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Laëtitia Hédouin, O. Pringault, Marc Metian, Paco Bustamante, Michel Warnau

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1                   **Nickel bioaccumulation in bivalves from the New Caledonia**  
2                   **lagoon: Seawater and food exposure**

3                   L. Hédouin<sup>1-3\*</sup>, O. Pringault<sup>2</sup>, M. Metian<sup>1,3</sup>, P. Bustamante<sup>3</sup> & M. Warnau<sup>1</sup>

4  
5                   <sup>1</sup> International Atomic Energy Agency – Marine Environment Laboratory, 4 Quai Antoine 1er,  
6                   MC-98000 Principality of Monaco

7                   <sup>2</sup> Institut de Recherche pour le Développement, BP A5, 98848 Nouméa, New-Caledonia

8                   <sup>3</sup> Centre de Recherche sur les Ecosystèmes Littoraux Anthropisés, UMR-6217  
9                   CNRS/IFREMER/Université de La Rochelle, 22, Avenue M. Crépeau, F-17042 La  
10                   Rochelle, France

11  
12                   \*Corresponding author:       Laetitia Hédouin  
13                                       IAEA - Marine Environment Laboratory  
14                                       4 Quai Antoine 1er  
15                                       MC-98000 Monaco  
16                                       Principality of Monaco  
17                                       E-mail: hedouinlaetitia@yahoo.fr  
18                                       Phone: +377 97 97 72 58  
19                                       Fax:   +377 97 97 72 76

21 **ABSTRACT**

22 The New Caledonian lagoon is submitted to intense heavy metal input from land-based Ni  
23 mining. Therefore, the use of sentinel species is strongly recommended in order to develop  
24 and implement coastal zone management programmes of the metal contamination. The  
25 tropical oysters *Isognomon isognomon* and *Malleus regula* and the clam *Gafrarium tumidum*  
26 were previously proposed as such possible sentinel organisms and were thus investigated in  
27 this context. The three species were exposed to Ni via seawater or food using radiotracer  
28 techniques. Results indicate that uptake and retention efficiencies of Ni are independent of the  
29 dissolved Ni concentrations in the surrounding seawater. Hence, for the three species, body  
30 concentrations of Ni taken up from the dissolved phase are directly proportional to the  
31 ambient dissolved concentrations. Biokinetic patterns indicated that the major part of Ni was  
32 rapidly lost from bivalves during the first days of depuration, whereas 7 to 47 % of <sup>63</sup>Ni were  
33 retained in tissues with a biological half-life not significantly different from infinity. Finally,  
34 feeding experiments showed that Ni ingested with food (phytoplankton) was assimilated more  
35 efficiently in clams (assimilation efficiency, AE = 61 %) than in oysters (AE = 17 %), and  
36 was strongly retained ( $T_{b/2} \geq 35$  d) in the tissues of both bivalves. It is concluded that the  
37 investigated species examined are efficient bioaccumulators of Ni from both the surrounding  
38 seawater and the food, and that they would be useful bioindicators for monitoring the status of  
39 Ni contamination in tropical coastal waters.

40

41 **Keywords:** Metal, Molluscs, Mining Activities, Bioindicator, Radiotracer

42

## 43 **1. Introduction**

44 Besides being the second largest lagoon in the world with a high rate of biodiversity species  
45 and endemism, the New Caledonian lagoon is also subject to enhanced contamination  
46 pressure (Labrosse et al., 2000; Bouchet et al., 2002). Indeed, the largest resources of Ni as  
47 laterites in the world (20-25 %) are present in New Caledonia, currently the third largest  
48 producer of Ni in the world (Dalvi et al., 2004).

49 Laterite deposits are made up of two ore sources available for mining extraction: limonite and  
50 saprolite ores which contain 1 to 1.6 % and 1.6 to 3 % of Ni, respectively. Due to their higher  
51 Ni content, saprolite ores have been traditionally exploited using pyrometallurgical process  
52 since the end of the nineteenth century. Future trend is expected to use limonite ores as well.  
53 INCO, one of the world largest Ni producers is currently developing a hydrometallurgical  
54 extraction plant in Goro (southern territory of New Caledonia) which will be based on acid  
55 extraction (viz. lixiviation).

56 Mining activities and their development represent an issue of concern in New Caledonia, due  
57 to a series of factors including increasing deforestation, soil erosion, extinction of endemic  
58 species and increasing Ni contamination in local waters (Bird et al., 1984; Labrosse et al.,  
59 2000). However, to the best of our knowledge, information available on impacts of open-cast  
60 mining in the marine coastal ecosystems of New Caledonia is extremely scarce (Monniot et  
61 al., 1994; Breau, 2003; Hédouin et al., 2006).

62 Among the common approaches used to survey environmental contamination, the use of  
63 bioindicator species has proven to be a valuable and informative tool (Goldberg et al., 1983;  
64 Phillips, 1990). In order to develop a biomonitoring programme to assess the levels of metal  
65 contamination in New Caledonia lagoon, studies have been recently undertaken to screen  
66 local species for their bioindicative potential (Breau, 2003). In particular, both laboratory and

67 field studies have shown that two oysters (*Isognomon isognomon* and *Malleus regula*) and  
68 one clam (*Gafrarium tumidum*) efficiently concentrate several elements (Ag, As, Cd, Co, Cr,  
69 Cu, Mn, Ni and Zn) and that they are able to discriminate among locations subject to  
70 contrasting levels of contamination (Hédouin, 2006). However, current information on Ni  
71 bioaccumulation is rather limited; in particular no data are available on the tissue distribution  
72 or bioaccumulation kinetics of Ni taken up through the dissolved and dietary pathways in  
73 these organisms. Therefore, the objective of the present study was to determine the  
74 bioaccumulation capacity of Ni from food and seawater in these three bivalves in order to  
75 assess their potential as bioindicators of environmental Ni contamination.

## 76 **2. Materials & methods**

### 77 **2.1. Collection and acclimation**

78 In October 2003, the clams *Gafrarium tumidum* were collected by sea-shore fishing in  
79 Dumbéa Bay (22°11'25 S, 166°24'38 E) and the two oysters *Isognomon isognomon* and  
80 *Malleus regula* were collected by SCUBA diving in Maa Bay (22°12'29, S166°19'42 E), in a  
81 similar environment, New Caledonia. Although both oysters are abundant in Maa Bay (Breau,  
82 2003), their quite close external appearance makes them difficult to differentiate one from the  
83 other during SCUBA sampling; hence a larger number of *M. regula* were collected compared  
84 to *I. isognomon*. However, as the oysters *I. isognomon* and *M. regula* are extremely close  
85 species from their ecophysiological characteristics too (Yonge, 1968), *M. regula* was used as  
86 a representative oyster in the seawater exposure experiments, whereas *I. isognomon* was used  
87 in the food exposure experiments. To ensure comparability of both oysters, some *I.*  
88 *isognomon* individuals were also included in the seawater experiments.

89 Oysters and clams were acclimated to laboratory conditions for one week prior to the  
90 experiments (close circuit aquarium; daily water renewal, salinity:  $35 \pm 1$  p.s.u.; temperature =

91  $25 \pm 1^\circ\text{C}$ ; pH =  $8.0 \pm 0.1$ ; light/dark cycle: 12 hrs/12 hrs). Since body size is known to affect  
92 metal concentrations in organisms (Boyden, 1977), only individuals with shell width  $\geq 35$  mm  
93 for *G. tumidum* and shell length  $\geq 70$  mm for *I. isognomon* and *M. regula* were used in the  
94 experiments (Metian, 2003; Hédouin et al., 2006).

## 95 **2.2. Testing influence of dissolved Ni concentrations**

96 Five groups of 51 *G. tumidum* clams (shell width from 35 to 44 mm; wet wt from 15 to 38 g),  
97 five groups of 51 *M. regula* oysters (shell length from 85 to 135 mm; wet wt from 14 to 48 g)  
98 and five groups of 5 *I. isognomon* oysters (shell length from 80 to 140 mm; wet wt from 13 to  
99 45 g) were placed in 5 aquaria of 50 l of natural seawater (close circuit aquarium; salinity: 35  
100  $\pm 1$  p.s.u.; temperature =  $25 \pm 1^\circ\text{C}$ ; pH =  $8.0 \pm 0.1$ ; light/dark cycle: 12 hrs/12 hrs). Seawater  
101 salinity, temperature and pH were checked twice daily during the experiment.

102 Each group of bivalves was exposed for 14 d to five added Ni concentrations (0, 15, 75, 350  
103 and 1,400 ng Ni l<sup>-1</sup>). The concentrations of Ni were adjusted using increasing amounts of  
104 Ni(NO<sub>3</sub>)<sub>2</sub> (Merck, synthesis quality) and a fixed activity (1 kBq l<sup>-1</sup>) of the corresponding  
105 radiotracer <sup>63</sup>Ni, as high specific activity <sup>63</sup>NiCl<sub>2</sub> (T<sub>1/2</sub> = 100 yrs) purchased from Amersham,  
106 UK. This radiotracer spike corresponded to 4.2 ng stable Ni l<sup>-1</sup>, a concentration at least 1 order  
107 of magnitude lower than the background concentrations of Ni in open seas (Bruland, 1983).  
108 No pH variation was detectable after stable metal and radiotracer addition. Seawater and  
109 spikes were renewed daily for 5 d, then every second day in order to keep exposure  
110 concentration and radioactivity levels as constant as possible. Activity of the radiotracer in  
111 seawater was checked daily, and before and after each seawater renewal, to determine its  
112 time-integrated activity (Warnau et al., 1996). For the entire experimental time course, the  
113 time-integrated <sup>63</sup>Ni activity in seawater was 0.81 kBq l<sup>-1</sup>.

114 During the exposure period, 3 individuals of *G. tumidum* and *M. regula* were collected after  
115 different time intervals; soft tissues were separated from shells and prepared for whole soft

116 tissues radioanalysis (see section II.4.). The last day ( $t_{14d}$ ), 5 individuals of *G. tumidum*,  
117 *M. regula* and *I. isognomon* were dissected to determine body distribution of incorporated  
118  $^{63}\text{Ni}$ . Dissected body compartments were digestive gland, gills, mantle, foot, muscle and  
119 remaining soft tissues for clams and visceral mass + mantle, gills and muscle for oysters.  
120 At the end of the 14-d exposure, the remaining organisms were placed in 60 x 60 x 60 cm  
121 plastic cages for 32 d in Sainte-Marie Bay, Nouméa, New Caledonia at 7 m depth. The  
122 selected area (22°18'55 S, 166°27'98 E) is characterized by relatively low Ni levels  
123 (Hédouin, 2006). At different time intervals of the depuration period, for each concentration  
124 tested, 3 *G. tumidum* and 3 *M. regula* individuals were collected, the soft tissues dissected  
125 from shells and then prepared for soft parts radioanalysis. The last day ( $t_{32d}$ ), 5 *G. tumidum*  
126 were dissected into different body compartments to determine the tissue distribution of the  
127 remaining  $^{63}\text{Ni}$ .

### 128 **2.3. Exposure via the food**

129 Cells of the Prymnesiophyceae *Isochrysis galbana* ( $10^3$  cell  $\text{ml}^{-1}$ ) which originated from  
130 axenic stock cultures were resuspended in an Erlenmeyer flask containing 5 l sterile-filtered  
131 (0.22  $\mu\text{m}$ ) seawater enriched with f/2 nutrients without EDTA and Si. Seawater was spiked  
132 with  $^{63}\text{Ni}$  (5 kBq  $\text{l}^{-1}$ ), and the cells were then incubated at 25°C (light/dark cycle: 12 hrs/12  
133 hrs). After 6 d of incubation, cell density increased from  $10^3$  to  $1.3 \cdot 10^6$  cell  $\text{ml}^{-1}$ . A sample of  
134 10 ml of the culture was then gently filtered (47 mm diameter Polycarbonate Nuclepore®  
135 filter, 1  $\mu\text{m}$  mesh size) and the radioactivity associated with *I. galbana* cells was measured  
136 before and after the filtration ( $3.4 \cdot 10^{-6}$  Bq  $^{63}\text{Ni}$  cell $^{-1}$ ).  
137 Bivalves (n = 196 *G. tumidum*, n = 196 *I. isognomon*) were placed in a 300 l aquarium (close  
138 circuit aquarium constantly aerated; salinity:  $35 \pm 1$  p.s.u.; temperature =  $25 \pm 1^\circ\text{C}$ ; pH =  $8.0$   
139  $\pm 0.1$ ) and fed the radiolabelled *I. galbana* for 2 hrs ( $10^4$  cell  $\text{ml}^{-1}$ ). Immediately after feeding,

140 14 individuals per species were collected and dissected to separate whole soft parts from  
141 shells.

142 The remaining bivalves were then placed in Sainte-Marie Bay in plastic cages as previously  
143 described. At different time intervals, 14 individuals of each species were collected in order to  
144 follow loss kinetics of  $^{63}\text{Ni}$  ingested with food. At 12 and 46 d, collected individuals were  
145 dissected to determine the distribution of  $^{63}\text{Ni}$  content among the different body  
146 compartments.

#### 147 **2.4. Sample preparation and Radioanalyses**

148 Seawater samples (2 ml) were directly transferred to 20-ml glass scintillation vials (Packard)  
149 and mixed with 10 ml of scintillation liquid (Ultima Gold<sup>®</sup>, Packard). The separated body  
150 compartments and whole soft parts of bivalves were weighed (wet wt), dried at 60°C until  
151 constant weight, and weighed again (dry wt). Clam and oyster tissues were then digested for  
152 one week (50°C) with 1 ml Soluene<sup>®</sup> (Packard) per 100 mg dry wt tissues, and then mixed  
153 with scintillation liquid (Hionic Fluor<sup>®</sup>, Packard) in proportion 1: 5 (v: v). Bivalve shells were  
154 leached in three successive baths (20 min) of 2N HCl in order to recover all the  $^{63}\text{Ni}$  adsorbed  
155 onto shells. Samples of 1 ml were transferred to 20-ml glass scintillation vials and mixed in  
156 proportion 1:10 (v: v) with scintillation liquid (Ultima Gold<sup>®</sup>).

157 The radioactivity of  $^{63}\text{Ni}$  was counted using a 1600 TR Packard Liquid Scintillation Analyzer.  
158 Counting time was adapted to obtain a propagated counting error less than 5 % (maximal  
159 counting duration 2 hrs). The radioactivity was determined by comparison with standards of  
160 known activities and measurements were corrected for counting efficiency, physical  
161 radioactive decay and quenching effects.



162        **2.5.      Data analyses**

163      Uptake of <sup>63</sup>Ni was expressed in terms of concentration factor (CF: ratio between activity of  
164      the radiotracer in the whole soft parts or in a body compartment -Bq g<sup>-1</sup> dry wt- and time-  
165      integrated activity of the radiotracer in seawater -Bq ml<sup>-1</sup>-). Radiotracer uptake kinetics were  
166      described using either a simple linear regression model (eq. 1) or, if the observed kinetics  
167      tended to reach a steady state, a saturation exponential model (eq. 2):

168       $CF_t = k_u t$  (eq. 1)

169       $CF_t = CF_{ss} (1 - e^{-k_e t})$  (eq. 2)

170      where  $CF_t$  and  $CF_{ss}$  are the concentration factors at time  $t$  (d) and at steady state (ml g<sup>-1</sup>),

171      ( $CF_{ss} = \frac{k_u}{k_e}$ );  $k_u$  is the uptake rate constant (ml g<sup>-1</sup> d<sup>-1</sup>) and  $k_e$  is the depuration rate constant

172      (d<sup>-1</sup>) (Whicker and Schultz, 1982; Thomann et al., 1995). Linearity of the uptake kinetics was

173      tested by a linearity test for regression with replication (Zar, 1996).

174      Loss of <sup>63</sup>Ni was expressed in term of percentage of remaining radioactivity (radioactivity at  
175      time  $t$  divided by initial radioactivity measured in the organisms immediately after the feeding

176      period). The loss kinetics were best fitted by either a single-component exponential equation

177      (eq. 3), a single-component exponential equation with an additional constant term (eq. 4), or a

178      double-component exponential equation (eq. 5):

179       $A_t = A_0 k_e t$  (eq. 3)

180       $A_t = A_{0s} e^{-k_{es} t} + A_{0l}$  (eq. 4)

181       $A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t}$  (eq. 5)

182      where  $A_t$  and  $A_0$  are the remaining activities (%) at time  $t$  (d) and 0, respectively;  $k_e$  is the

183      depuration rate constant (d<sup>-1</sup>); 's' and 'l' are the subscripts for the 'short-lived' and 'long-

184      lived' components. For each exponential component (s and l), a biological half-life can be

185      calculated ( $T_{b/2s}$  and  $T_{b/2l}$ ) from the corresponding depuration rate constant ( $k_{es}$  and  $k_{el}$ ,

186 respectively) according to the relation  $T_{b\frac{1}{2}} = \frac{\ln 2}{k_e}$ . The additional constant term of equation 4

187 represents a fraction  $A_{01}$  of the radiotracer incorporated that is sequestered in the organism  
188 tissues.

189 Regarding feeding experiments, the ‘long-lived’ exponential term describes the proportion of  
190 the radiotracer ingested with food that is actually absorbed by the organism and slowly  
191 eliminated. The corresponding  $A_{01}$  represents the assimilation efficiency (AE) of the  
192 considered element.

193 Model constants and their statistics were estimated by iterative adjustment of the model and  
194 Hessian matrix computation, respectively using the nonlinear curve-fitting routines in the  
195 Statistica 5.2.1 software. Best fitting models were selected according to the highest  
196 determination coefficient and examination of residuals.

197 In order to assess possible effect of dissolved Ni concentration on bioconcentration behaviour,  
198 estimated kinetic parameters ( $k_u$ ,  $CF_{ss}$ ,  $A_{01}$ ,  $k_{el}$ ) were plotted against the concentration of total  
199 Ni (stable + stable equivalent of added radiotracer) in seawater and were fitted using simple  
200 linear regression. Statistical comparisons were also performed using 1-way ANOVA followed  
201 by the multiple comparison test of Tukey (Zar, 1996).

202 The level of significance for statistical analyses was always set at  $\alpha = 0.05$ .

## 203 **3. Results**

### 204 **3.1. Seawater exposure to different Ni concentrations**

205 Uptake of  $^{63}\text{Ni}$  in whole soft parts of the clam *G. tumidum* and the oyster *M. regula* displayed  
206 linear kinetics at all of the five exposure concentrations tested ( $p < 0.0001$ ,  $R^2$ : 0.79-0.92 for  
207 *G. tumidum* and 0.63-0.72 for *M. regula*) (Table 1 and Fig. 1-A1). Statistical analysis

208 indicated that uptake rate constants  $k_u$  in both clams and oysters did not differ significantly  
209 over the range of concentrations tested.

210 After 14 d of exposure, the concentration factors ( $CF_{14d}$ ) of  $^{63}Ni$  were calculated in the  
211 different body compartments of the clam as well as in both oysters (*M. regula* and *I.*  
212 *isognomon*) (Table 2). In all three bivalves, whole soft part  $CF_{14d}$  was one to three orders of  
213 magnitude higher than those calculated for shells. Table 2 indicates that  $^{63}Ni$  was concentrated  
214 selectively by the different body compartments in each species, according to the following  
215 order:

216 - *G. tumidum*: digestive gland ( $CF_{14d}$  up to 620) > gills > remaining tissues > mantle > muscle  
217  $\approx$  foot,

218 - *I. isognomon*: gills ( $CF_{14d}$  up to 660) > visceral mass + mantle > muscle,

219 - *M. regula*: visceral mass + mantle ( $CF_{14d}$  up to 270) > gills  $\approx$  muscle.

220 In general, no significant difference was found among  $CF_{14d}$  in whole soft parts as well as in  
221 body compartments over the range of concentrations tested. The only exception was  
222 *I. isognomon*, for which the  $CF_{14d}$  calculated in whole soft parts, gills and visceral mass +  
223 mantle for the highest Ni concentration (1,400 ng added Ni  $l^{-1}$ ) were found to be significantly  
224 different from the ones calculated for 75 ng added Ni  $l^{-1}$  ( $p_{Tukey} = 0.026, 0.046$  and  $0.043$ ,  
225 respectively).

226 Comparisons of  $CF_{14d}$  in the whole soft parts and body compartments between *I. isognomon*  
227 and *M. regula* indicated that no significant difference was found for the whole soft parts,  
228 except for 15 ng added Ni  $l^{-1}$ , for which *I. isognomon* displayed a significantly higher CF than  
229 *M. regula* ( $p_{Tukey} = 0.002$ ). Regarding body compartments, no significant difference was found  
230 for visceral mass + mantle and muscle between the two species ( $p_{Tukey}$  always > 0.05),  
231 whereas  $CF_{14d}$  in gills of *I. isognomon* were significantly higher than those of *M. regula* at  
232 each concentration tested ( $p_{Tukey}$  always  $\leq 0.04$ ).

233 In terms of body load distribution,  $^{63}\text{Ni}$  was mainly found in the digestive gland for clams (36  
234 to 47 % of total body load; Fig. 2-A1) and in the visceral mass + mantle for both oysters (67  
235 to 82 % of total body load; Fig. 2-A2). The body distribution of  $^{63}\text{Ni}$  was similar over the  
236 entire range of concentrations tested.

237 At the end of the exposure time, non-contaminating conditions were restored and loss kinetics  
238 of  $^{63}\text{Ni}$  were followed in the field for 32 d. Loss kinetics from whole soft parts were best  
239 described by a double exponential model in *G. tumidum*, whereas a single-component  
240 exponential equation with an additional constant term best fitted the loss kinetics in *M. regula*  
241 (Table 1 and Fig. 1-A2).

242 A relatively small fraction (< 14 %) of  $^{63}\text{Ni}$  was lost from the long-lived component in *M.*  
243 *regula*, whereas this component contained 27 to 47 % of  $^{63}\text{Ni}$  in *G. tumidum* (Table 1).  
244 However, in both species, the estimated loss rate constants of the long-lived components ( $k_{el}$ )  
245 were not significantly different from 0 ( $p > 0.05$ ), and therefore the derived biological half-  
246 lives of  $^{63}\text{Ni}$  in clams and oysters were virtually infinite at all the exposure concentrations  
247 tested. In addition, in both species, linear regressions established between estimated  $A_{0I}$  and  
248 exposure concentrations displayed slopes not significantly different from 0 for both clams ( $p$   
249 = 0.71) and oysters ( $p = 0.34$ ), indicating that  $^{63}\text{Ni}$  was assimilated similarly (in relative %) in  
250 each species regardless of the exposure concentration.

251 The distribution of  $^{63}\text{Ni}$  among the body compartments of the clam was determined at the end  
252 of the depuration period (Fig. 2-A1).  $^{63}\text{Ni}$  was mainly associated with the mantle (27 to 44 %)  
253 and the muscle (22 to 32 %). Distributions were similar for the different exposure treatments,  
254 but differed from the distributions observed at the end of the exposure period, with lower  
255 fraction associated with digestive gland and higher fractions associated with mantle and  
256 muscle.

### 257 **3.2. Food exposure**

258 The loss kinetics of  $^{63}\text{Ni}$  ingested with food in both the clam *G. tumidum* and the oyster *I.*  
259 *isognomon* were best fitted using a double exponential model ( $R^2 = 0.49$  and  $0.59$ ,  
260 respectively) (Table 3 and Fig. 1-B). A substantial part of the  $^{63}\text{Ni}$  activity (39 % for the clam  
261 and 83 % for the oyster) was rapidly lost via defecation ( $T_{b/2} < 2$  d). Assimilation efficiency  
262 (AE) of  $^{63}\text{Ni}$  was 61 % and 17 % in clams and oysters, respectively. These assimilated  
263 fractions were retained with  $T_{b/2}$  of 35 d in clams and a time not different from the infinite in  
264 oysters. During the depuration period, the highest proportion of  $^{63}\text{Ni}$  (74 %) was associated  
265 with visceral mass + mantle in *I. isognomon* whereas in *G. tumidum* each organ contributed  
266 similarly to the total  $^{63}\text{Ni}$  content (Fig. 2-B). In both clam and oyster,  $^{63}\text{Ni}$  body distribution  
267 showed no major difference between the two sampling times (12 and 46 d).

## 268 **4. Discussion**

269 Bivalves are well known for their capacity to accumulate metals to quite high levels (e.g.  
270 Phillips, 1976). However, few studies have been devoted to Ni in marine bivalves (Friedrich  
271 and Fillice, 1976; Hardy and Roesijadi, 1982; Zaroogian and Johnson, 1984; Punt et al.,  
272 1998), and particularly in tropical areas.

### 273 **4.1. Seawater pathway**

274 Ideally, a bioindicator should bioconcentrate contaminants in direct proportion to the  
275 dissolved metal concentration occurring in the environment. This implies that the  
276 concentration factor (CF) of a contaminant would remain constant over the concentration  
277 range to which the organism could be exposed in its environment (Phillips, 1980, 1990).

278 Previous experimental investigations of Ni bioaccumulation in marine organisms generally  
279 considered exposure concentrations (e.g. up to  $80 \text{ mg Ni l}^{-1}$ , Friedrich and Fillice, 1976) that  
280 were far above (several orders of magnitude) the natural background ones. The concentrations

281 tested in the present study (up to 1,400 ng added Ni l<sup>-1</sup>) were selected in order to include the  
282 entire concentration range which can be measured in the coastal waters of the New  
283 Caledonian lagoon (Fernandez et al., 2002). Results showed that the bioconcentration of Ni in  
284 the clam *G. tumidum* and in both oysters *M. regula* and *I. isognomon* was directly  
285 proportional to the Ni concentration in seawater virtually over the entire range of Ni  
286 concentrations tested. Similar observations were made for other elements accumulated by *I.*  
287 *isognomon* and *G. tumidum* such as Co, Cr, Mn also present in Ni ores (Hédouin, 2006). The  
288 capacity of Ni bioconcentration reported here in clams and oysters (CF<sub>14d</sub> ranging from 70 to  
289 280 ml g<sup>-1</sup> dry wt) was quite lower than that determined in comparable experimental  
290 conditions for some metals such as Ag and Zn, which reached CF values up to 200,000 ml g<sup>-1</sup>  
291 dry wt (Hédouin, 2006). However, the observed CFs are in the range of those reported in  
292 previous studies related to Ni in bivalves (4 ml g<sup>-1</sup> dry wt in the clam *Prototheca staminea*,  
293 Hardy and Roesijadi, 1982; from 10 to 607 ml g<sup>-1</sup> dry wt in the mussel *Mytilus edulis*,  
294 Friedrich and Fillice, 1976; from 156 to 336 ml g<sup>-1</sup> wet wt in *Crassostrea virginica* and *M.*  
295 *edulis*, Zaroogian and Johnson, 1984).

296 Results indicated that, in relative units, loss kinetics of Ni from the soft parts of *G. tumidum*  
297 and *M. regula* were also independent on the Ni concentrations to which the organisms were  
298 previously exposed. These observations are in agreement with those of Zaroogian and  
299 Johnson, (1984) who reported that the loss rate of Ni was similar in oysters exposed to two  
300 different Ni treatments (5,000 and 10,000 ng Ni l<sup>-1</sup>). In addition, our study showed that Ni was  
301 efficiently retained in both bivalve species, with biological half-lives not significantly  
302 different from infinity. These values should of course be considered with caution due to the  
303 relatively short duration of the experiment (32 d). Nevertheless, they clearly indicate that both  
304 clams and oysters would be able to preserve information regarding their contamination history  
305 over a very long timescale (probably several months).

306 The very close resemblance of the two oyster species *I. isognomon* and *M. regula*, both in  
307 their appearance and ecophysiology (Yonge, 1968) was also reflected in their  
308 bioaccumulation and depuration capacities for Co, Cr, Zn and, to a lesser extent, Cd  
309 (Hédouin, 2006). Similarly, the present work indicated that bioconcentration of dissolved Ni  
310 was quite similar in both species on a whole-body basis, although a slight decrease in CF was  
311 observed in *I. isognomon* at the highest Ni concentration tested. However, examination of CF  
312 values at the organ level indicated that gills of *I. isognomon* concentrate Ni much more  
313 efficiently (up to one order of magnitude) than those of *M. regula*. This suggests that although  
314 these two species are very closely related in many aspects at a macroscopic scale, mechanisms  
315 controlling Ni uptake could be quite different in *I. isognomon* and *M. regula*. Furthermore,  
316 previous studies have observed that gills are generally a major site of Ni intake in bivalves  
317 (e.g. Hardy and Roesijadi, 1982; Wilson, 1983), indicating that this dissimilarity between  
318 these two oysters deserves further investigation.

#### 319 **4.2. Food pathway**

320 Although it is now well documented that assimilation of metals ingested with food plays an  
321 important role in their bioaccumulation in marine organisms (e.g. Wang and Fisher, 1999b),  
322 very few studies have been devoted to trophic transfer of Ni. Kumblad et al. (2005) have  
323 shown that assimilation efficiency (AE) of sediment-associated  $^{63}\text{Ni}$  (II) ranged from 43 to 49  
324 % in the clam *Macoma balthica*, the amphipod *Monoporeia affinis* and the priapulid  
325 *Halicryptus spinulosus*. In the present study, the estimated AE of  $^{63}\text{Ni}$  was much higher in the  
326 clam *G. tumidum* (61 %) than in the oyster *I. isognomon* (17 %). Such a low assimilation in *I.*  
327 *isognomon* compared to *G. tumidum* as well as to the species studied by Kumblad et al.  
328 (2005) could be related to the differences in feeding physiology (e.g. digestion efficiency) of  
329 these different organisms (Lee II, 1991; Mayer et al., 2001). However, in a recent study on  
330 other metals, AEs reaching values up to 77 % were found for Ag in *I. isognomon* (Hédouin,

331 2006), suggesting that low AE is not a physiological characteristic of this species. Rather, the  
332 low assimilation of Ni in *I. isognomon* is likely related to the oyster behaviour towards Ni  
333 and/or how it is able to deal with the way Ni is bound to algal cells.

334 To the best of our knowledge, the estimated AEs for Ni are the first published for these  
335 species. However, it should be kept in mind that these Ni AEs were obtained in controlled  
336 feeding conditions (mono-specific culture of *I. galbana* at  $10^4$  cell ml<sup>-1</sup>). These conditions are  
337 quite different from those found in the field which are much more complex and variable and  
338 which could result in actual AEs somewhat different from those estimated here. It is indeed  
339 well known that AE may be influenced by the type of food ingested (Wang and Fisher, 1999a;  
340 Chong and Wang, 2000). This has also been recently observed for Co, Mn and Zn in *G.*  
341 *tumidum* and *I. isognomon*: AEs estimated for these 3 metals with 3 different species of  
342 phytoplankton were found to vary over a factor of 4 (Hédouin, 2006). Hence, Ni AEs  
343 estimated in the present study should be considered as preliminary and other feeding  
344 conditions should be investigated before making generalisations about Ni assimilation under  
345 environmental conditions.

### 346 **4.3. Conclusions**

347 Within a range of dissolved Ni concentrations that cover the natural range occurring in New  
348 Caledonia seawaters, (1) Ni bioconcentration was directly proportional to the Ni  
349 concentration in seawater for *G. tumidum*, *M. regula* and, to a lesser extent, *I. isognomon*, and  
350 (2) the retention efficiency of Ni in clams and oysters was independent on the total Ni  
351 concentration previously accumulated by the organisms. In addition, clams and oysters were  
352 shown to efficiently assimilate Ni ingested with their food (in particular in clams) and retain it  
353 very efficiently (in particular in oysters). These characteristics suggest that these New  
354 Caledonian bivalves could be used to detect Ni contamination in their surrounding  
355 environment and to preserve this information over long periods of time. In addition, the clams



356 and oysters displayed different bioaccumulation behaviour for Ni, especially when exposed  
357 via the food. Both groups would thus be worth further characterization with regard to their  
358 values as bioindicators for Ni contamination. Indeed, both bivalve groups could provide  
359 complementary ecotoxicological information as they interact differently with their  
360 environment.

361

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453

454

455 **Figure 1.** Uptake and loss kinetics of  $^{63}\text{Ni}$  in whole soft parts of the investigated bivalves.  
456 (A) Uptake kinetics (mean concentration factor,  $\text{CF} \pm \text{SD}$ ,  $n = 3$ ) (A-1) and loss kinetics  
457 (mean % remaining activity  $\pm \text{SD}$ ,  $n = 3$ ) (A-2) in the clam *Gafrarium tumidum* and the oyster  
458 *Malleus regula* exposed to 5 different dissolved Ni concentrations.  
459 (B) Loss kinetics (mean % remaining activity  $\pm \text{SD}$ ,  $n = 14$ ) in the clam *G. tumidum* and the  
460 oyster *Isognomon isognomon* after a 2-hr feeding on  $^{63}\text{Ni}$ -labelled *Isochrysis galbana*.  
461  
462 **Figure 2.** Distribution of  $^{63}\text{Ni}$  (mean %) among the body compartments of clams and oysters.  
463 (A) Body distribution ( $n = 5$ ) in the seawater experiments in (A-1) the clam *Gafrarium*  
464 *tumidum* after a 14-d exposure to 5 different dissolved Ni concentrations (end of uptake) and  
465 a subsequent 32-d depuration period (end of depuration), and (A-2) the oysters *Isognomon*  
466 *isognomon* and *Malleus regula* after a 14-d exposure to the 5 dissolved Ni concentrations.  
467 (B) Body distribution ( $n = 14$ ) in the clam *G. tumidum* and the oyster *I. isognomon*, 12 and 46  
468 d after a 2-hr feeding on  $^{63}\text{Ni}$ -labelled *Isochrysis galbana* cells.  
469  
470

471 **Table 1.** Estimated uptake rate constant ( $k_{\text{u}}$ , ml g<sup>-1</sup> dry wt d<sup>-1</sup>), absorption efficiency ( $A_{01}$ , %)   
472 and loss rate constant ( $k_{\text{el}}$ , d<sup>-1</sup>) of <sup>63</sup>Ni in the whole soft parts of the clam *Gafrarium tumidum*   
473 and the oyster *Malleus regula* exposed to five different dissolved Ni concentrations via   
474 seawater for 14 d (uptake period) and then maintained for 32 d in the field in a clean site   
475 (depuration period).

476 ASE: asymptotic standard error; R<sup>2</sup>: determination coefficient of the uptake and loss kinetics

477

478 **Table 2.** Concentration factors (mean CF ± SD, ml g<sup>-1</sup> dry wt; n = 5 per species per   
479 concentrations tested) of <sup>63</sup>Ni in the clam *Gafrarium tumidum* and the oysters *Isognomon*   
480 *isognomon* and *Malleus regula* exposed for 14 d to 5 different dissolved Ni concentrations   
481 (C<sub>0</sub>-C<sub>4</sub>).

482

483 **Table 3.** Assimilation efficiency (AE, %), loss rate constant ( $k_{\text{el}}$ , d<sup>-1</sup>) and biological half life   
484 ( $T_{\text{b}^{1/2}}$ , d) of <sup>63</sup>Ni in whole soft parts of the clam *Gafrarium tumidum* and the oyster *Isognomon*   
485 *isognomon*, after a 2-hr feeding on radiolabelled *Isochrysis galbana* cells.

486 ASE: asymptotic standard error; R<sup>2</sup>: determination coefficient of the loss kinetics

487 **Table 1.** Estimated uptake rate constant ( $k_u$ , ml g<sup>-1</sup> dry wt d<sup>-1</sup>), absorption efficiency ( $A_{0l}$ , %)
 488 and loss rate constant ( $k_{el}$ , d<sup>-1</sup>) of <sup>63</sup>Ni in the whole soft parts of the clam *Gafrarium tumidum*
 489 and the oyster *Malleus regula* exposed to five increasing dissolved Ni concentrations via
 490 seawater for 14 d (uptake period) and then maintained for 32 d in the field in a clean site
 491 (depuration period).
 492 ASE: asymptotic standard error; R<sup>2</sup>: determination coefficient of the uptake and loss kinetics

Concentrations (ng added Ni l <sup>-1</sup> )	Uptake period		Depuration period		
	$k_u \pm \text{ASE}$	R <sup>2</sup>	$A_{0l} \pm \text{ASE}$	$k_{el} \pm \text{ASE}$	R <sup>2</sup>
<b><i>G. tumidum</i></b>					
C <sub>0</sub> : 0	9.1 ± 0.6 <sup>c</sup>	0.79	44 ± 11 <sup>b</sup>	0.010 ± 0.013*	0.72
C <sub>1</sub> : 15	7.2 ± 0.4 <sup>c</sup>	0.83	27 ± 20*	0.002 ± 0.028*	0.84
C <sub>2</sub> : 75	7.7 ± 0.4 <sup>c</sup>	0.82	45 ± 18 <sup>a</sup>	0.002 ± 0.017*	0.60
C <sub>3</sub> : 350	7.1 ± 0.4 <sup>c</sup>	0.82	47 ± 7 <sup>c</sup>	0.019 ± 0.009*	0.70
C <sub>4</sub> : 1400	5.2 ± 0.2 <sup>c</sup>	0.92	35 ± 3 <sup>c</sup>	0.001 ± 0.006*	0.77
<b><i>M. regula</i></b>					
C <sub>0</sub> : 0	11.5 ± 1.0 <sup>c</sup>	0.72	14 ± 2.3 <sup>c</sup>	#	0.90
C <sub>1</sub> : 15	10.1 ± 0.8 <sup>c</sup>	0.68	7 ± 3.6 <sup>a</sup>	#	0.90
C <sub>2</sub> : 75	14.0 ± 1.3 <sup>c</sup>	0.67	13 ± 2.6 <sup>c</sup>	#	0.90
C <sub>3</sub> : 350	10.7 ± 1.0 <sup>c</sup>	0.63	11 ± 2.1 <sup>c</sup>	#	0.94
C <sub>4</sub> : 1400	8.1 ± 0.7 <sup>c</sup>	0.70	9 ± 5.5*	#	0.78

493 # According to the equation fitting the loss kinetics ( $A_t = A_{0s} e^{-k_{es} t} + A_{0l}$ ) this parameter = 0

494 Significance of the estimated parameters: <sup>a</sup> p < 0.05, <sup>b</sup> p < 0.001, <sup>c</sup> p < 0.0001, \* p > 0.05

495



496 **Table 2.** Concentration factors (mean CF  $\pm$  SD, ml g<sup>-1</sup> dry wt; n = 5 per species per  
 497 concentrations tested) of <sup>63</sup>Ni in the clam *Gafrarium tumidum* and the oysters *Isognomon*  
 498 *isognomon* and *Malleus regula* exposed for 14 d to 5 increasing dissolved Ni concentrations  
 499 (C<sub>0</sub>-C<sub>4</sub>).

Body compartments	C <sub>0</sub>	C <sub>1</sub>	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>
	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD
<b><i>G. tumidum</i></b>					
Shell	2.0 $\pm$ 0.4	2.0 $\pm$ 0.3	2.2 $\pm$ 0.1	2.0 $\pm$ 0.2	1.6 $\pm$ 0.3
Whole soft parts	129 $\pm$ 61	94 $\pm$ 19	87 $\pm$ 7.3	90 $\pm$ 25	70 $\pm$ 11
Digestive gland	620 $\pm$ 200	306 $\pm$ 126	286 $\pm$ 31	356 $\pm$ 43	235 $\pm$ 129
Gills	206 $\pm$ 11	151 $\pm$ 43	176 $\pm$ 41	164 $\pm$ 12	112 $\pm$ 24
Mantle	114 $\pm$ 52	69 $\pm$ 13	62 $\pm$ 9.4	75 $\pm$ 47	58 $\pm$ 7.1
Muscle	52 $\pm$ 18	36 $\pm$ 3.2	32 $\pm$ 8.8	42 $\pm$ 33	32 $\pm$ 3.1
Foot	17 $\pm$ 11	27 $\pm$ 6.4	29 $\pm$ 13	18 $\pm$ 4.2	16 $\pm$ 2.1
Remaining tissues	123 $\pm$ 50	96 $\pm$ 34	62 $\pm$ 11	93 $\pm$ 48	64 $\pm$ 20
<b><i>I. isognomon</i></b>					
Shell	1.4 $\pm$ 0.1	1.6 $\pm$ 0.4	1.2 $\pm$ 0.4	1.2 $\pm$ 0.2	0.9 $\pm$ 0.1
Whole soft parts	213 $\pm$ 70	262 $\pm$ 20	284 $\pm$ 105	171 $\pm$ 61	143 $\pm$ 52
Visceral mass + Mantle	232 $\pm$ 74	295 $\pm$ 35	305 $\pm$ 116	175 $\pm$ 71	155 $\pm$ 70
Gills	456 $\pm$ 177	519 $\pm$ 93	660 $\pm$ 265	460 $\pm$ 148	338 $\pm$ 91
Muscle	110 $\pm$ 8.3	77 $\pm$ 30	85 $\pm$ 34	113 $\pm$ 19	75 $\pm$ 47
<b><i>M. regula</i></b>					
Shell	42 $\pm$ 29	47 $\pm$ 35	53 $\pm$ 36	60 $\pm$ 48	67 $\pm$ 24
Whole soft parts	183 $\pm$ 76	133 $\pm$ 53	205 $\pm$ 107	136 $\pm$ 19	106 $\pm$ 24
Visceral mass + Mantle	249 $\pm$ 98	1901 $\pm$ 93	265 $\pm$ 161	186 $\pm$ 12	178 $\pm$ 43
Gills	176 $\pm$ 134	77 $\pm$ 15	94 $\pm$ 12	79 $\pm$ 13	38 $\pm$ 15
Muscle	69 $\pm$ 63	54 $\pm$ 23	101 $\pm$ 61	67 $\pm$ 48	43 $\pm$ 28

**Table 3.** Assimilation efficiency (AE, %), loss rate constant ( $k_e$ ,  $d^{-1}$ ) and biological half life ( $T_{b\frac{1}{2}}$ , d) of  $^{63}\text{Ni}$  in whole soft parts of the clam *Gafrarium tumidum* and the oyster *Isognomon isognomon*, after a 2-hr feeding on radiolabelled *Isochrysis galbana* cells.

ASE: asymptotic standard error;  $R^2$ : determination coefficient of the loss kinetics

Species	AE $\pm$ ASE	$k_e \pm$ ASE	$T_{b\frac{1}{2}} \pm$ SD	$R^2$
<i>G. tumidum</i>	61.2 $\pm$ 4.5 <sup>b</sup>	0.019 $\pm$ 0.04 <sup>b</sup>	35 $\pm$ 7	0.49
<i>I. isognomon</i>	17.1 $\pm$ 6.9 <sup>a</sup>	0.0001 $\pm$ 0.015 <sup>*</sup>	n.s.i.	0.59

Significance of the estimated parameters <sup>a</sup>  $p < 0.05$ , <sup>b</sup>  $p < 0.0001$ , \*  $p > 0.05$

n.s.i.:  $T_{b\frac{1}{2}}$  not significantly different from infinite

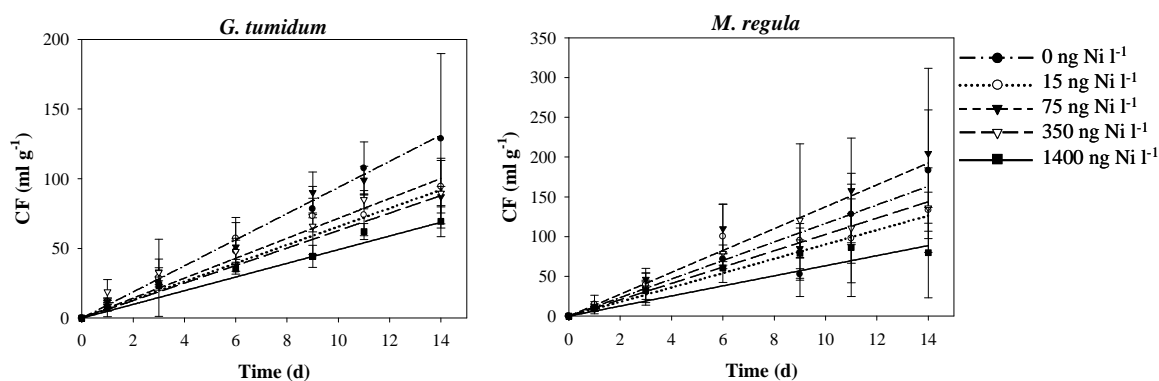
**Figure 1.** Uptake and loss kinetics of  $^{63}\text{Ni}$  in whole soft parts of the investigated bivalves.

(A) Uptake kinetics (mean concentration factor,  $\text{CF} \pm \text{SD}$ ,  $n = 3$ ) (A-1) and loss kinetics (mean % remaining activity  $\pm \text{SD}$ ,  $n = 3$ ) (A-2) in the clam *Gafrarium tumidum* and the oyster *Malleus regula* exposed to 5 increasing dissolved Ni concentrations.

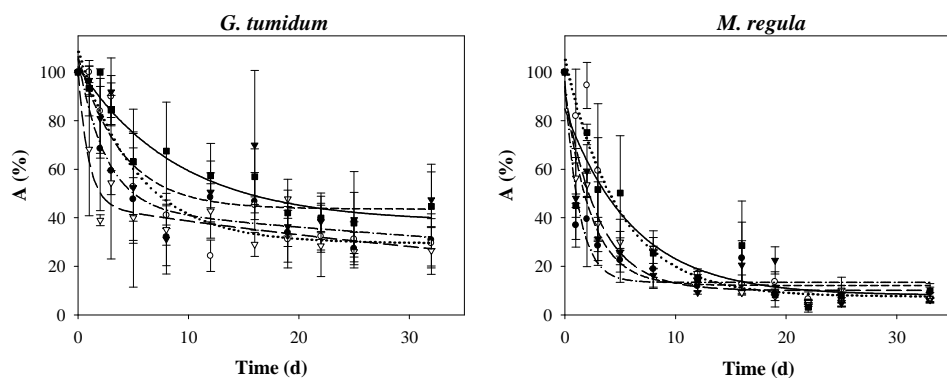
(B) Loss kinetics (mean % remaining activity  $\pm \text{SD}$ ,  $n = 14$ ) in the clam *G. tumidum* and the oyster *Isognomon isognomon* after a 2-hr feeding on  $^{63}\text{Ni}$ -labelled *Isochrysis galbana*.

## A- SEAWATER

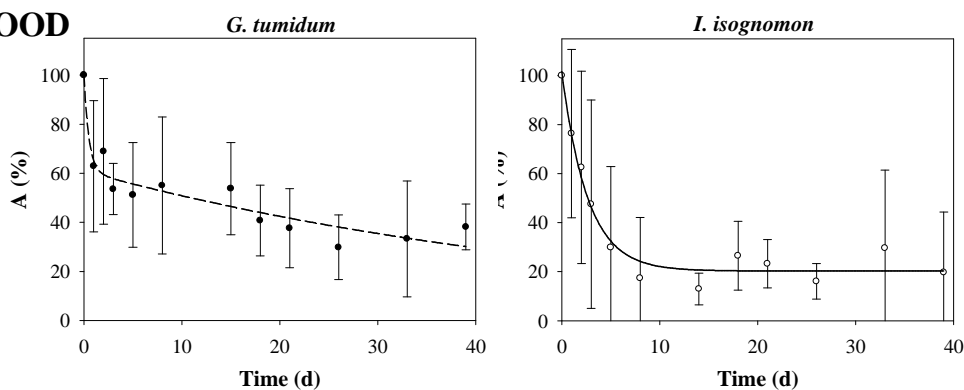
### A-1. UPTAKE



### A-2. LOSS



## B- FOOD



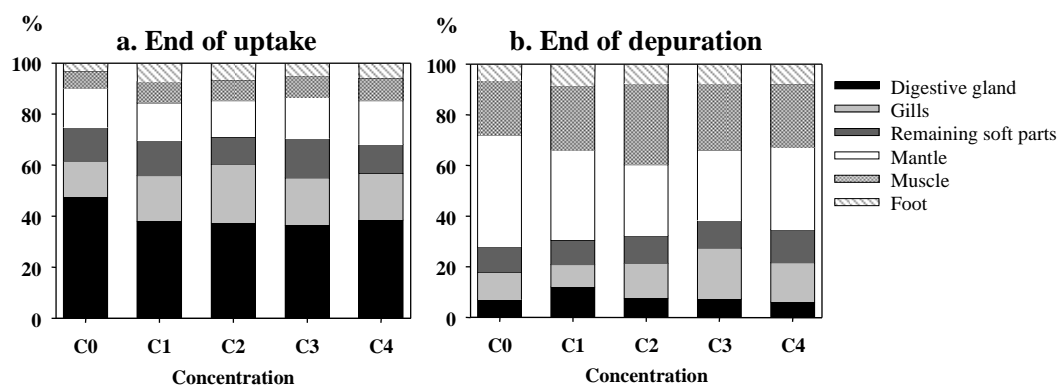
**Figure 2.** Distribution of  $^{63}\text{Ni}$  (mean %) among the body compartments of clams and oysters.

(A) Body distribution (n = 5) in the seawater experiments in (A-1) the clam *Gafrarium tumidum* after a 14-d exposure to 5 increasing dissolved Ni concentrations (end of uptake) and a subsequent 32-d depuration period (end of depuration), and (A-2) the oysters *Isognomon isognomon* and *Malleus regula* after a 14-d exposure to the 5 dissolved Ni concentrations.

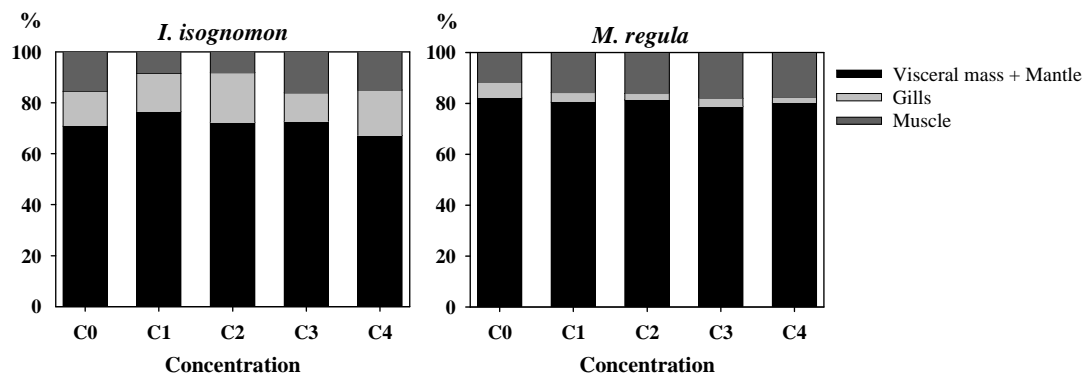
(B) Body distribution (n = 14) in the clam *G. tumidum* and the oyster *I. isognomon*, 12 and 46 d after a 2-hr feeding on  $^3\text{Ni}$ -labelled *Isochrysis galbana* cells.

## A- SEAWATER

### A-1. Clams



### A-2. Oysters



## B- FOOD

