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Abstract

The present study aimed at examining the influence of food quality and quantity on the assimilation efficiency (AE) of metals in two abundant bivalves in the New Caledonia lagoon, the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum*. Bivalves were exposed via their food to the radiotracers of three metals of concern in New Caledonia (^{54}Mn, ^{57}Co and ^{65}Zn) under different feeding conditions (phytoplankton species, cell density, and cell-associated metal concentration). When bivalves were fed *Heterocapsa triquetra, Emiliania huxleyi* and *Isochrysis galbana*, AE of Mn, Co and Zn was strongly influenced by the phytoplankton species and by the metal considered. In contrast, when fed one given phytoplankton species previously exposed to different concentrations of Co, phytoplankton-associated Co load had no influence on the AE and on the retention time of the metal in both bivalves. Metals ingested with *I. galbana* displayed generally the highest AE in both bivalve species, except for Mn in clams for which the highest AE was observed for *H. triquetra*. Influence of food quantity was investigated by exposing bivalves to different cell densities of *I. galbana* (5×10^3, 10^4 or 5×10^4 cell ml^{-1}). As for food quality, food quantity was found to influence AE of Mn, Co and Zn, the highest AE being observed when bivalves were fed the lowest cell density. Overall, results indicate that the two bivalve species are able to adjust their feeding strategies according to the food conditions prevailing in their environment.

Keywords: Mollusks, *Isognomon isognomon*, *Gafrarium tumidum*, New Caledonia, Radiotracer, Feeding

1. Introduction

Changes in coastal ecosystem functioning due to anthropogenic metal inputs is a worldwide issue of concern especially as metals are not biodegradable and enter biogeochemical cycles (Tessier and Turner, 1995). In the coral reef lagoon of New-Caledonia, metal contamination is a critical problem in relation with its extreme biodiversity (Labrosse et al., 2000). Indeed, the lagoon is subject to an increasing environmental pressure imposed by urban development and intensive mining activities. In addition, the use of hydrometallurgic process employing heated and pressured sulphuric acid (lixiviation) has been recently developed in New Caledonia and is expected to be implemented at industrial scale early 2010 (Goro-Nickel, 2001, 2003). Such a process will provide new potential to exploit laterite soils that display lower nickel (Ni) contents than garnierite ores currently used in pyrometallurgic plants, such as at the Société Le Nickel, and will allow recovering the cobalt (Co) as a by-product (Mihaylov et al., 2000; Dalvi et al., 2004). However, the Ni and Co extraction based on lixiviation is an unselective process that may result in additional discharges of by-product metals such as chromiun (Cr), iron (Fe), manganese (Mn) or zinc (Zn) (Goro-Nickel, 2001; Baroudi et al., 2003).

Although long lasting contamination exists in New Caledonia (Laganier, 1991; Ambatsian et al., 1997) with high levels of metals reported in coastal marine sediments (e.g. Fernandez et al., 2006), few data on contamination levels in marine organisms and possible local marine ecosystem impairments are available so far in the open literature

(e.g. Monniot et al., 1994; Dalto et al., 2006; Hédouin et al., 2008a,b; Metian and Warnau, 2008; Chouvelon et al., 2009). Therefore, programmes for monitoring possible impact of the land-based mining activities in the New Caledonia lagoon are needed. Such programmes should largely rely on the use of biomonitor species, as already developed and implemented in temperate areas (e.g. US and EU Mussel Watches; see e.g. Goldberg et al., 1983; Warnau and Acuña, 2007; Thébault et al., 2008). Indeed, the main advantage of the biomonitoring approach compared to direct measurement in water or sediment is to provide a direct and time-integrated assessment of the metal fraction that is actually available to the organisms (bioavailable fraction) (e.g., Phillips, 1991; Coteur et al., 2003; Danis et al., 2004; Metian et al., 2008b).

In this context, both experimental and field studies have recently identified the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* as promising candidates biomonitoring metal contamination in New Caledonia lagoon waters (Metian et al., 2005; Hédouin et al., 2006, 2007, 2008a; Chouvelon et al., 2009).

It is now well established that food is often a dominant pathway for metal bioaccumulation in marine invertebrates and that food composition and/or quantity can strongly influence metal assimilation efficiency (AE) (e.g., Borchardt, 1983; Riisgard et al., 1987; Wang and Fisher, 1999a). Furthermore, feeding processes such as filtration rate are flexible in marine filter-feeding organisms and may be adapted according to the changes in environmental conditions such as food quantity and/or composition (e.g., Widdows and Donkin, 1992; Navarro and Iglesias, 1993). For example, Cd assimilation in the mussel *Mytilus edulis* is inversely related to food quantity (Borchardt, 1983). In the scallop *Pecten maximus*, food is the main bioaccumulation pathway for Ag (~98%) when

95 diet is composed of Bacillariophyceae phytoplankton whereas dietary contribution drops
96 below 40% when the scallop is fed Prymnesiophyceae phytoplankton (Metian et al.,
97 2008a). Furthermore, heterorhabdic bivalves (those which gills are composed of 2
98 different filament types) are also able to select the particles that they are ingesting (Ward
99 et al., 1998), which results in a preferential ingestion of nutritionally-rich particles that
100 may also affect metal influx from food (e.g., Bayne, 1993; Wang and Fisher, 1997).
101 The objective of this study was thus to investigate the possible influence of food
102 quality (i.e. phytoplankton species) and quantity on the assimilation efficiency of three
103 metals of concern in New Caledonia lagoon waters (Co, Mn and Zn) in the oyster
104 *Isognomon isognomon* and the clam *Gafrarium tumidum*. The variations in the feeding
105 conditions that were considered are: (1) the phytoplankton species used as food, (2) the
106 phytoplankton density and (3) the metal concentration associated with phytoplankton.
107 Radiotracer techniques were used to enhance the detection sensitivity of metals and to
108 allow for measuring metal flux at environmentally realistic contaminant concentrations
109 (Warnau and Bustamante, 2007).
110
111 **2. Materials and Methods**
112
113 *2.1. Collection and Acclimation*
114
115 The organisms (n = 100 per species) were collected by SCUBA diving in Maa
116 Bay (oysters *Isognomon isognomon*) or by hand-picking in Dumbea Bay (clams
117 *Gafrarium tumidum*) in October 2003. Both locations are located 15 to 20 km north of

Nouméa City, New Caledonia. Body size is known to affect bioaccumulation of metals in marine organisms (e.g., Boyden, 1974; Warnau et al., 1995); hence, according to previous preliminary studies (Metian, 2003; Hédouin et al., 2006, 2008a), only individuals with a shell longer than 70 mm (*I. isognomon*) or a shell wider than 35 mm (*G. tumidum*) were used in the experiments. After collection, clams and oysters clams were shipped to IAEA-MEL premises in Monaco, where they were acclimated for 2 months to laboratory conditions (open circuit aquarium; water renewal: 30% hr^{-1}; salinity: 36 p.s.u.; temperature T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) simulating the conditions prevailing in the New Caledonia lagoon. During acclimation, bivalves were fed phytoplankton using the Prymnesiophyceae *Isochrysis galbana* (10^4 cells ml^{-1}). Recorded mortality was lower than 5% over the acclimation period.

2.2. Radiotracers and Counting

Investigated elements (Co, Mn and Zn) were introduced into the experimental microcosms as radiotracers of high specific activity, purchased from Amersham, UK (^{57}Co in 0.1M HCl, $T_{½}$ = 271.8 d) and Isotope Product Lab., USA (^{54}Mn in 0.1M HCl, $T_{½}$ = 312.2 d; ^{65}Zn in 0.5M HCl; $T_{½}$ = 243.9 d).

Radioactivity was measured using a high-resolution γ-spectrometer system composed of three Germanium -N or P type- detectors (EGNC 33-195-R, Eurysis®) connected to a multi-channel analyzer 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 of appropriate geometry.

Measurements were corrected for counting efficiency, background and physical radioactive decay. Counting times were adapted to obtain counting rates with propagated errors less than 5% (Rodriguez y Baena et al., 2006a).

2.3. Experimental Procedures

2.3.1. Testing the influence of Co concentration in food

Isochrysis galbana cells from an axenic stock culture were resuspended into 4 erlenmeyer flasks (light/dark cycle: 12 hrs/12 hrs at 25°C). Each flask contained 500 ml sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si (Guillard, 1975). Flasks were spiked with 4 increasing Co concentrations (0, 5, 50, 500 ng l^{-1}) and phytoplankton was allowed to grow under these conditions for 6 d. Added Co concentrations were realized using increasing amount of $Co(NO_3)_2$ (synthesis quality, Merck) and a fixed activity of the corresponding radiotracer ^{57}Co (2.5 kBq l^{-1}, corresponding to 0.13 ng Co l^{-1}). The range of concentrations selected covers those encountered in the New Caledonia lagoon waters (Fernandez et al., 2002; Goro-Nickel, 2004). After 6 d of incubation, cell density increased from 10^3 to 1.5×10^6 cell ml^{-1}. The cells were gently filtered (1 µm-mesh size, Nuclepore® Polycarbonate filters) and resuspended in clean seawater. The radioactivity of the radiolabelled *I. galbana* in each flask was γ-counted before and after the filtration. The radioactivity of algal cells used in feeding experiments was not significantly different among the different flasks, with an average calculated activity of 0.49 ± 0.14 µBq $cell^{-1}$.

For each added Co concentration, 4 groups of 9 oysters (shell length from 71 to 94 mm) and 4 groups of 9 clams (shell width from 35 to 40 mm) were placed in 4 aquaria containing 16 l of 0.45-µm filtered natural seawater (close circuit aquaria constantly aerated; other parameters as previously described). Oysters were acclimated for one week to these conditions and seawater was renewed daily. Bivalves from each aquarium were then allowed to feed for 2 hrs on one out of the 4 batches of previously radiolabelled *Isochrysis galbana* (10^4 cell ml^{-1}) (pulse-chase feeding method; see e.g. Warnau et al., 1996b).

Empty shells were placed as control in each aquarium to check for any direct uptake of radiotracers from seawater due to possible recycling from phytoplankton cells during the 2-hr feeding period (Metian et al., 2007). These control shells were radioanalysed at regular intervals of time.

At the end of the feeding period, all organisms were γ-counted and open circuit conditions were restored (water renewal rate: 30% hr^{-1}; salinity: 36 p.s.u.; T° = 25 ± 0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). From that time on, all individuals were γ-counted at different time intervals over a 25-d period in order to determine the whole-body depuration kinetics of the radiotracers ingested with food. Throughout the depuration period, bivalves were fed daily for 1 hr non-radiolabelled phytoplankton (*Isochrysis galbana*, 10^4 cell ml^{-1}).

2.3.2. Testing the influence of phytoplankton species

186 Two batches of 9 oysters (shell length from 73 to 90 mm) and 2 batches of 9
187 clams (shell width from 35 to 44 mm) were placed in 2 aquaria containing 16 l of 0.45-
188 µm filtered natural seawater (close circuit aquaria constantly aerated; other parameters as
189 previously described). Clams and oysters were acclimated to these conditions for 1 week
190 (daily seawater renewal) and then fed either radiolabelled *Emiliania huxleyi* or
191 *Heterocapsa triquetra* (10^4 cell ml^{-1}) for 2 hrs (pulse-chase feeding) in order to asses the
192 possible influence of the phytoplankton species on metal assimilation efficiency and
193 retention capacity in the bivalves. Both phytoplankton species occur naturally in several
194 bays of the New Caledonia lagoon where the clams and oysters are living (Jacquet et al.,
195 2006).
196 For radiolabelling phytoplankton species, experimental approaches conducted on
197 *I. galbana* were applied to the Prymnesiophyceae *E. huxleyi* and to the Dinophyceae *H.*
198 *triquetra*. Cells from axenic stock cultures were re-suspended in two different erlenmeyer
199 flasks (10^3 cell ml^{-1}), containing 4.5 l sterile-filtered seawater enriched with f/50 for *E.*
200 *huxleyi* and enriched with f/2 nutrients without EDTA and Si for *H. triquetra* (Guillard,
201 1975). The two cultures were spiked with 5 kBq l^{-1} of ^{54}Mn, ^{57}Co and ^{65}Zn,
202 corresponding to 3.6 ng Mn l^{-1}, 25 ng Co l^{-1} and 60 ng Zn l^{-1}. The cultures were then
203 incubated for 6 d at 25°C (light/dark cycle: 12 hrs/12 hrs). After incubation, the cell
204 densities were 7×10^5 cell ml^{-1} for *E. huxleyi* and 1.6×10^5 cell ml^{-1} for *H. triquetra*. The
205 cells were then gently filtered, resuspended in clean seawater and γ-counted as described
206 above (section 2.3.1.). The radioactivity of algal cells used in the feeding experiments
207 was 0.26 ± 0.18 µBq $cell^{-1}$ for *E. huxleyi* and 0.96 ± 0.11 µBq $cell^{-1}$ for *H. triquetra* for

208 ^{54}Mn, 2.1 ± 0.8 and 20.8 ± 12.1 µBq cell^{-1} for ^{57}Co and 3.2 ± 1.3 and 3.3 ± 0.1 µBq cell^{-1}
209 for ^{65}Zn, respectively.

210 Empty bivalve shells were used as controls for possible metal recycling and
211 whole-body depuration kinetics of radiotracer ingested with the food were determined in
212 both bivalve species as described in section 2.3.1.

213

214 2.3.3. Testing the influence of cellular density

215

216 Three groups of 9 oysters (shell length from 71 to 92 mm) and 3 groups of 9
217 clams (shell width from 36 to 45 mm) were placed in 3 aquaria containing 16 l of 0.45-
218 µm filtered natural seawater (close circuit aquaria constantly aerated; other parameters as
219 previously described), and acclimated for one week (daily seawater renewal) during
220 which time their food was prepared.

221 To do this, cells of *I. galbana* from an axenic stock culture were resuspended in an
222 erlenmeyer flask containing 4.5 l sterile-filtered seawater enriched with f/2 nutrients
223 without EDTA and Si. The culture was then spiked with 5 kBq l^{-1} of ^{54}Mn, ^{57}Co and ^{65}Zn
224 and incubated for 6 d at 25°C (light/dark cycle: 12 hrs/12 hrs). After incubation, the cell
225 density had increased from 10^3 to 1.4 x 10^6 cell ml^{-1}. Three sub-samples of 58, 115 and
226 580 ml of the culture were then gently filtered and resuspended in clean seawater. These
227 3 batches were prepared to obtain final cell density of 5 x 10^3, 10^4 and 5 x 10^4 cell ml^{-1} in
228 the 16-l exposure aquaria. The radioactivity of the radiolabelled *I. galbana* was measured
229 before and after the cellular filtration. The radioactivity of algal cells ranged from 1.11 to
230 1.80 µBq cell^{-1} for ^{54}Mn, 0.83 to 1.37 µBq cell^{-1} for ^{57}Co, 2.69 to 4.38 µBq cell^{-1} for ^{65}Zn.

Each group of clams and oysters was then fed for 2 hrs one of the radiolabelled *I. galbana* batches (5×10^3, 10^4 or 5×10^4 cell ml^{-1}). Whole-body depuration kinetics of the radiotracers ingested with the food were then followed as described in section 2.3.1 and controls (empty shells) were placed in the aquaria for assessing possible radiotracer recycling.

2.4. Data Analysis

Depuration of the radiotracers was expressed as the percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in the organisms just after the feeding period * 100) (Warnau et al., 1996b; Rodriguez y Baena et al., 2006b).

Depuration kinetics for all experiments were fitted using kinetic models and statistical methods as described by Warnau et al. (1996a,b) and Lacoue-Labarthe et al. (2008). Depuration kinetics were always best fitted by a double-component exponential equation (decision based on F test and ANOVA tables for two fitted model objects):

$$A_t = A_{0s}\, e^{-k_{es}\, t} + A_{0l}\, e^{-k_{el}\, t}$$

where k_e is the depuration rate constant (d^{-1}), A_t and A_0 are the remaining activities (%) at time *t* (d) and 0, respectively, and 's' and 'l' are the subscripts for the short-lived and long-lived components. The short-lived component represents the loss of the radiotracer fraction that remains associated with the faeces and is rapidly eliminated with them, whereas the long-lived component describes the loss of the radiotracer fraction that is actually absorbed by the organism and slowly eliminated (Whicker and Schultz, 1982;

Warnau et al., 1996b). The long-lived component allows assessing the assimilation efficiency (AE) of the radiotracer ingested with food (AE = A_{0l}). Also, for each exponential component (s and l), a biological half-life can be calculated ($T_{b½s}$ and $T_{b½l}$) from the corresponding depuration rate constant (k_{es} and k_{el}) according to the relation $T_{b½} = \ln 2 / k_e$.

Constants of the models and their statistics were estimated by iterative adjustments of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica® 5.2.1 software. Differences among the estimated kinetic parameters for the different feeding conditions were tested using comparison tests of the means and possible trends linking metal concentrations to cell densities were assessed using simple linear regression techniques (Zar, 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

Depuration kinetics of the radiotracers were followed in the organisms which ingested enough food to display sufficient radioactivity to be accurately counted. Most oysters met this requirement; however some clams displaying very low activities were discarded. No activity was detected on control shells, indicating that no detectable recycling of phytoplankton-associated tracers occurred in the experimental microcosms.

3.1. Effect of Co Concentration in Phytoplankton

277 Fitting of the whole-body depuration kinetics of ^{57}Co in oysters fed Co-loaded *I.*
278 *galbana* by a double-exponential model was quite satisfactory (R^2: 0.86-0.90) for all the
279 food-associated Co concentrations tested (Table 1, Fig. 1). The major fraction (80-85%)
280 of the total radioactivity in oysters was rapidly lost ($T_{b½s}$ < 1d) whereas the long-lived
281 component accounted for only 15-20% of the ^{57}Co ingested with food that was eliminated
282 with a biological half life ($T_{b½l}$) ranging from 13 to 25 d.
283 Similarly, the fit of the whole-body depuration of ^{57}Co in clams was quite good
284 (R^2: 0.27-0.64) for all the Co concentrations tested (Table 1, Fig. 1). However, the
285 estimated AE of ^{57}Co ingested with food was much higher than in oysters (i.e., 76-84%)
286 and this fraction was retained with a $T_{b½}$ ranging from 36 to 39 d.
287 In both bivalve species, no significant difference (p always > 0.05) was found
288 among the estimated kinetic parameters (A_{os}, k_{es}, A_{ol}, k_{el}) determined for the 4 different
289 food-associated Co concentrations.
290
291 *3.2. Effect of Phytoplankton Species*
292
293 In oysters, depuration kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn ingested with *I. galbana*
294 (ISO), *E. huxleyi* (EMI) or *H. triquetra* (HET) (10^4 cell ml^{-1}) were best described by a
295 double-exponential model (R^2: 0.23- 0.63 for ISO, 0.11-0.83 for EMI and 0.57-0.92 for
296 HET) (Table 2, Fig. 2). No significant difference was found among estimated $T_{b½}$ for all
297 radiotracers and all phytoplankton species tested. In addition, no significant difference
298 was found between AEs for Co and Mn in oysters fed EMI and HET, and for Zn in
299 oysters fed ISO and EMI. In contrast, significant differences (p < 0.02) among AEs were

observed for Co and Mn (ISO > HET = EMI, $p < 0.004$) and fro Zn (ISO = EMI > HET, $p < 0.02$).

In clams, fitting of the whole-body depuration of the radiotracers ingested with *I. galbana*, *E. huxleyi or H. triquetra* (10^4 cell ml^{-1}) were genrally somewhat better than in the oyster (R^2: 0.47-0.98 for ISO, 0.47-0.93 for EMI and 0.61-0.89 for HET) (Table 2, Fig. 2). $T_{b½}$ of ^{54}Mn was significantly longer when it was assimilated from HET than from EMI or ISO ($p = 0.03$ and 0.004, respectively). Significant differences were also observed among AEs calculated for Co, Mn and Zn ingested with the three phytoplankton strains (Mn: ISO < EMI = HET, $p < 0.03$; Co: ISO = EMI > HET, $p < 0.0004$; and Zn: ISO ≥ EMI ≥ HET, $p = 0.04$).

3.3. Effect of Cellular Density

When oysters were fed 10^4 and 5×10^4 cells ml^{-1} of radiolabelled *I. galbana*, whole-body depuration kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn were fitted with R^2 ranging from 0.23 to 0.63 and 0.38 to 0.60, respectively (Table 2, Fig. 3). No significant difference in $T_{b½}$ and AE between cell densities was found for Co. In contrast, significant differences in AE were found for Mn and Zn, with higher AE calculated at the low cell density ($p = 0.001$ and 0.0003, respectively).

For clams, examination depuration kinetics of the radiotracers (R^2: 0.33-0.65 at 5×10^3 cell ml^{-1} and 0.47-0.98 at 10^4 cell ml^{-1}) indicated that $T_{b½}$ was not significantly different between the two food densities for all three radiotracers (Table 2, Fig. 3).

However, when fed the low cell density, clams incorporated Co, Mn and Zn with significantly higher AE ($p = 0.003$, 0.047 and 0.0003, respectively).

4. Discussion

During the last two decades, dietary pathway has been increasingly recognized as a major source of contaminant accumulation in marine invertebrates (e.g. Wang et al., 1996; Reinfelder et al., 1998; Wang and Fisher, 1999b). The assimilation efficiency (AE) and retention time ($T_{b½}$) are the critical parameters in assessing and modelling the dietary uptake of contaminants and numerous studies have been devoted to assess these parameters in different marine organisms (e.g., Wang et al., 1996; Warnau et al., 1996b, 1999; Pan and Wang, 2008). However, as almost a rule in tropical environments (e.g., Phillips, 1991; Chong and Wang, 2000; Metian et al., 2005), only very few data are yet available regarding AE and $T_{b½}$ parameters for organisms from New Caledonia (e.g. Hédouin et al., 2006, 2007).

Ideally, the concentrations of metals in the tissues of a biomonitor species should reflect those occurring in the ambient environment. This essential criterion has been previously experimentally assessed for *I. isognomon* and *G. tumidum* for the dissolved pathway (Hédouin et al., 2007, *this issue*). Exposures to a range of dissolved concentrations of As, Cr, Co, Cd, Mn, Ni and Zn indicated that, over a realistic range of concentrations, these elements were generally bioconcentrated in direct proportion to their concentration in seawater (*ibid.*). The results presented here are complementary with

344 these previous studies as they expand the available knowledge regarding metal
345 accumulation in *I. isognomon* and *G. tumidum* to the dietary pathway.

346 When ingested with phytoplankton previously exposed to a range of increasing
347 Co concentration (up to 500 ng added Co l^{-1}) Co was shown to be assimilated in the same
348 proportion (AE) and retained with similar relative strength ($T_{b½}$) whatever the food-
349 associated Co concentration was (see Fig.1 and Table1). The experimental conditions
350 were designed to cover the whole range of Co concentrations that can be encountered in
351 New Caledonia waters, from pristine up to extremely contaminated areas (Fernandez et
352 al., 2002; Goro-Nickel, 2004). Similar trends have been previously reported by Chong
353 and Wang (2000) who observed that concentration of Cd, Cr and Zn in sediment had little
354 effect on the assimilation efficiency of sediment-bound metals in the green mussel *Perna*
355 *viridis* and in the Manila clam *Ruditapes philippinarum*. However, the response to metal
356 concentration variation in ingested food appears to depend on the element as well as on
357 the species investigated. Indeed, whereas AE of Se in the mussel *Mytilus edulis* was not
358 affected by the Se concentration in the ingested diatoms (*Thalassiosira pseudonana*), AE
359 of Zn and Cd did respectively decrease and increased with increasing contamination of
360 the diatom used as food (Wang and Fisher, 1996). Nevertheless, along with data obtained
361 from exposures to increasing dissolved Co concentrations in the same species (Hédouin
362 et al., *this issue*), the present results on Co AE indicate that in the field Co concentrations
363 in both *I. isognomon* and *G. tumidum* would be reflecting the level of Co in their
364 environment, both in the dissolved and particulate phases.

365 Whereas food quality and quantity were shown to have limited influence on the
366 retention time of Co, Mn and Zn in clam and oyster tissues, metal AE generally differed

367 according to the feeding conditions. Metals were generally better assimilated when
368 bivalves were fed *I. galbana* than the two other phytoplankton species (*E. huxleyi or H.*
369 *triquetra*). *I. galbana* cells have comparable cell length (c.l.: 4-6 µm) and cell width
370 (c.w.: 2-4 µm) than *E. huxleyi* cells (c.l.: 3-4 µm; c.w.: 3-4 µm), but are much smaller
371 than *H. triquetra* cells (c.l.: 20-28 µm; c.w.: 14-18 µm). Hence, the differences and
372 similarities in AE observed among the feeding conditions indicate that phytoplankton size
373 would not be the major factor driving metal AEs in the two bivalves. Bivalves are able to
374 feed selectively on particles of different size and of different nature (various
375 phytoplankton species as well as inorganic particles) (e.g., Newell et al., 1989) and
376 species-related selectivity and/or dietary preferences could at least partly explain the
377 specific differences observed in AE. Alternatively or complementarily, specific
378 difference in metal speciation such as storage of the metal under bioavailable forms in the
379 cytoplasm of the phytoplankton cells (e.g., Reinfelder and Fisher, 1991; Wang et al.,
380 1996; Metian et al., 2008a) could also explain the AE differences that were observed.
381 Food availability is another key factor that is well known to influence feeding
382 behaviour of filter-feeding bivalves (e.g. Bayne et al., 1987; Bayne, 1993; Pan and Wang,
383 2008). Generally, filter-feeders can adjust their filtration rate to ambient phytoplankton
384 density and thereby are able to maintain a stable ingestion rate even at high food
385 concentrations (Jin et al., 1996; Dong et al., 2000; Zhuang and Wang, 2004). Although no
386 conclusion on the influence of this adaptive feeding behaviour could be directly drawn
387 from our results, it is clear that food availability notably influenced the AE of the metals
388 examined in *I. isognomon* and *G. tumidum*.

389 It is nowadays well documented that the dietary pathway is an important
390 contributor to the global bioaccumulation of metals in marine organisms (e.g. Wang and
391 Fisher, 1997; Metian et al., 2008a). Since the present study has shown that the feeding
392 behaviour of *I. isognomon* and *G. tumidum* is influenced by the feeding conditions
393 (quality and/or quantity of food), it is strongly recommended that future studies take into
394 account these parameters so as to refine the prediction of biodynamic models (e.g.,
395 Thomann et al., 1995; Metian et al, 2008a; Pan and Wang, 2008). The consideration of
396 such data is also needed to explain bioaccumulation data obtained in the framework of
397 biomonitoring programmes. For example, Bendell-Young and Arifin (2004)
398 demonstrated the influence of mussel feeding behaviour on their predicted tissue
399 concentrations in Cd, especially under conditions of highly variable quantity and quality
400 of suspended particles.
401 In conclusion, our experimental results suggest that food quality (phytoplankton
402 composition) and quantity (cell density) may play a significant role in the assimilation of
403 metals ingested with food in *I. isognomon* and *G. tumidum*. Because of the major
404 importance of the dietary contribution to global metal bioaccumulation in marine
405 organisms, it is thus recommended to pay great attention to factors influencing AE. This
406 would help refining both bioaccumulation model predictions and interpretation of data
407 from field surveys and biomonitoring programmes.
408
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410

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420

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638 **Captions to Figures**

639

640 **Figure 1.** Influence of phytoplankton-associated Co concentrations on whole-body
641 depuration kinetics of ^{57}Co in the oyster *Isognomon isognomon* and the clam *Gafrarium*
642 *tumidum* fed radiolabelled *Isochrysis galbana* (10^4 cells ml^{-1}). A(%): remaining activity
643 (%) ± SD (n = 9 oysters; n = 6 clams for 0 and 5 ng l^{-1} and n = 8 clams for 50 and 500 ng
644 l^{-1}).

645

646 **Figure 2.** Influence of phytoplankton species (*Isochrysis galbana*, *Emiliania huxleyi* and
647 *Heterocapsa triquetra*; 10^4 cells ml^{-1}) used as food on whole-body depuration kinetics of
648 ^{54}Mn, ^{57}Co and ^{65}Zn in the oyster *Isognomon isognomon* (n = 9 for *I. galbana* and 8 for *E.*
649 *huxleyi* and *H. triquetra*) and the clam *Gafrarium tumidum* (n = 8 for *I. galbana* and 7 for
650 *E. huxleyi* and *H. triquetra*). A(%): remaining activity (%) ± SD.

651

652 **Figure 3.** Influence of phytoplankton cell density (5×10^3, 10^4 or 5×10^4 cells ml^{-1}) on
653 whole-body depuration kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn in the oyster *Isognomon*
654 *isognomon* (n = 9 for 10^4 cells ml^{-1} and 5×10^4 cells ml^{-1}) and the clam *Gafrarium*
655 *tumidum* (n = 6 for 5×10^3 cells ml^{-1} and n = 8 for 10^4 cells ml^{-1}) fed radiolabelled
656 *Isochrysis galbana*. A(%): remaining activity (%) ± SD.

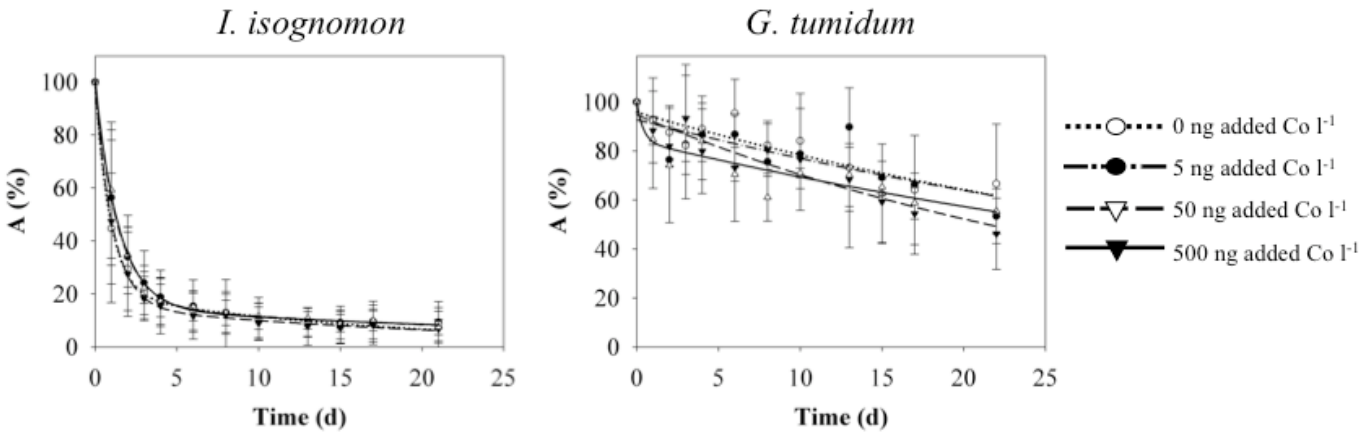
I. isognomon
G. tumidum
A (%)
Time (d)
0 ng added Co l-1
5 ng added Co l-1
50 ng added Co l-1
500 ng added Co l-1

Fig. 1.

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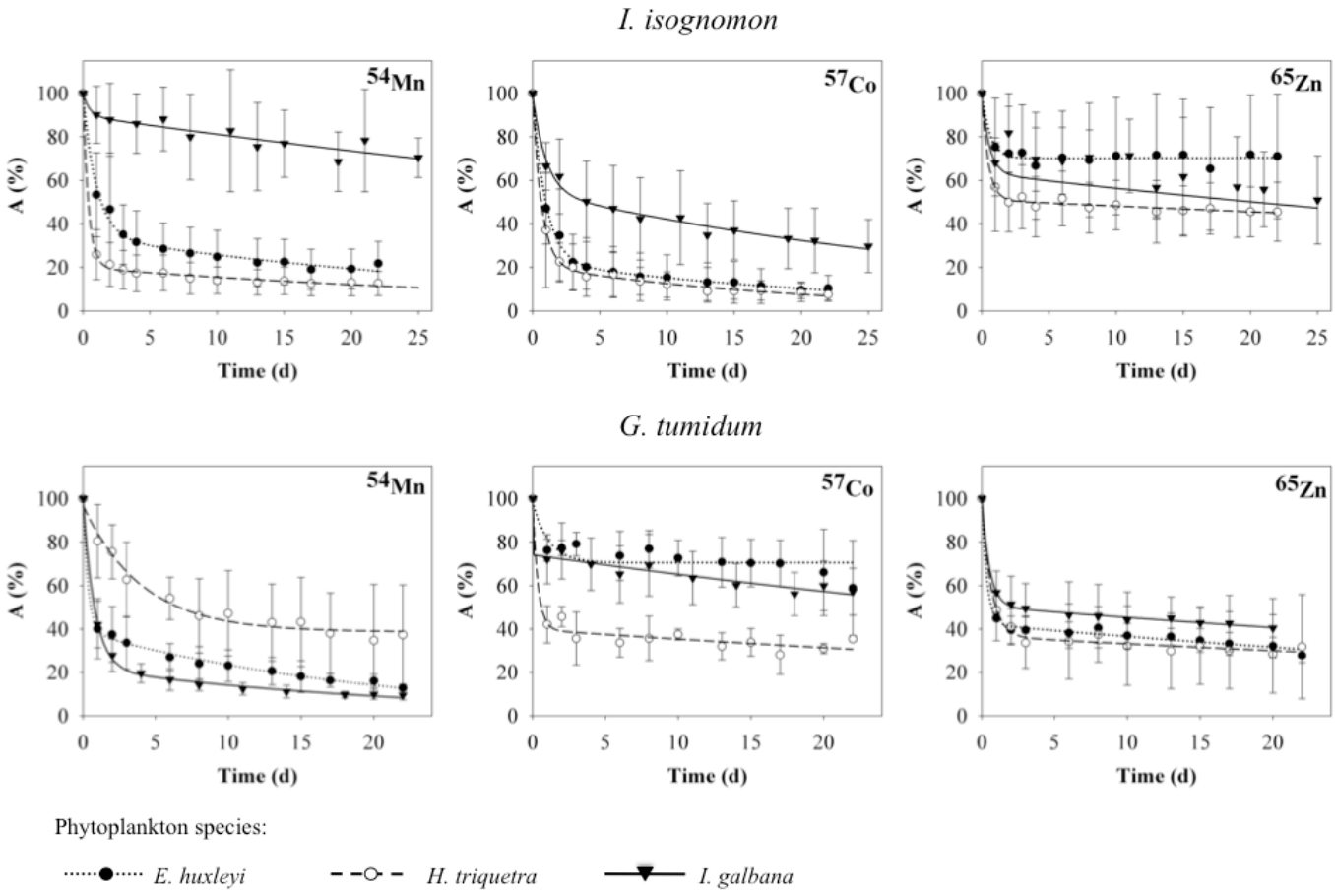

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675 Fig. 2.

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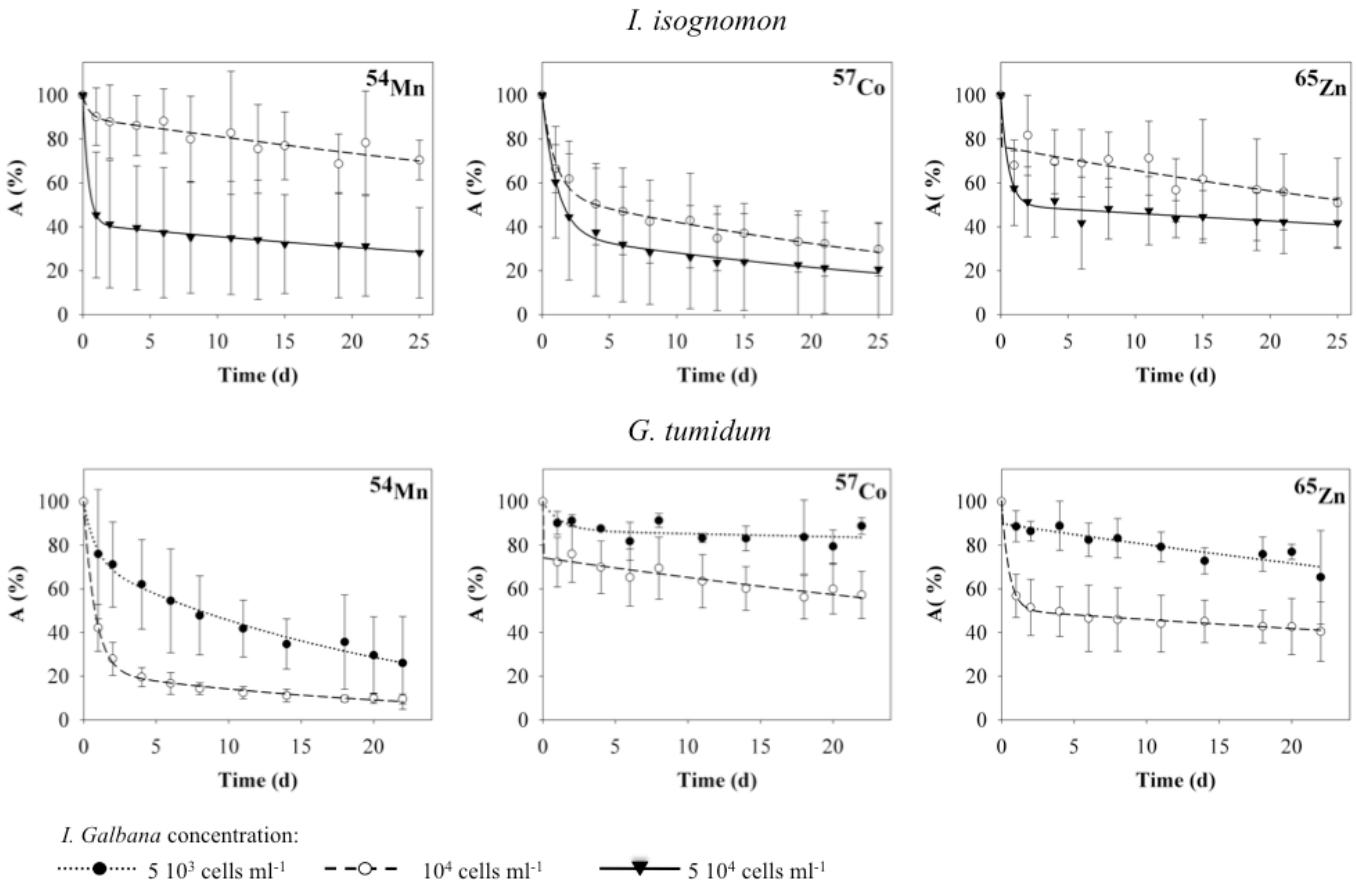

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682 Fig. 3

Table 1. Assimilation efficiency (AE, %), depuration rate constant (k_{el}, d^{-1}) and biological half-life ($T_{b½l}$, d) of ^{57}Co in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* fed radiolabelled *Isochrysis galbana* (10^4 cell ml^{-1}) previously exposed to four increasing Co concentrations (n = 9 oysters per concentration tested, n = 6 clams for 0 and 5 ng l^{-1} and n = 8 clams for 50 and 500 ng l^{-1}). ASE: asymptotic standard error; R^2: determination coefficient.

Species	**Co concentration added** (ng l^{-1})	**AE ± ASE**	**k_{el} ± ASE**	**$T_{b½l}$ ± ASE**	**R^2**
I. isognomon	0	15.8 ± 7.0 [a]	0.032 ± 0.036*	22 ± 24*	0.87
	5	19.6 ± 5.4 [c]	0.054 ± 0.028*	13 ± 7*	0.88
	50	16.6 ± 6.2 [b]	0.050 ± 0.036*	14 ± 10*	0.86
	500	14.7 ± 6.0 [a]	0.027 ± 0.033*	25 ± 30*	0.90
G. tumidum	0	77.2 ± 3.9 [d]	0.018 ± 0.006 [b]	37. ± 11 [b]	0.64
	5	77.4 ± 3.9 [d]	0.019 ± 0.006 [c]	36 ± 10 [c]	0.27
	50	75.7 ± 3.9 [d]	0.018 ± 0.006 [b]	39 ± 12 [b]	0.51
	500	84.1 ± 5.9 [d]	0.019 ± 0.007 [a]	36 ± 13 [a]	0.51

Significance of the estimated parameters: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, [d] $p < 0.0001$, * not significant ($p > 0.05$)

Table 2. Assimilation efficiency (AE, %), depuration rate constant (k_{el}, d^{-1}) and biological half-life, ($T_{b½l}$, d) of ^{54}Mn, ^{57}Co and ^{65}Zn in the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* fed radiolabelled *Emiliania huxleyi* (10^4 cell ml^{-1}), *Heterocapsa triquetra* (10^4 cell ml^{-1}) and *Isochrysis galbana* (10^4 cell ml^{-1} and 5 10^4 cell ml^{-1} for *I. isognomon;* 5 10^3 cell ml^{-1} and 10^4 cell ml^{-1} for *G. tumidum*) (n = 8 oysters; n = 7 clams per phytoplankton species tested). ASE: asymptotic standard error; R^2: determination coefficient.

Species	Phytoplankton strain	Cell density (cells ml^{-1})	Isotope	AE ± ASE	k_{el} ± ASE	$T_{b½l}$ ± ASE	R^2
I. isognomon	***E. huxleyi***	10^4	^{54}Mn	34 ± 6.5 ^{d}	0.028 ± 0.015*	24 ± 13*	0.74
		10^4	^{57}Co	22 ± 5.6 ^{c}	0.039 ± 0.021*	18 ± 10*	0.83
		10^4	^{65}Zn	70 ± 6.5 ^{d}	0.0002 ± 0.007*	2783*	0.11
	H. triquetra	10^4	^{54}Mn	20 ± 2.6 ^{d}	0.025 ± 0.012 ^{a}	28 ± 13 ^{a}	0.92
		10^4	^{57}Co	21 ± 4.1 ^{d}	0.050 ± 0.020 ^{a}	14 ± 6 ^{a}	0.88
		10^4	^{65}Zn	51 ± 3.2 ^{d}	0.006 ± 0.005*	123 ± 109*	0.57
	I. galbana	10^4	^{54}Mn	90 ± 5.6 ^{d}	0.010 ± 0.005 ^{a}	70 ± 32 ^{a}	0.23
		10^4	^{57}Co	55 ± 7.1 ^{d}	0.026 ± 0.010 ^{b}	26 ± 10 ^{b}	0.63
		10^4	^{65}Zn	76 ± 4.1 ^{d}	0.015 ± 0.004 ^{b}	45 ± 13 ^{b}	0.36
		5 10^4	^{54}Mn	41 ± 7.8 ^{d}	0.015 ± 0.014*	47 ± 46*	0.38
		5 10^4	^{57}Co	37 ± 21*	0.027 ± 0.043*	26 ± 42*	0.51
		5 10^4	^{65}Zn	52 ± 3.5 ^{d}	0.010 ± 0.005^{a}	70 ± 34 ^{a}	0.60
G. tumidum	***E. huxleyi***	10^4	^{54}Mn	39 ± 4.3 ^{d}	0.051 ± 0.011^{d}	14 ± 3 ^{d}	0.92
		10^4	^{57}Co	80 ± 3.5 ^{d}	0.010 ± 0.004 ^{a}	70 ± 26 ^{a}	0.47
		10^4	^{65}Zn	42 ± 2.3 ^{d}	0.014 ± 0.004 ^{b}	48 ± 14 ^{b}	0.93
	H. triquetra	10^4	^{54}Mn	56 ± 23 ^{a}	0.021 ± 0.026*	34 ± 10*	0.61
		10^4	^{57}Co	41 ± 3.1^{d}	0.014 ± 0.006 ^{a}	49 ± 9 ^{a}	0.89
		10^4	^{65}Zn	33 ± 7.3 ^{d}	0.005 ± 0.016*	143 ± 474*	0.71
	I. galbana	10^4	^{54}Mn	22 ± 3.7 ^{d}	0.044 ± 0.015 ^{b}	16 ± 5 ^{b}	0.65
		10^4	^{57}Co	73 ± 2.7 ^{d}	0.010 ± 0.003 ^{b}	68 ± 21 ^{b}	0.33
		10^4	^{65}Zn	51 ± 3.8 ^{d}	0.013 ± 0.006 ^{a}	55 ± 25 ^{a}	0.52
		5 10^3	^{54}Mn	72 ± 17.9 ^{c}	0.046 ± 0.021^{a}	15 ± 7 ^{a}	0.98
		5 10^3	^{57}Co	87 ± 6.1^{d}	0.002 ± 0.005*	416 ± 135*	0.47
		5 10^3	^{65}Zn	90 ± 3.0 ^{d}	0.011 ± 0.003 ^{c}	61 ± 16 ^{c}	0.68

Significance of the estimated parameters: ^{a} $p < 0.05$, ^{b} $p< 0.01$, ^{c} $p< 0.001$, ^{d} $p< 0.0001$, * not significant ($p > 0.05$)