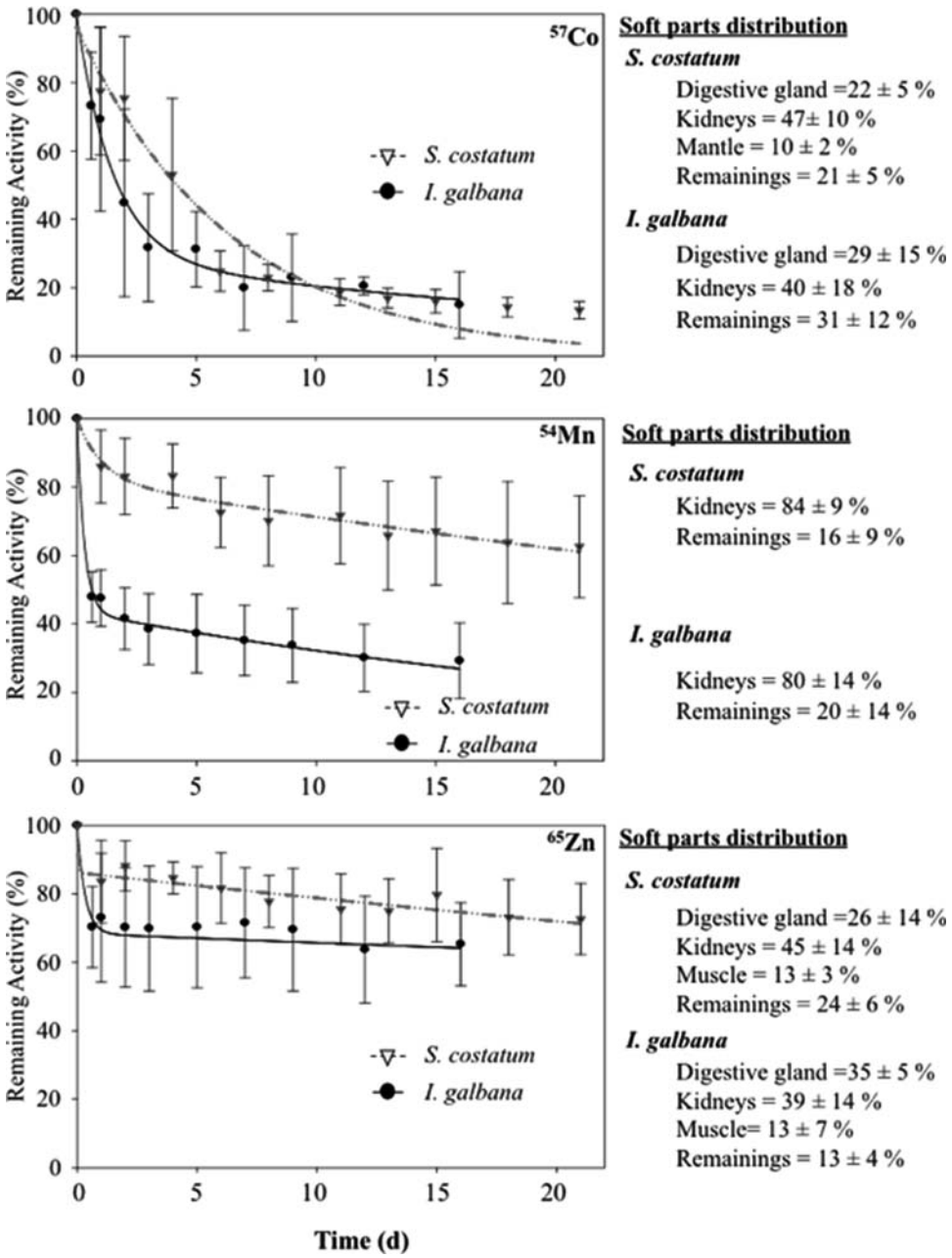

Fig. 4 Influence of phytoplankton food (*Skeletonema costatum* and *Isochrysis galbana*) on whole-body depuration kinetics [remaining activity (%); mean ± SD; $n = 9$ for *S. costatum* and $n = 6$ for *I. galbana*] of ingested ^{57}Co, ^{54}Mn and ^{65}Zn in *Pecten maximus* and body distribution of radiotracers determined 16 days (*I. galbana*) or 21 days (*S. costatum*) after feeding

scallops would readily bioconcentrate the essential elements required for their metabolic needs.

Our dietary exposure experiments with a diatom, *Skeletonema costatum*, and a flagellate, *Isochrysis galbana*, showed that food quality played an important role in both the assimilation and retention of metals ingested with the food. For Co, the AE was higher when ingested with *I. galbana* than with *S. costatum*, whereas an opposite trend was observed for Mn and Zn. Several factors related to food quality could explain these differences, such as the presence (*S. costatum*) or lack (*I. galbana*) of a Si-wall, the carbon composition of the algal species and/or the proportion of the metals stored in bioavailable forms in the cytoplasm of the phytoplankton cells (see e.g. Reinfelder and Fisher 1991; Reinfelder et al. 1997; Ng et al. 2005; Metian et al. 2008a). The physico-chemical conditions within the gut and the digestive processes also play a major role in efficiency of metal assimilation from food (e.g. Reinfelder et al. 1997). Hence, it has been proposed that the higher AE observed for Ag in *P. maximus* when fed *S. costatum* than when fed *I. galbana*, could be due to a difference in gut transit time for the two phytoplankton species, the transit being longer for *S. costatum* (Metian et al. 2008a). Although a similar longer gut transit time for *S. costatum* was observed in this study (see the much slower initial decrease in depuration kinetics and the corresponding longer $T_{b½\ s}$ after feeding on *S. costatum*;

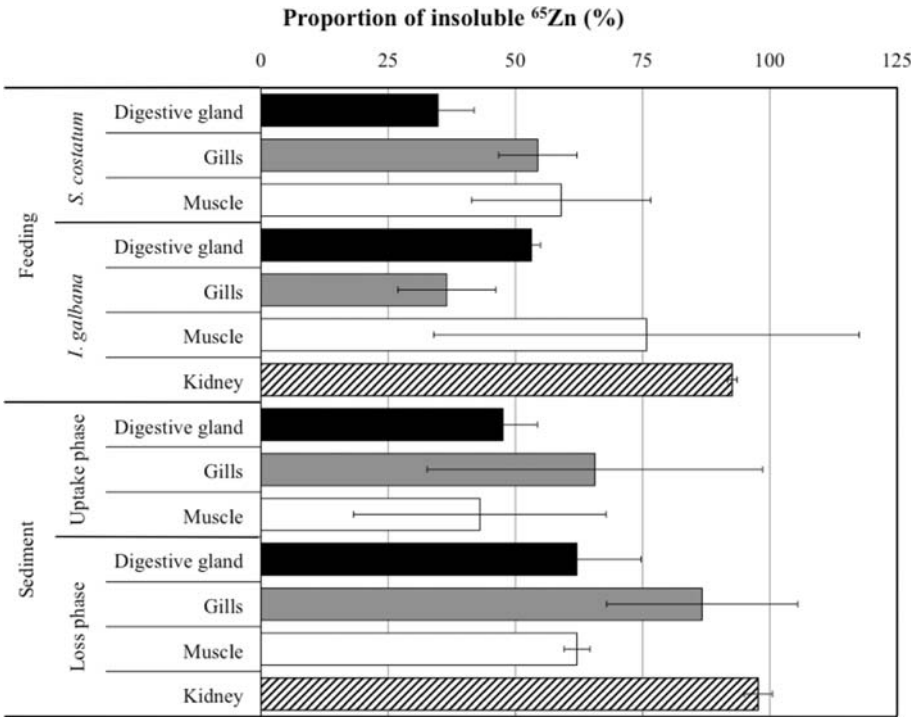

Fig. 5 Proportion of ^{65}Zn (%; mean ± SD, $n = 4$) associated with insoluble fraction of cells in selected tissues of *Pecten maximus* exposed via food or sediment

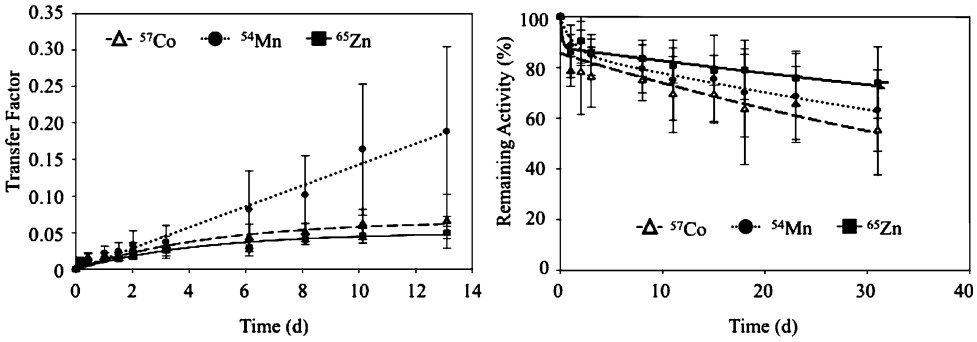

Fig. 6 Uptake (**a**) and depuration (**b**) kinetics of ^{57}Co, ^{54}Mn and ^{65}Zn in *Pecten maximus* exposed for 13 days to sediment-bound radiotracers [transfer factors (TF); mean ± SD; $n = 6$], then maintained for 31 days in clean conditions [remaining activity (%); mean ± SD; $n = 6$]

Table 3 Transfer factors (mean TF ± SD, $n = 4$) of ^{57}Co, ^{54}Mn and ^{65}Zn in *Pecten maximus* and its tissues following 13-day exposure to sediment-bound radiotracers

Compartment	^{57}Co	^{54}Mn	^{65}Zn
Whole body	0.07 ± 0.04	0.19 ± 0.12	0.05 ± 0.01
Shell	0.09 ± 0.02	0.17 ± 0.08	0.02 ± 0.00
Soft tissues	0.07 ± 0.01	0.04 ± 0.02	0.32 ± 0.02
Digestive gland	0.23 ± 0.08	0.03 ± 0.02	1.90 ± 0.59
Gills	0.01 ± 0.00	0.03 ± 0.01	0.12 ± 0.01
Kidneys	2.38 ± 1.51	5.65 ± 2.10	12.7 ± 4.2
Intestine	0.09 ± 0.05	0.02 ± 0.01	0.22 ± 0.17
Gonad	0.08 ± 0.08	0.15 ± 0.15	0.54 ± 0.54
Foot	0.03 ± 0.01	0.02 ± 0.01	0.18 ± 0.04
Mantle	0.01 ± 0.00	0.01 ± 0.00	0.12 ± 0.01
Adductor muscle	< 0.01	0.01 ± 0.01	0.13 ± 0.00
Remainder	0.06 ± 0.02	0.04 ± 0.04	0.30 ± 0.09

Fig. 4), the correlation with a higher AE does not appear obvious. Indeed, in the case of Co, its AE when ingested with *I. galbana* was much higher (by a factor of 7) than when ingested with *S. costatum*.

Since *P. maximus* lives buried in the sediment and sediment particles have been reported in scallop stomachs (e.g. Mikulich and Tsikhon-Lukamina 1981; Shumway et al. 1987), metal transfer via this pathway was assessed. For the three essential metals studied, estimated TFs in *P. maximus* were lower by 3–4 orders of magnitude than CFs calculated after 7 days of seawater exposure. This indicates a much lower bioavailability of metals when bound to sediment than when dissolved in seawater. However, metal concentrations reported in sediments are generally far higher than those in seawater (e.g. Danis et al. 2004), and sediment could therefore contribute significantly to the total metal uptake in scallops despite a low

Table 4 Body distribution of ^{57}Co, ^{54}Mn and ^{65}Zn in *Pecten maximus* (mean % ± SD; $n = 4$) after 13-day exposure to radiolabelled sediment (uptake phase) and after a subsequent 31-day depuration (depuration phase)

	^{57}Co		^{54}Mn		^{65}Zn	
	Uptake phase	Depuration phase	Uptake phase	Depuration phase	Uptake phase	Depuration phase
Digestive gland	22 ± 4	20 ± 7	2 ± 1	1 ± 0	24 ± 7	12 ± 5
Gills	9 ± 3	8 ± 1	5 ± 1	2 ± 1	6 ± 4	4 ± 0
Kidneys	51 ± 10	39 ± 14	78 ± 9	83 ± 3	37 ± 3	51 ± 5
Intestine	1 ± 0	4 ± 2	<1	<1	<1	<1
Gonad	4 ± 4	9 ± 3	4 ± 4	4 ± 1	3 ± 3	4 ± 0
Foot	1 ± 0	4 ± 1	<1	<1	<1	<1
Mantle	7 ± 2	9 ± 2	3 ± 1	1 ± 0	13 ± 1	7 ± 0
Adductor muscle	4 ± 1	4 ± 1	6 ± 3	7 ± 1	16 ± 3	21 ± 1
Remainder	2 ± 1	2 ± 1	1 ± 1	2 ± 2	1 ± 0	1 ± 0

transfer efficiency. Moreover, once incorporated, more than 85% of the metals taken up from sediment were incorporated and strongly retained ($T_{b½} > 1$ month) in scallop tissues.

In order to assess the relative contribution of the different exposure pathways (i.e. seawater, food, and sediment) to total metal bioaccumulation in the scallop, a biodynamic model was applied, considering three different uptake pathways (seawater, food and sediment) as described by Metian et al. (2008a). Originally proposed by Thomann (1981), this model has been used and adapted by others (e.g. Landrum et al. 1992; Thomann et al. 1995; Reinfelder et al. 1998; Metian et al. 2008a, 2009). Uptake and depuration kinetic parameters estimated for Co and Zn were used in the model (the lack of depuration data for Mn did not allow global computations for this element). Besides the kinetic parameters, the model also required other parameters that were either calculated from the results of our experiments or taken from the literature. Calculated parameters were the partitioning coefficient between food and seawater ($K_{d,f}$: 8.47×10^5 and 1.35×10^3 for Co and 6.62×10^6 and 1.12×10^5 for Zn in *S. costatum* and *I. galbana*, respectively). Literature-derived parameters were the partitioning coefficients between sediment and seawater ($K_{d,s}$: 3×10^5 for Co, and 7×10^4 for Zn; IAEA 2004) and the ingestion rate of the scallop *P. maximus* (IR: 0.0404 g g^{-1} day^{-1}; Metian et al. 2008a).

Model results highlight the predominant role of the particulate pathway (i.e. food and/or sediment) in the total bioaccumulation of Co and Zn in *P. maximus*. Food was found to be the main route of Zn bioaccumulation and contributed from 88 (*I. galbana*) to 100% (*S. costatum*) to total Zn bioaccumulation in the scallop. Although generally less important than in *P. maximus*, preferential mobilization of Zn from food has also been shown experimentally for other bivalves such as the mussel *Mytilus edulis* (e.g. contribution of 67%; Wang and Fisher 1999), the scallop *Chlamys varia* (contribution of ca. 100%; Metian et al. 2009) and tropical oysters *Isognomon isognomon* and *Malleus regula* (contribution of 57–75%; Hédouin et al. 2009). In the case of Co, sediment was the major source of the metal in *P. maximus* regardless of the phytoplankton species that was used as food (89 and 99% with *S. costatum* and *I. galbana*, respectively). Such an elevated contribution from this pathway might be partly related to Co speciation in sediments. Indeed, microorganisms are known to biotransform inorganic Co into organic Co (e.g. cobalamine), leading to high concentrations of cobalamine in estuarine mud (up to 3 μg g^{-1} DW) and in activated sewage sludge (up to 50 μg g^{-1} DW) (e.g. White et al. 1973; Hamilton 1994). Cobalamine is a vitamin (vit. B12) for marine organisms, including many phytoplankton species (Croft et al. 2006). It is much more efficiently bioaccumulated and strongly retained than inorganic Co (e.g. Nolan et al. 1992). However, the quite low Co TFs that we measured in *P. maximus* indicate that if any such biotransformation occurred in our experiments, it was limited.

Determining the relative importance of different exposure pathways for metal accumulation in marine organisms is important for interpreting field observations and for better understanding environmental effects. Indeed, it permits us to relate possible differences in metal concentrations in organisms to differences in metal concentrations in different compartments of the ecosystem (seawater, food or sediment). For example, in *P. maximus*, the sediment pathway could be identified as the major bioaccumulation pathway for Co, and food as the main source of Zn. These results are of particular interest in the field of toxicology; toxicological tests using scallops are generally limited to seawater exposures, and sometimes to dietary exposures (e.g. Nelson et al. 1976; Pesch et al. 1979; Gould et al. 1985; Metayer et al. 1990; Berthet et al. 1992). In the light of our results, more appropriate toxicity tests (i.e.

considering sediment and food as metal sources) would be more environmentally relevant and would reduce the risk of underestimating metal toxicity in Pectinidae.

Regardless of the exposure pathway, the target organs of the three essential metals were always the kidneys and then the digestive gland. In addition, the similarity in subcellular distribution of the elements whether exposure was via seawater, food or sediment suggests that the processes governing their accumulation and detoxification were similar regardless of exposure mode. Changes in metal concentrations during depuration following seawater exposure highlighted the importance of the kidneys as storage organs. Mn concentration linearly increased in the kidneys over 5 weeks of depuration, indicating that this metal was transferred from other tissues to the kidneys where Mn was stored. Storage of Mn in the kidneys of *P. maximus* might be related to the precipitation and/or co-precipitation of this metal into mineral ($CaPO_4$) granules (George et al. 1980). The subcellular distribution of Mn in kidneys of scallops exposed via the food (*I. galbana*) and via the sediment strongly supports this assumption as more than 96% of the metal was associated with the insoluble fraction which contains the metal-enriched $CaPO_4$ granules. In contrast, translocation of Zn in the kidneys during the seawater depuration period reached a steady-state equilibrium after 10 days, which may represent an equilibrium between translocation of Zn into the kidneys and its subsequent renal excretion. It is striking that even though Mn and Zn showed different elimination dynamics, they were both mainly stored in the insoluble cellular fraction. In contrast to Mn and Zn, renal Co did not change significantly during depuration. Since Co did decrease in the other organs, either a very low Co transfer to the kidneys occurred or the scallop kidneys have a very efficient excretion capacity for this metal. To the best of our knowledge, the incorporation of Co into renal mineral granules has not been demonstrated in Pectinidae (George et al. 1980). In the digestive gland of *P. maximus*, we found that Co was always mainly associated with the soluble cellular fraction for the three exposure pathways. Although information on cellular detoxification of Co in mollusks is scarce, our data agree with field observations: more than 65% of the Co was localized in the cytosolic fraction of the digestive gland of the scallop *Chlamys varia* and the cuttlefish *Sepia officinalis* (Bustamante and Miramand 2005; Bustamante et al. 2006, respectively). Hence, although requiring confirmation, these data do not support a preferential storage of Co in the kidneys as mineral granule-precipitated forms, and rather substantiate a high renal elimination capacity.

Acknowledgments Authors are grateful to F. Oberhänsli and J.L. Teyssié (IAEA-MEL) for skilful technical assistance. We also thank the anonymous reviewers for critical review of the manuscript and fruitful suggestions. MW is an Honorary Senior Research Associate of the National Fund for Scientific Research (NFSR, Belgium) and holds a 2008–2009 Invited Expert position at LIENSs (CNRS-Université de La Rochelle) supported by the Conseil Régional de Poitou-Charente. This work was supported by the IAEA, the Conseil Général de la Charente-Maritime and the GIP Seine-Aval Programme. The IAEA is grateful for the support provided to its Marine Environment Laboratories by the Government of the Principality of Monaco. The experiments described herein comply with the current laws of the Principality of Monaco and France.

References

Berthet B, Amiard JC, Amiard-Triquet C, Martoja R, Jeantet AY (1992) Bioaccumulation, toxicity and physico-chemical speciation of silver in bivalve molluscs: ecotoxicological and health consequences. Sci Total Environ 125:97–122

Bryan GW (1973) The occurence and seasonal variation of trace metals in the scallops *Pecten maximus* (L.) and *Chlamys opercularis* (L.). J Mar Biol Assoc UK 53:145–166

Bustamante P, Miramand P (2004) Interspecific and geographical variations of trace element concentrations in Pectinidae from European waters. Chemosphere 57:1355–1362

Bustamante P, Miramand P (2005) Subcellular and body distributions of 17 trace elements in the variegated scallop *Chlamys varia* from the French coast of the Bay of Biscay. Sci Total Environ 337:59–73

Bustamante P, Bertrand M, Boucaud-Camou E, Miramand P (2006) Subcellular distribution of Ag, Cd, Co, Cu, Fe, Mn, Pb and Zn in the digestive gland of the common cuttlefish *sepia officinialis*. J Shellfish Res 25:987–993

Coombs TL (1972) The distribution of zinc in the oyster *Ostrea edulis* and its relation with enzymatic activity and to other metals. Mar Biol 12:170–178

Croft MT, Warren MJ, Smith AG (2006) Algae need their vitamins. Eukaryotic Cell 5:1175–1183

Danis B, Cotret O, Teyssié JL, Fowler SW, Bustamante P, Warnau M (2003) Delineation of PCB uptake pathways in a benthic sea star using a radiolabelled congener. Mar Ecol Prog Ser 253:155–163

Danis B, Wantier P, Flammang R, Dutrieux S, Dubois P, Warnau M (2004) Contaminant levels in sediment and asteroids (*Asterias rubens*, Echinoderm) from the Belgian coast and Scheldt estuary: polychlorinated biphenyls and metals. Sci Total Environ 333:149–165

Danis B, Bustamante P, Cotret O, Teyssié JL, Fowler SW, Warnau M (2005) Bioaccumulation of PCBs in the cuttlefish *Sepia officinalis* from sea water, sediment and food pathways. Environ Pollut 134:113–122

Förstner U, Wittmann GT (1983) Marine pollution in the aquatic environment, 2nd edn. Springer, Berlin

Fowler BA, Gould E (1988) Ultrastructural and biochemical studies of intracellular metal-binding patterns in kidney tubule cells of the scallop *Placopecten magellanicus* following prolonged exposure to cadmium or copper. Mar Biol 97:207–216

George SG, Pirie BJS, Coombs TL (1980) Isolation and elemental analysis of metal-rich granules from kidney of the scallop *Pecten maximum* (L.). J Exp Mar Biol Ecol 42:143–156

Gould E, Greig RA, Rusanowsky D, Marks BC (1985) Metal-exposed sea scallops, *Placopecten magellanicus* (Gmelin): a comparison of the effects and uptake of cadmium and copper. In: Vernberg FJ, Thurberg FP, Callabrese A, Vernberg WB (eds) Marine pollution and physiology, recent advances. University of South Carolina Press, Columbia, pp 157–186

Hamilton EI (1994) The geochemistry of cobalt. SciTotal Environ 150:7–39

Hédouin L, Metian M, Teyssié JL, Fichez R, Warnau M (2009) Delineation of heavy metal contamination pathways (seawater, food and sediment) in tropical oysters from New Caledonia using radiotracer techniques. Mar Pollut Bull (in press)

IAEA (2004) Sediments distribution coefficients and concentration factors for biota in the marine environment. International Atomic Energy Agency, Vienna

Lacoue-Labarthe T, Warnau M, Oberhänsli F, Teyssié JL, Kouéta N, Bustamante P (2008) Differential bioaccumulation behaviour of Ag and Cd during the early development of the cuttlefish *Sepia officinalis*. Aquat Toxicol 86:437–446

Landrum PF, Lee H, Lydy MJ (1992) Toxicokinetics in aquatic systems: model comparisons and use in hazard assessment. Environ Toxicol Chem 11:1709–1725

Mauri M, Orlando E, Nigro M, Regoli F (1990) Heavy metals in the Antarctic scallop *Adamussium colbecki*. Mar Ecol Prog Ser 67:27–33

Metayer C, Amiard-Triquet C, Baud JP (1990) Variations interspécifiques de la bioaccumulation et de la toxicité de l'argent à l'égard de trois mollusques bivalves marins: species-related variations of silver bioaccumulation and toxicity to three marine bivalves. Water Res 24:995–1001

Metian M, Warnau M, Oberhänsli F, Teyssié JL, Bustamante (2007) Interspecific comparison of Cd bioaccumulation in European Pectinidae (*Chlamys varia* and *Pecten maximus*). J Exp Mar Biol Ecol 353:58–67

Metian M, Bustamante P, Cosson RP, Hédouin L, Warnau M (2008a) Investigation of Ag in the king scallop *Pecten maximus* using field and laboratory approaches. J Exp Mar Biol Ecol 367:53–60

Metian M, Bustamante P, Hédouin L, Warnau M (2008b) Accumulation of nine metals and one metalloid in the tropical scallop *Comptopallium radula* from coral reefs in New Caledonia. Environ Pollut 152:543–552

Metian M, Bustamante P, Hédouin L, Oberhänsli F, Warnau M (2009) Delineation of heavy metal uptake pathways (seawater and food) in the variegated scallop *Chlamys varia* using radiotracer techniques. Mar Ecol Prog Ser 375:161–171

Mikulich LV, Tsikhon-Lukamina A (1981) Food of the scallop. Oceanology 21:633–635

Nelson DA, Calabrese A, Nelson BA, Maclnnes JR, Wenzloff DR (1976) Biological effects of heavy metals on juvenile bay scallops, *Argopecten irradians*, in short term exposures. Bull Environ Contam Toxicol 16:275–282

Ng TYT, Amiard-Triquet C, Rainbow PS, Amiard JC, Wang WX (2005) Physicochemical form of trace metals accumulated by phytoplancton and their assimilation by filter feeder. Mar Ecol Prog Ser 299:179–191

Nolan CV, Fowler SW, Teyssié JL (1992) Cobalt speciation and bioavailability in marine organisms. Mar Ecol Prog Ser 88:105–116

Pecquenat JE, Fowler SW, Small LF (1969) Estimates of the zinc requirements of marine organisms. J Fish Res Board Can 26:145–150

Pesch GG, Steward NE, Pesch C (1979) Copper toxicity to the bay scallop (*Argopecten irradians*). Bull Environ Contam Toxicol 23:759–765

Rainbow PS (2002) Trace metal concentrations in aquatic invertebrates: why and so what? Environ Pollut 120:497–507

Reinfelder JR, Fisher NS (1991) The assimilation of elements ingested by marine copepods. Science 251:794–796

Reinfelder JR, Wang WX, Luoma SN, Fisher NS (1997) Assimilation efficiencies and turnover rates of trace elements in marine bivalves: a comparison of oysters, clams and mussels. Mar Biol 129:443–452

Reinfelder JR, Fisher NS, Luoma SN, Nichols JW, Wang WX (1998) Trace element trophic transfer in aquatic organisms: a critique of the kinetic model approach. Sci Total Environ 219:117–135

Rodriguez y Baena AM, Miquel JC, Masqué P, Povinec P, La Rosa J (2006a) A single vs. double spike approach to improve the accuracy of ^{234}Th measurements in small-volume seawater samples. Mar Chem 100:269–281

Rodriguez y Baena AM, Metian M, Teyssié JL, De Broyer C, Warnau M (2006b) Experimental evidence for ^{234}Th bioaccumulation in three Antarctic crustaceans: potential implications for particle flux studies. Mar Chem 100:354–365

Shumway SE, Selvin R, Schick DF (1987) Food resources related to habitat in the scallop *Placopecten magellanicus* (Gmelin, 1791): a qualitative study. J Shellfish Res 6:89–95

Simkiss K (1979) Metal ions in cells. Endeavour New Ser 3:2–6

Temara A, Ledent G, Warnau M, Paucot H, Jangoux M, Dubois P (1996) Experimental cadmium contamination of *Asterias rubens* (Echinodermata). Mar Ecol Prog Ser 140:83–90

Thomann RV (1981) Equilibrium model of fate of microcontaminants in diverse aquatic food chains. Can J Fish Aquat Sci 38:280–296

Thomann RV, Mahony JD, Mueller R (1995) Steady-state model of biota sediment accumulation factor for metals in two marine bivalves. Environ Toxicol Chem 14:1989–1998

Wang WX, Fisher NS (1999) Delineating metal accumulation pathways for marine invertebrates. Sci Total Environ 237(238):459–472

Warnau M, Teyssié JL, Fowler SW (1996) Biokinetics of selected heavy metals and radionuclides in the common Mediterranean echinoid *Paracentrotus lividus:* sea water and food exposures. Mar Ecol Prog Ser 141:83–94

Whicker FW, Schultz V (1982) Radioecology: nuclear energy and the environment. CRC Press, Florida

White A, Handler P, Smith EL (1973) Principles of biochemistry. McGraw-Hill, New York

Williams RJP (1981) Physico-chemical aspects of inorganic element transfer through membranes. Phil Trans Royal Soc B 294:57–74