1 **Influence of food on the assimilation of selected metals in tropical bivalves from the**

2 **New Caledonia lagoon: qualitative and quantitative aspects**

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24

25   **Abstract**

27   The present study aimed at examining the influence of food quality and quantity

28   on the assimilation efficiency (AE) of metals in two abundant bivalves in the New

29   Caledonia lagoon, the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum*.

30   Bivalves were exposed via their food to the radiotracers of three metals of concern in

31   New Caledonia (^{54}Mn, ^{57}Co and ^{65}Zn) under different feeding conditions (phytoplankton

32   species, cell density, and cell-associated metal concentration). When bivalves were fed

33   *Heterocapsa triquetra, Emiliania huxleyi* and *Isochrysis galbana*, AE of Mn, Co and Zn

34   was strongly influenced by the phytoplankton species and by the metal considered. In

35   contrast, when fed one given phytoplankton species previously exposed to different

36   concentrations of Co, phytoplankton-associated Co load had no influence on the AE and

37   on the retention time of the metal in both bivalves. Metals ingested with *I. galbana*

38   displayed generally the highest AE in both bivalve species, except for Mn in clams for

39   which the highest AE was observed for *H. triquetra*. Influence of food quantity was

40   investigated by exposing bivalves to different cell densities of *I. galbana* (5×10^3, 10^4 or

41   5×10^4 cell ml^{-1}). As for food quality, food quantity was found to influence AE of Mn, Co

42   and Zn, the highest AE being observed when bivalves were fed the lowest cell density.

43   Overall, results indicate that the two bivalve species are able to adjust their feeding

44   strategies according to the food conditions prevailing in their environment.

46   **Keywords**: Mollusks, *Isognomon isognomon*, *Gafrarium tumidum,* New Caledonia,

47   Radiotracer, Feeding

49

1. Introduction

51

52   Changes in coastal ecosystem functioning due to anthropogenic metal inputs is a

53 worldwide issue of concern especially as metals are not biodegradable and enter

54 biogeochemical cycles (Tessier and Turner, 1995). In the coral reef lagoon of New-

55 Caledonia, metal contamination is a critical problem in relation with its extreme

56 biodiversity (Labrosse et al., 2000). Indeed, the lagoon is subject to an increasing

57 environmental pressure imposed by urban development and intensive mining activities. In

58 addition, the use of hydrometallurgic process employing heated and pressured sulphuric

59 acid (lixiviation) has been recently developed in New Caledonia and is expected to be

60 implemented at industrial scale early 2010 (Goro-Nickel, 2001, 2003). Such a process

61 will provide new potential to exploit laterite soils that display lower nickel (Ni) contents

62 than garnierite ores currently used in pyrometallurgic plants, such as at the Société Le

63 Nickel, and will allow recovering the cobalt (Co) as a by-product (Mihaylov et al., 2000;

64 Dalvi et al., 2004). However, the Ni and Co extraction based on lixiviation is an

65 unselective process that may result in additional discharges of by-product metals such as

66 chromiun (Cr), iron (Fe), manganese (Mn) or zinc (Zn) (Goro-Nickel, 2001; Baroudi et

67 al., 2003).

68   Although long lasting contamination exists in New Caledonia (Laganier, 1991;

69 Ambatsian et al., 1997) with high levels of metals reported in coastal marine sediments

70 (e.g. Fernandez et al., 2006), few data on contamination levels in marine organisms and

71 possible local marine ecosystem impairments are available so far in the open literature

72   (e.g. Monniot et al., 1994; Dalto et al., 2006; Hédouin et al., 2008a,b; Metian and

73   Warnau, 2008; Chouvelon et al., 2009). Therefore, programmes for monitoring possible

74   impact of the land-based mining activities in the New Caledonia lagoon are needed. Such

75   programmes should largely rely on the use of biomonitor species, as already developed

76   and implemented in temperate areas (e.g. US and EU Mussel Watches; see e.g. Goldberg

77   et al., 1983; Warnau and Acuña, 2007; Thébault et al., 2008). Indeed, the main advantage

78   of the biomonitoring approach compared to direct measurement in water or sediment is to

79   provide a direct and time-integrated assessment of the metal fraction that is actually

80   available to the organisms (bioavailable fraction) (e.g., Phillips, 1991; Coteur et al., 2003;

81   Danis et al., 2004; Metian et al., 2008b).

82   In this context, both experimental and field studies have recently identified the

83   oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* as promising candidates

84   biomonitoring metal contamination in New Caledonia lagoon waters (Metian et al., 2005;

85   Hédouin et al., 2006, 2007, 2008a; Chouvelon et al., 2009).

86   It is now well established that food is often a dominant pathway for metal

87   bioaccumulation in marine invertebrates and that food composition and/or quantity can

88   strongly influence metal assimilation efficiency (AE) (e.g., Borchardt, 1983; Riisgard et

89   al., 1987; Wang and Fisher, 1999a). Furthermore, feeding processes such as filtration rate

90   are flexible in marine filter-feeding organisms and may be adapted according to the

91   changes in environmental conditions such as food quantity and/or composition (e.g.,

92   Widdows and Donkin, 1992; Navarro and Iglesias, 1993). For example, Cd assimilation

93   in the mussel *Mytilus edulis* is inversely related to food quantity (Borchardt, 1983). In the

94   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

118   Nouméa City, New Caledonia. Body size is known to affect bioaccumulation of metals in

119   marine organisms (e.g., Boyden, 1974; Warnau et al., 1995); hence, according to previous

120   preliminary studies (Metian, 2003; Hédouin et al., 2006, 2008a), only individuals with a

121   shell longer than 70 mm (*I. isognomon*) or a shell wider than 35 mm (*G. tumidum*) were

122   used in the experiments. After collection, clams and oysters clams were shipped to IAEA-

123   MEL premises in Monaco, where they were acclimated for 2 months to laboratory

124   conditions (open circuit aquarium; water renewal: 30% hr^{-1}; salinity: 36 p.s.u.;

125   temperature $T° = 25 \pm 0.5°C$; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs) simulating

126   the conditions prevailing in the New Caledonia lagoon. During acclimation, bivalves

127   were fed phytoplankton using the Prymnesiophyceae *Isochrysis galbana* (10^4 cells ml^{-1}).

128   Recorded mortality was lower than 5% over the acclimation period.

129

130   *2.2. Radiotracers and Counting*

131

132   Investigated elements (Co, Mn and Zn) were introduced into the experimental

133   microcosms as radiotracers of high specific activity, purchased from Amersham, UK

134   (^{57}Co in 0.1M HCl, $T_{1/2}$ = 271.8 d) and Isotope Product Lab., USA (^{54}Mn in 0.1M HCl, $T_{1/2}$

135   = 312.2 d; ^{65}Zn in 0.5M HCl; $T_{1/2}$ = 243.9 d).

136   Radioactivity was measured using a high-resolution γ-spectrometer system

137   composed of three Germanium -N or P type- detectors (EGNC 33-195-R, Eurysis[®])

138   connected to a multi-channel analyzer and a computer equipped with a spectra analysis

139   software (Interwinner[®] 6). The radioactivity of the samples was determined by

140   comparison with standards of known activities and of appropriate geometry.

141   Measurements were corrected for counting efficiency, background and physical

142   radioactive decay. Counting times were adapted to obtain counting rates with propagated

143   errors less than 5% (Rodriguez y Baena et al., 2006a).

144

145   *2.3. Experimental Procedures*

146

147   2.3.1. Testing the influence of Co concentration in food

148

149   *Isochrysis galbana* cells from an axenic stock culture were resuspended into 4

150   erlenmeyer flasks (light/dark cycle: 12 hrs/12 hrs at 25°C). Each flask contained 500 ml

151   sterile-filtered seawater enriched with f/2 nutrients without EDTA and Si (Guillard,

152   1975). Flasks were spiked with 4 increasing Co concentrations (0, 5, 50, 500 ng l^{-1}) and

153   phytoplankton was allowed to grow under these conditions for 6 d. Added Co

154   concentrations were realized using increasing amount of $Co(NO_3)_2$ (synthesis quality,

155   Merck) and a fixed activity of the corresponding radiotracer ^{57}Co (2.5 kBq l^{-1},

156   corresponding to 0.13 ng Co l^{-1}). The range of concentrations selected covers those

157   encountered in the New Caledonia lagoon waters (Fernandez et al., 2002; Goro-Nickel,

158   2004). After 6 d of incubation, cell density increased from 10^3 to 1.5 x 10^6 cell ml^{-1}. The

159   cells were gently filtered (1 µm-mesh size, Nuclepore® Polycarbonate filters) and

160   resuspended in clean seawater. The radioactivity of the radiolabelled *I. galbana* in each

161   flask was γ-counted before and after the filtration. The radioactivity of algal cells used in

162   feeding experiments was not significantly different among the different flasks, with an

163   average calculated activity of 0.49 ± 0.14 µBq $cell^{-1}$.

164   For each added Co concentration, 4 groups of 9 oysters (shell length from 71 to 94

165   mm) and 4 groups of 9 clams (shell width from 35 to 40 mm) were placed in 4 aquaria

166   containing 16 l of 0.45-µm filtered natural seawater (close circuit aquaria constantly

167   aerated; other parameters as previously described). Oysters were acclimated for one week

168   to these conditions and seawater was renewed daily. Bivalves from each aquarium were

169   then allowed to feed for 2 hrs on one out of the 4 batches of previously radiolabelled

170   *Isochrysis galbana* (10^4 cell ml^{-1}) (pulse-chase feeding method; see e.g. Warnau et al.,

171   1996b).

172   Empty shells were placed as control in each aquarium to check for any direct

173   uptake of radiotracers from seawater due to possible recycling from phytoplankton cells

174   during the 2-hr feeding period (Metian et al., 2007). These control shells were

175   radioanalysed at regular intervals of time.

176   At the end of the feeding period, all organisms were γ-counted and open circuit

177   conditions were restored (water renewal rate: 30% hr^{-1}; salinity: 36 p.s.u.; T° = 25 ±

178   0.5°C; pH = 8.0 ± 0.1; light/dark cycle: 12 hrs/12 hrs). From that time on, all individuals

179   were γ-counted at different time intervals over a 25-d period in order to determine the

180   whole-body depuration kinetics of the radiotracers ingested with food. Throughout the

181   depuration period, bivalves were fed daily for 1 hr non-radiolabelled phytoplankton

182   (*Isochrysis galbana*, 10^4 cell ml^{-1}).

183

184   2.3.2. Testing the influence of phytoplankton species

185

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 x 10^5 cell ml^{-1} for *E. huxleyi* and 1.6 x 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.

231   Each group of clams and oysters was then fed for 2 hrs one of the radiolabelled *I.*

232   *galbana* batches (5 x 10^3, 10^4 or 5 x 10^4 cell ml^{-1}). Whole-body depuration kinetics of the

233   radiotracers ingested with the food were then followed as described in section 2.3.1 and

234   controls (empty shells) were placed in the aquaria for assessing possible radiotracer

235   recycling.

236

237   *2.4. Data Analysis*

238

239   Depuration of the radiotracers was expressed as the percentage of remaining

240   radioactivity (radioactivity at time t divided by initial radioactivity measured in the

241   organisms just after the feeding period * 100) (Warnau et al., 1996b; Rodriguez y Baena

242   et al., 2006b).

243   Depuration kinetics for all experiments were fitted using kinetic models and

244   statistical methods as described by Warnau et al. (1996a,b) and Lacoue-Labarthe et al.

245   (2008). Depuration kinetics were always best fitted by a double-component exponential

246   equation (decision based on F test and ANOVA tables for two fitted model objects):

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

248   where k_e is the depuration rate constant (d^{-1}), A_t and A_0 are the remaining activities (%) at

249   time *t* (d) and 0, respectively, and 's' and 'l' are the subscripts for the short-lived and

250   long-lived components. The short-lived component represents the loss of the radiotracer

251   fraction that remains associated with the faeces and is rapidly eliminated with them,

252   whereas the long-lived component describes the loss of the radiotracer fraction that is

253   actually absorbed by the organism and slowly eliminated (Whicker and Schultz, 1982;

254   Warnau et al., 1996b). The long-lived component allows assessing the assimilation

255   efficiency (AE) of the radiotracer ingested with food (AE = A_{0l}). Also, for each

256   exponential component (s and l), a biological half-life can be calculated ($T_{b\frac{1}{2}s}$ and $T_{b\frac{1}{2}l}$)

257   from the corresponding depuration rate constant (k_{es} and k_{el}) according to the relation $T_{b\frac{1}{2}}$

258    $= \ln2 / k_e$.

259   Constants of the models and their statistics were estimated by iterative

260   adjustments of the model and Hessian matrix computation using the nonlinear curve-

261   fitting routines in the Statistica® 5.2.1 software. Differences among the estimated kinetic

262   parameters for the different feeding conditions were tested using comparison tests of the

263   means and possible trends linking metal concentrations to cell densities were assessed

264   using simple linear regression techniques (Zar, 1996). The level of significance for

265   statistical analyses was always set at $\alpha = 0.05$.

266

267   **3. Results**

268

269   Depuration kinetics of the radiotracers were followed in the organisms which

270   ingested enough food to display sufficient radioactivity to be accurately counted. Most

271   oysters met this requirement; however some clams displaying very low activities were

272   discarded. No activity was detected on control shells, indicating that no detectable

273   recycling of phytoplankton-associated tracers occurred in the experimental microcosms.

274

275   *3.1. Effect of Co Concentration in Phytoplankton*

276

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 (R²: 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 ($T_{b\frac{1}{2}s}$ < 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 ($T_{b\frac{1}{2}l}$) ranging from 13 to 25 d.

283   Similarly, the fit of the whole-body depuration of ^{57}Co in clams was quite good

284   (R²: 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 $T_{b\frac{1}{2}}$ 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 (R²: 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 $T_{b\frac{1}{2}}$ 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

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

301 p < 0.02).

302   In clams, fitting of the whole-body depuration of the radiotracers ingested with *I.*

303 *galbana*, *E. huxleyi or H. triquetra* (10^4 cell ml^{-1}) were genrally somewhat better than in

304 the oyster (R²: 0.47-0.98 for ISO, 0.47-0.93 for EMI and 0.61-0.89 for HET) (Table 2,

305 Fig. 2). $T_{b½}$ of ^{54}Mn was significantly longer when it was assimilated from HET than

306 from EMI or ISO (p = 0.03 and 0.004, respectively). Significant differences were also

307 observed among AEs calculated for Co, Mn and Zn ingested with the three phytoplankton

308 strains (Mn: ISO < EMI = HET, p < 0.03; Co: ISO = EMI > HET, p < 0.0004; and Zn:

309 ISO ≥ EMI ≥ HET, p = 0.04).

310

311 *3.3. Effect of Cellular Density*

312

313   When oysters were fed 10^4 and 5 x 10^4 cells ml^{-1} of radiolabelled *I. galbana*,

314 whole-body depuration kinetics of ^{54}Mn, ^{57}Co and ^{65}Zn were fitted with R² ranging from

315 0.23 to 0.63 and 0.38 to 0.60, respectively (Table 2, Fig. 3). No significant difference in

316 $T_{b½}$ and AE between cell densities was found for Co. In contrast, significant differences

317 in AE were found for Mn and Zn, with higher AE calculated at the low cell density (p =

318 0.001 and 0.0003, respectively).

319   For clams, examination depuration kinetics of the radiotracers (R²: 0.33-0.65 at 5

320 x 10^3 cell ml^{-1} and 0.47-0.98 at 10^4 cell ml^{-1}) indicated that $T_{b½}$ was not significantly

321 different between the two food densities for all three radiotracers (Table 2, Fig. 3).

322   However, when fed the low cell density, clams incorporated Co, Mn and Zn with

323   significantly higher AE (p = 0.003, 0.047 and 0.0003, respectively).

324

325   **4. Discussion**

326

327   During the last two decades, dietary pathway has been increasingly recognized as

328   a major source of contaminant accumulation in marine invertebrates (e.g. Wang et al.,

329   1996; Reinfelder et al., 1998; Wang and Fisher, 1999b). The assimilation efficiency (AE)

330   and retention time ($T_{b\frac{1}{2}}$) are the critical parameters in assessing and modelling the dietary

331   uptake of contaminants and numerous studies have been devoted to assess these

332   parameters in different marine organisms (e.g., Wang et al., 1996; Warnau et al., 1996b,

333   1999; Pan and Wang, 2008). However, as almost a rule in tropical environments (e.g.,

334   Phillips, 1991; Chong and Wang, 2000; Metian et al., 2005), only very few data are yet

335   available regarding AE and $T_{b\frac{1}{2}}$ parameters for organisms from New Caledonia (e.g.

336   Hédouin et al., 2006, 2007).

337   Ideally, the concentrations of metals in the tissues of a biomonitor species should

338   reflect those occurring in the ambient environment. This essential criterion has been

339   previously experimentally assessed for *I. isognomon* and *G. tumidum* for the dissolved

340   pathway (Hédouin et al., 2007, *this issue*). Exposures to a range of dissolved

341   concentrations of As, Cr, Co, Cd, Mn, Ni and Zn indicated that, over a realistic range of

342   concentrations, these elements were generally bioconcentrated in direct proportion to

343   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$_{b\frac{1}{2}}$) 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

409 **5. Acknowledgements**

410

420

421 **6. References**

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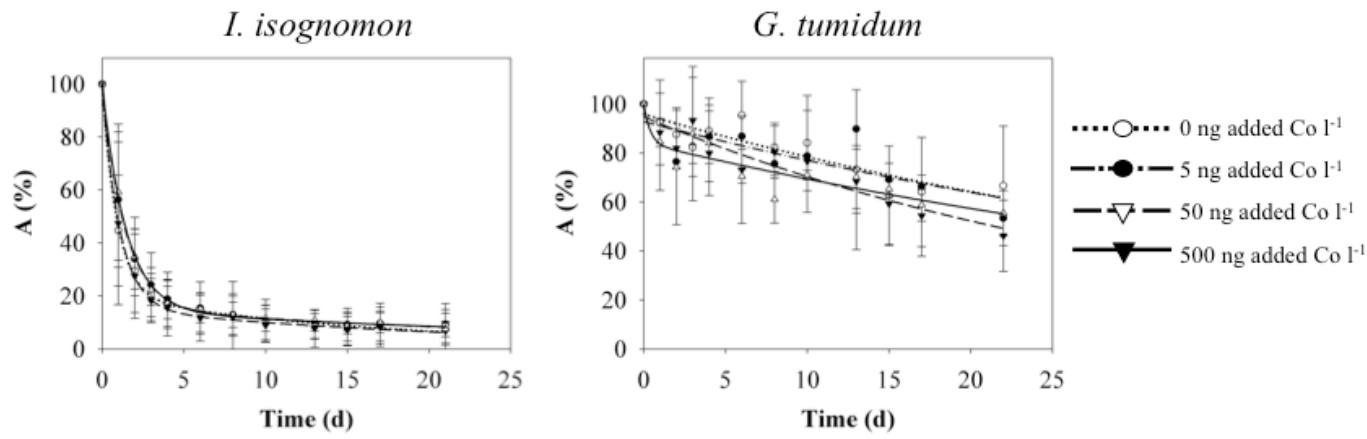
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Captions to Figures

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

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

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

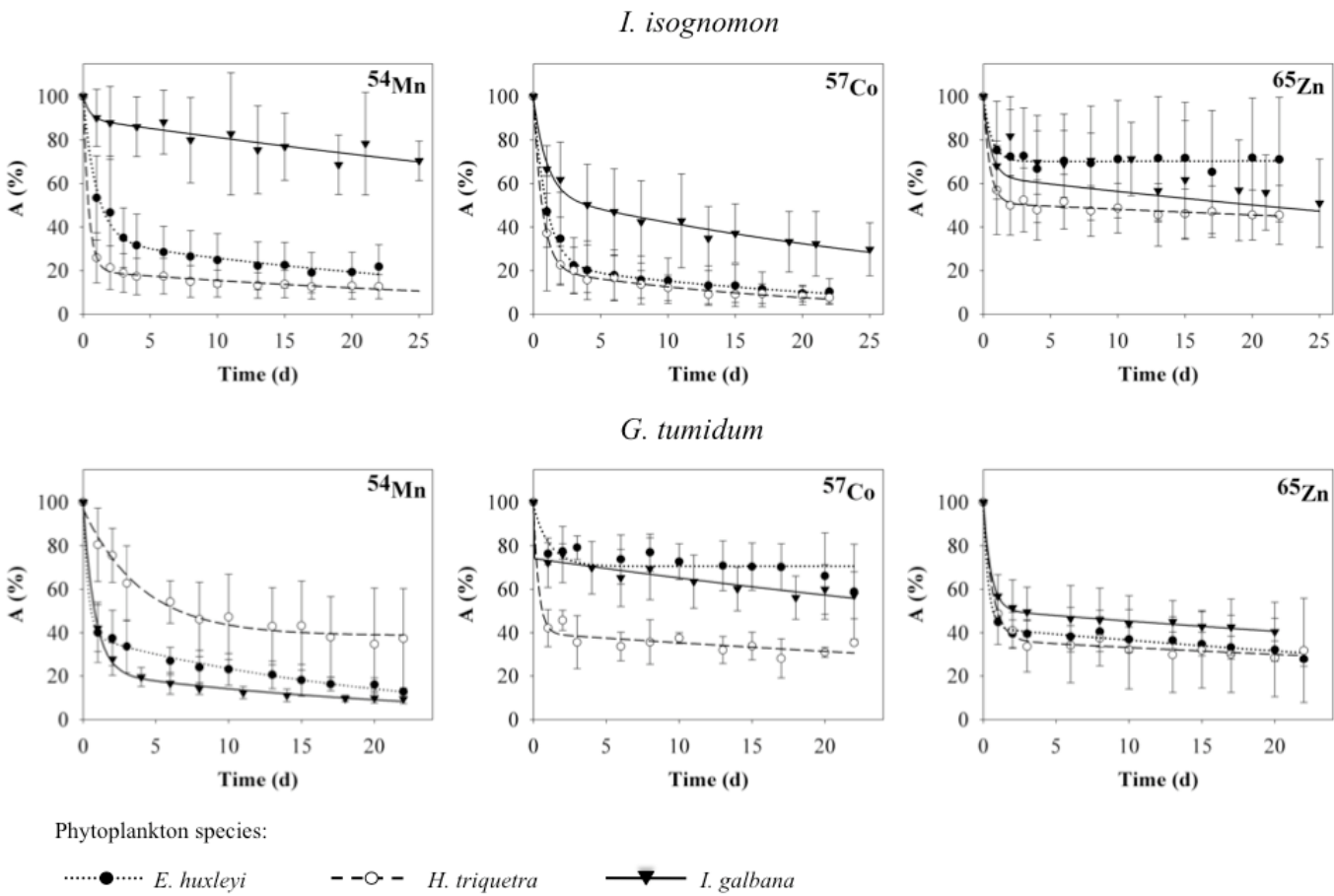657

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665   Fig. 1.

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I. isognomon

G. tumidum

Phytoplankton species:

········●········ E. huxleyi   – –○– – H. triquetra   ▼ I. galbana

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675   Fig. 2.

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I. isognomon

G. tumidum

I. Galbana concentration:

········●········ 5 10³ cells ml⁻¹   — –○ – — 10⁴ cells ml⁻¹   ▬▼▬ 5 10⁴ cells ml⁻¹

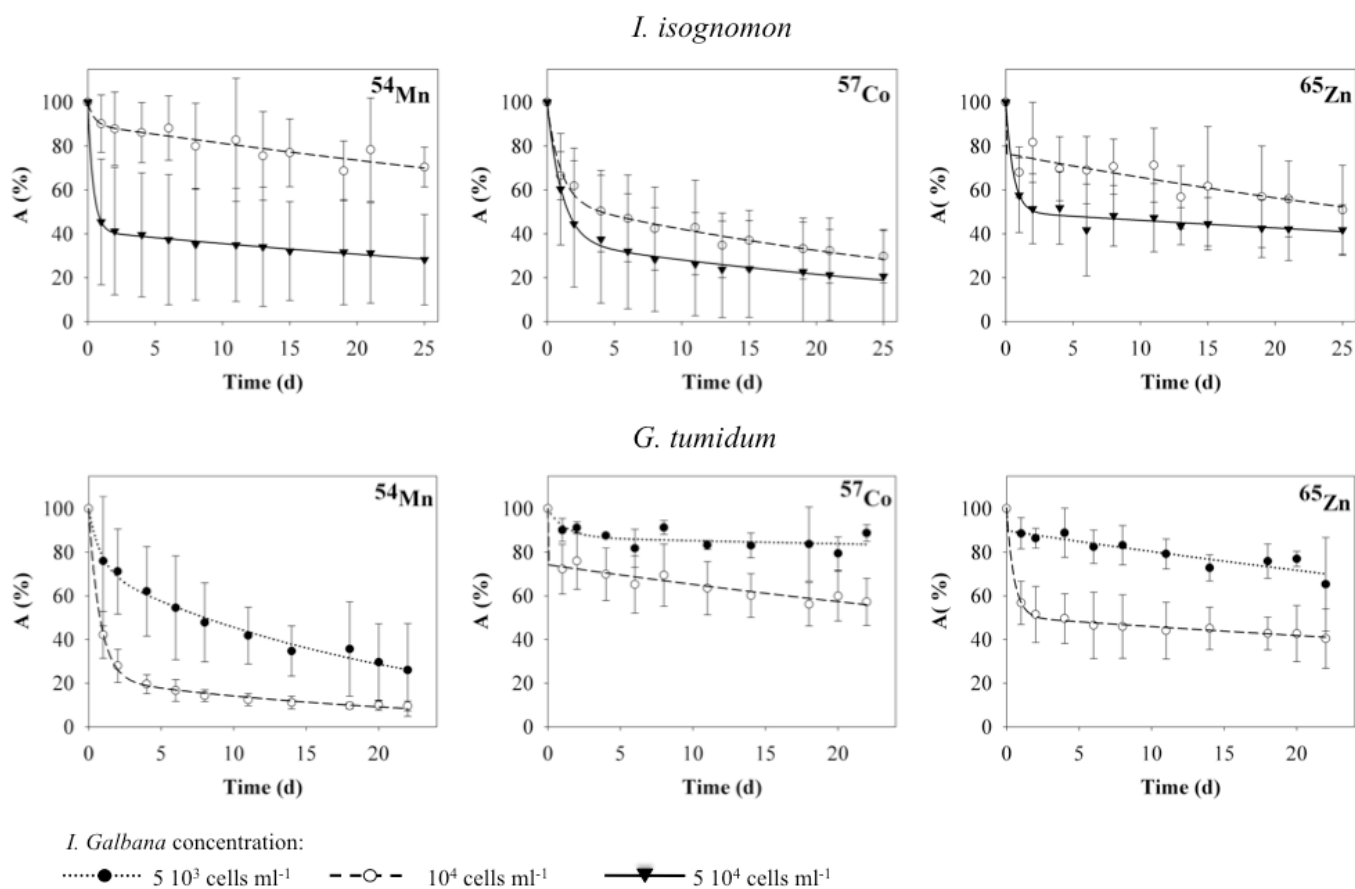677

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682   Fig. 3

Table 1. Assimilation efficiency (AE, %), depuration rate constant (k_{el}, d^{-1}) and biological half-life ($T_{b\frac{1}{2}l}$, 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; R²: determination coefficient.

Species	Co concentration added (ng l^{-1})	AE ± ASE	k_{el} ± ASE	$T_{b\frac{1}{2}l}$ ± 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, ($T_{b\frac{1}{2}l}$, 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; R²: determination coefficient.

Species	Phytoplankton strain	Cell density (cells ml^{-1})	Isotope	AE ± ASE	k_{el} ± ASE	$T_{b\frac{1}{2}l}$ ± ASE	R²
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 ($p > 0.05$)