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

L. Hédouin [a,b,c,*], O. Pringault [b], M. Metian [a,c], P. Bustamante [c], M. Warnau [a]

[a] *International Atomic Energy Agency, Marine Environment Laboratory, 4 Quai Antoine Ier, MC-98000, Monaco*
[b] *Institut de Recherche pour le Développement, BP A5, 98848 Nouméa, New Caledonia*
[c] *Centre de Recherche sur les Ecosystèmes Littoraux Anthropisés, UMR-6217 CNRS/IFREMER/Université de La Rochelle, 22, Avenue M. Crépeau, F-17042 La Rochelle, France*

Abstract

The New Caledonian lagoon is submitted to intense heavy metal input from land-based Ni mining. Therefore, the use of sentinel species is strongly recommended in order to develop and implement coastal zone management programmes of the metal contamination. The tropical oysters *Isognomon isognomon* and *Malleus regula* and the clam *Gafrarium tumidum* were previously proposed as such possible sentinel organisms and were thus investigated in this context. The three species were exposed to Ni via seawater or food using radiotracer techniques. Results indicate that uptake and retention efficiencies of Ni are independent of the dissolved Ni concentrations in the surrounding seawater. Hence, for the three species, body concentrations of Ni taken up from the dissolved phase are directly proportional to the ambient dissolved concentrations. Biokinetic patterns indicated that the major part of Ni was rapidly lost from bivalves during the first days of depuration, whereas 7 to 47% of [63]Ni were retained in tissues with a biological half-life not significantly different from infinity. Finally, feeding experiments showed that Ni ingested with food (phytoplankton) was assimilated more efficiently in clams (assimilation efficiency, AE = 61%) than in oysters (AE = 17%), and was strongly retained ($T_{b1/2} \geqslant 35$ d) in the tissues of both bivalves. It is concluded that the investigated species examined are efficient bioaccumulators of Ni from both the surrounding seawater and the food, and that they would be useful bioindicators for monitoring the status of Ni contamination in tropical coastal waters.
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1. Introduction

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

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

Mining activities and their development represent an issue of concern in New Caledonia, due to a series of factors including increasing deforestation, soil erosion, extinction of endemic species and increasing Ni contamination in

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* Corresponding author. Address: International Atomic Energy Agency, Marine Environment Laboratory, 4 Quai Antoine Ier, MC-98000, Monaco. Tel.: +377 97 97 72 58; fax: +377 97 97 72 76.
   E-mail address: hedouinlaetitia@yahoo.fr (L. Hédouin).

local waters (Bird et al., 1984; Labrosse et al., 2000). However, to the best of our knowledge, information available on impacts of open-cast mining in the marine coastal ecosystems of New Caledonia is extremely scarce (Monniot et al., 1994; Breau, 2003; Hédouin et al., 2006).

Among the common approaches used to survey environmental contamination, the use of bioindicator species has proven to be a valuable and informative tool (Goldberg et al., 1983; Phillips, 1990). In order to develop a biomonitoring programme to assess the levels of metal contamination in New Caledonia lagoon, studies have been recently undertaken to screen local species for their bioindicative potential (Breau, 2003). In particular, both laboratory and field studies have shown that two oysters (*Isognomon isognomon* and *Malleus regula*) and one clam (*Gafrarium tumidum*) efficiently concentrate several elements (Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn) and that they are able to discriminate among locations subject to contrasting levels of contamination (Hédouin, 2006). However, current information on Ni bioaccumulation is rather limited; in particular no data are available on the tissue distribution or bioaccumulation kinetics of Ni taken up through the dissolved and dietary pathways in these organisms. Therefore, the objective of the present study was to determine the bioaccumulation capacity of Ni from food and seawater in these three bivalves in order to assess their potential as bioindicators of environmental Ni contamination.

2. Materials and methods

2.1. Collection and acclimation

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

Oysters and clams were acclimated to laboratory conditions for one week prior to the experiments (close circuit aquarium; daily water renewal, salinity: 35 ± 1 psu; temperature = 25 ± 1 °C; pH = 8.0 ± 0.1; light/dark cycle: 12 h/ 12 h). Since body size is known to affect metal concentrations in organisms (Boyden, 1977), only individuals with

shell width $\geqslant 35$ mm for *G. tumidum* and shell length $\geqslant 70$ mm for *I. isognomon* and *M. regula* were used in the experiments (Metian, 2003; Hédouin et al., 2006).

2.2. Testing influence of dissolved Ni concentrations

Five groups of 51 *G. tumidum* clams (shell width from 35 to 44 mm; wet wt from 15 to 38 g), five groups of 51 *M. regula* oysters (shell length from 85 to 135 mm; wet wt from 14 to 48 g) and five groups of 5 *I. isognomon* oysters (shell length from 80 to 140 mm; wet wt from 13 to 45 g) were placed in 5 aquaria of 50 l of natural seawater (close circuit aquarium; salinity: 35 ± 1 psu; temperature = 25 ± 1 °C; pH = 8.0 ± 0.1; light/dark cycle: 12 h/12 h). Seawater salinity, temperature and pH were checked twice daily during the experiment.

Each group of bivalves was exposed for 14 d to five added Ni concentrations (0, 15, 75, 350 and 1400 ng Ni l^{-1}). The concentrations of Ni were adjusted using increasing amounts of Ni(NO$_3$)$_2$ (Merck, synthesis quality) and a fixed activity (1 kBq l^{-1}) of the corresponding radiotracer ^{63}Ni, as high specific activity ^{63}NiCl$_2$ ($T_{1/2} = 100$ yrs) purchased from Amersham, UK. This radiotracer spike corresponded to 4.2 ng stable Ni l^{-1}, a concentration at least 1 order of magnitude lower than the background concentrations of Ni in open seas (Bruland, 1983). No pH variation was detectable after stable metal and radiotracer addition. Seawater and spikes were renewed daily for 5 d, then every second day in order to keep exposure concentration and radioactivity levels as constant as possible. Activity of the radiotracer in seawater was checked daily, and before and after each seawater renewal, to determine its time-integrated activity (Warnau et al., 1996). For the entire experimental time course, the time-integrated ^{63}Ni activity in seawater was 0.81 kBq l^{-1}.

During the exposure period, 3 individuals of *G. tumidum* and *M. regula* were collected after different time intervals; soft tissues were separated from shells and prepared for whole soft tissues radioanalysis (see section 2.4.). The last day (t_{14d}), 5 individuals of *G. tumidum, M. regula* and *I. isognomon* were dissected to determine body distribution of incorporated ^{63}Ni. Dissected body compartments were digestive gland, gills, mantle, foot, muscle and remaining soft tissues for clams and visceral mass + mantle, gills and muscle for oysters.

At the end of the 14-d exposure, the remaining organisms were placed in $60 \times 60 \times 60$ cm plastic cages for 32 d in Sainte-Marie Bay, Nouméa, New Caledonia at 7 m depth. The selected area (22°18′55 S, 166°27′98 E) is characterized by relatively low Ni levels (Hédouin, 2006). At different time intervals of the depuration period, for each concentration tested, 3 *G. tumidum* and 3 *M. regula* individuals were collected, the soft tissues dissected from shells and then prepared for soft parts radioanalysis. The last day (t_{32d}), 5 *G. tumidum* were dissected into different body compartments to determine the tissue distribution of the remaining ^{63}Ni.

2.3. Exposure via the food

Cells of the Prymnesiophyceae *Isochrysis galbana* (10^3 cell ml^{-1}) which originated from axenic stock cultures were resuspended in an Erlenmeyer flask containing 5 l sterile-filtered (0.22 μm) seawater enriched with f/2 nutrients without EDTA and Si. Seawater was spiked with ^{63}Ni (5 kBq l^{-1}), and the cells were then incubated at 25 °C (light/dark cycle: 12 hrs/12 hrs). After 6 d of incubation, cell density increased from 10^3 to $1.3 \ 10^6$ cell ml^{-1}. A sample of 10 ml of the culture was then gently filtered (47 mm diameter Polycarbonate Nuclepore$^{®}$ filter, 1 μm mesh size) and the radioactivity associated with *I. galbana* cells was mesured before and after the filtration ($3.4 \ 10^{-6}$ Bq ^{63}Ni cell^{-1}).

Bivalves ($n = 196$ *G. tumidum*, $n = 196$ *I. isognomon*) were placed in a 300 l aquarium (close circuit aquarium constantly aerated; salinity: 35 ± 1 psu; temperature = 25 ± 1 °C; pH = 8.0 ± 0.1) and fed the radiolabelled *I. galbana* for 2 hrs (10^4 cell ml^{-1}). Immediately after feeding, 14 individuals per species were collected and dissected to separate whole soft parts from shells.

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

2.4. Sample preparation and Radioanalyses

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

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

2.5. Data analyses

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

$$CF_t = k_u t \tag{1}$$
$$CF_t = CF_{ss}(1 - e^{-k_e t}) \tag{2}$$

where CF_t and CF_{ss} are the concentration factors at time $t(d)$ and at steady state (ml g^{-1}), ($CF_{ss} = \frac{k_u}{k_e}$); k_u is the uptake rate constant (ml g^{-1} d^{-1}) and k_e is the depuration rate constant (d^{-1}) (Whicker and Schultz, 1982; Thomann et al., 1995). Linearity of the uptake kinetics was tested by a linearity test for regression with replication (Zar, 1996).

Loss of ^{63}Ni was expressed in term of percentage of remaining radioactivity (radioactivity at time t divided by initial radioactivity measured in the organisms immediately after the feeding period). The loss kinetics were best fitted by either a single-component exponential equation (Eq. (3)), a single-component exponential equation with an additional constant term (Eq. (4)), or a double-component exponential equation (Eq. (5)):

$$A_t = A_0 k_e t \tag{3}$$
$$A_t = A_{0s}e^{-k_{es}t} + A_{0l} \tag{4}$$
$$A_t = A_{0s}e^{-k_{es}t} + A_{0l}e^{-k_{el}t} \tag{5}$$

where A_t and A_0 are the remaining activities (%) at time t (d) and 0, respectively; k_e is the depuration rate constant (d^{-1}); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components. For each exponential component (s and l), a biological half-life can be calculated ($T_{b1/2s}$ and $T_{b1/2l}$) from the corresponding depuration rate constant (k_{es} and k_{el}, respectively) according to the relation $T_{b1/2} = \frac{\ln 2}{k_e}$. The additional constant term of Eq. (4) represents a fraction A_{0l} of the radiotracer incorporated that is sequestrated in the organism tissues.

Regarding feeding experiments, the 'long-lived' exponential term describes the proportion of the radiotracer ingested with food that is actually absorbed by the organism and slowly eliminated. The corresponding A_{0l} represents the assimilation efficiency (AE) of the considered element.

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

In order to assess possible effect of dissolved Ni concentration on bioconcentration behaviour, estimated kinetic parameters (k_u, CF_{ss}, A_{0l}, k_{el}) were plotted against the concentration of total Ni (stable + stable equivalent of added radiotracer) in seawater and were fitted using simple linear regression. Statistical comparisons were also performed

using 1-way ANOVA followed by the multiple comparison test of Tukey (Zar, 1996).

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

3. Results

3.1. Seawater exposure to different Ni concentrations

Uptake of ^{63}Ni in whole soft parts of the clam *G. tumidum* and the oyster *M. regula* displayed linear kinetics at all of the five exposure concentrations tested ($p < 0.0001$, R^2: 0.79–0.92 for *G. tumidum* and 0.63–0.72 for *M. regula*) (Table 1 and Fig. 1-A-1). Statistical analysis indicated that uptake rate constants k_u in both clams and oysters did not differ significantly over the range of concentrations tested.

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

- *G. tumidum*: digestive gland (CF_{14d} up to 620) > gills > remaining tissues > mantle > muscle ≈ foot,
- *I. isognomon*: gills (CF_{14d} up to 660) > visceral mass + mantle > muscle,
- *M. regula*: visceral mass + mantle (CF_{14d} up to 270) > gills ≈ muscle.

In general, no significant difference was found among CF_{14d} in whole soft parts as well as in body compartments over the range of concentrations tested. The only exception was *I. isognomon*, for which the CF_{14d} calculated in whole soft parts, gills and visceral mass + mantle for the highest Ni concentration (1400 ng added Ni l^{-1}) were found to be significantly different from the ones calculated for 75 ng added Ni l^{-1} ($p_{Tukey} = 0.026$, 0.046 and 0.043, respectively).

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

In terms of body load distribution, ^{63}Ni was mainly found in the digestive gland for clams (36–47% of total body load; Fig. 2-A-1) and in the visceral mass + mantle for both oysters (67–82% of total body load; Fig. 2-A-2). The body distribution of ^{63}Ni was similar over the entire range of concentrations tested.

At the end of the exposure time, non-contaminating conditions were restored and loss kinetics of ^{63}Ni were followed in the field for 32d. Loss kinetics from whole soft parts were best described by a double exponential model in *G. tumidum*, whereas a single-component exponential equation with an additional constant term best fitted the loss kinetics in *M. regula* (Table 1 and Fig. 1-A-2).

Table 1
Estimated uptake rate constant (k_u, ml g^{-1} dry wt d^{-1}), absorption efficiency (A_{0l},%) and loss rate constant (k_{el}, d^{-1}) of ^{63}Ni in the whole soft parts of the clam *Gafrarium tumidum* and the oyster *Malleus regula* exposed to five different dissolved Ni concentrations via seawater for 14 d (uptake period) and then maintained for 32 d in the field in a clean site (depuration period)

Concentrations (ng added Ni l^{-1})	Uptake period		Depuration period		
	$k_u \pm ASE$	R^2	$A_{0l} \pm ASE$	$k_{el} \pm ASE$	R^2
G. tumidum					
C_0: 0	9.1 ± 0.6^c	0.79	44 ± 11^b	$0.010 \pm 0.013^*$	0.72
C_1: 15	7.2 ± 0.4^c	0.83	$27 \pm 20^*$	$0.002 \pm 0.028^*$	0.84
C_2: 75	7.7 ± 0.4^c	0.82	45 ± 18^a	$0.002 \pm 0.017^*$	0.60
C_3: 350	7.1 ± 0.4^c	0.82	47 ± 7^c	$0.019 \pm 0.009^*$	0.70
C_4: 1400	5.2 ± 0.2^c	0.92	35 ± 3^c	$0.001 \pm 0.006^*$	0.77
M. regula					
C_0: 0	11.5 ± 1.0^c	0.72	14 ± 2.3^c	#	0.90
C_1: 15	10.1 ± 0.8^c	0.68	7 ± 3.6^a	#	0.90
C_2: 75	14.0 ± 1.3^c	0.67	13 ± 2.6^c	#	0.90
C_3: 350	10.7 ± 1.0^c	0.63	11 ± 2.1^c	#	0.94
C_4: 1400	8.1 ± 0.7^c	0.70	$9 \pm 5.5^*$	#	0.78

According to the equation fitting the loss kinetics ($A_t = A_{0s}e^{-k_{es}t} + A_{0l}$) this parameter = 0. Significance of the estimated parameters.
ASE: asymptotic standard error; R^2: determination coefficient of the uptake and loss kinetics.
[*] $p > 0.05$.
[a] $p < 0.05$.
[b] $p < 0.001$.
[c] $p < 0.0001$.

A- SEAWATER

A-1. UPTAKE
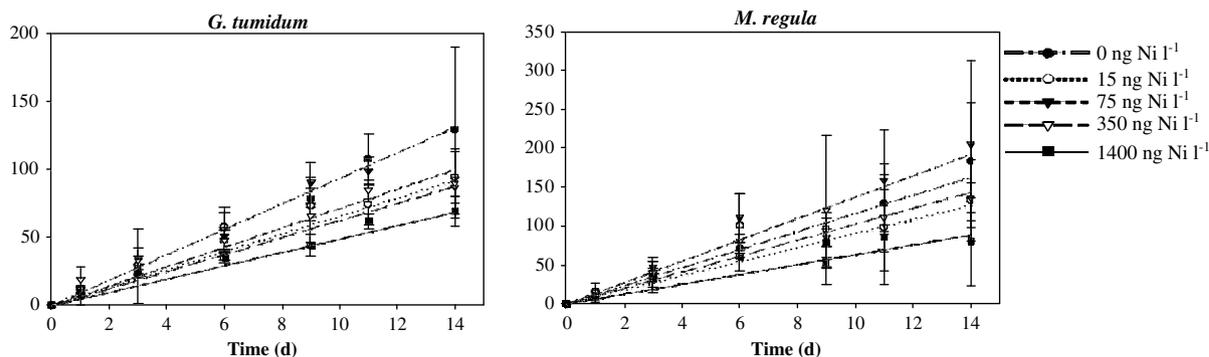
A-2. LOSS
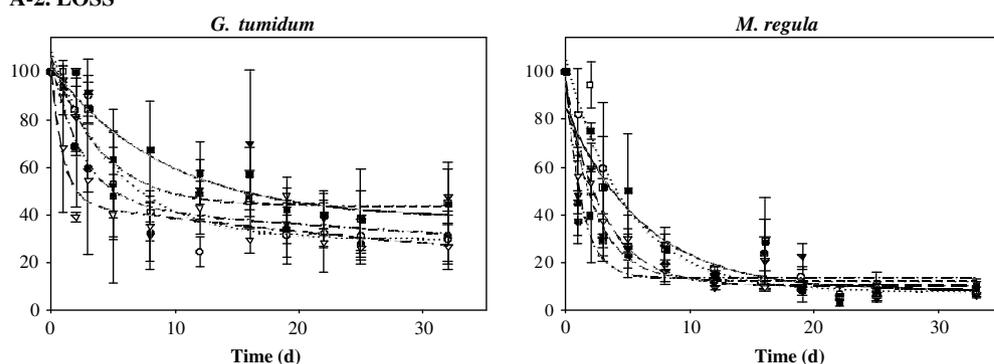
B- FOOD
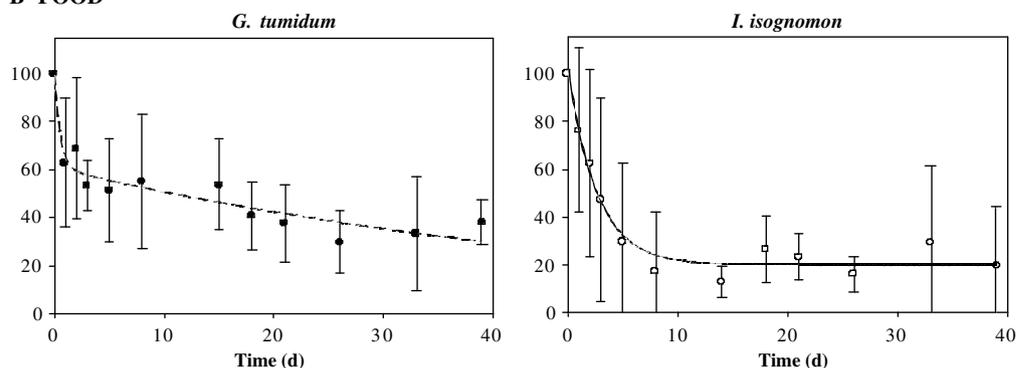
Fig. 1. Uptake and loss kinetics of [63]Ni in whole soft parts of the investigated bivalves. (A) Uptake kinetics (mean concentration factor, CF ± SD, $n = 3$) (A-1) and loss kinetics (mean% remaining activity ± SD, $n = 3$) (A-2) in the clam *Gafrarium tumidum* and the oyster *Malleus regula* exposed to 5 different dissolved Ni concentrations. (B) Loss kinetics (mean% remaining activity ± SD, $n = 14$) in the clam *G. tumidum* and the oyster *Isognomon isognomon* after a 2-hr feeding on [63]Ni-labelled *Isochrysis galbana*.

A relatively small fraction (<14%) of [63]Ni was lost from the long-lived component in *M. regula*, whereas this component contained 27–47% of [63]Ni in *G. tumidum* (Table 1). However, in both species, the estimated loss rate constants of the long-lived components (k_{el}) were not significantly different from 0 ($p > 0.05$), and therefore the derived biological half-lives of [63]Ni in clams and oysters were virtually infinite at all the exposure concentrations tested. In addition, in both species, linear regressions established between estimated A_{0l} and exposure concentrations displayed slopes not significantly different from 0 for both clams ($p = 0.71$) and oysters

($p = 0.34$), indicating that [63]Ni was assimilated similarly (in relative%) in each species regardless of the exposure concentration.

The distribution of [63]Ni among the body compartments of the clam was determined at the end of the depuration period (Fig. 2-A-1). [63]Ni was mainly associated with the mantle (27–44%) and the muscle (22–32%). Distributions were similar for the different exposure treatments, but differed from the distributions observed at the end of the exposure period, with lower fraction associated with digestive gland and higher fractions associated with mantle and muscle.

Table 2
Concentration factors (mean CF $\pm$ SD, ml g^{-1} dry wt; $n = 5$ per species per concentrations tested) of ^{63}Ni in the clam *Gafrarium tumidum* and the oysters *Isognomon isognomon* and *Malleus regula* exposed for 14 d to 5 different dissolved Ni concentrations (C$_0$–C$_4$)

Body compartments	C$_0$	C$_1$	C$_2$	C$_3$	C$_4$
	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD	mean $\pm$ SD
G. tumidum					
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	240 $\pm$ 130
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
I. isognomon					
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	280 $\pm$ 110	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	460 $\pm$ 180	519 $\pm$ 93	660 $\pm$ 270	460 $\pm$ 150	338 $\pm$ 91
Muscle	110 $\pm$ 8.3	77 $\pm$ 30	85 $\pm$ 34	113 $\pm$ 19	75 $\pm$ 47
M. regula					
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	210 $\pm$ 110	136 $\pm$ 19	106 $\pm$ 24
Visceral mass + Mantle	249 $\pm$ 98	1901 $\pm$ 93	270 $\pm$ 160	186 $\pm$ 12	178 $\pm$ 43
Gills	180 $\pm$ 130	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

3.2. Food exposure

The loss kinetics of ^{63}Ni ingested with food in both the clam *G. tumidum* and the oyster *I. isognomon* were best fitted using a double exponential model ($R^2 = 0.49$ and 0.59, respectively) (Table 3 and Fig. 1-B). A substantial part of the ^{63}Ni activity (39% for the clam and 83% for the oyster) was rapidly lost via defecation ($T_{b1/2} < 2$ d). Assimilation efficiency (AE) of ^{63}Ni was 61% and 17% in clams and oysters, respectively. These assimilated fractions were retained with $T_{b1/2}$ of 35 d in clams and a time not different from the infinite in oysters. During the depuration period, the highest proportion of ^{63}Ni (74%) was associated with visceral mass + mantle in *I. isognomon* whereas in *G. tumidum* each organ contributed similarly to the total ^{63}Ni content (Fig. 2-B). In both clam and oyster, ^{63}Ni body distribution showed no major difference between the two sampling times (12 and 46 d).

4. Discussion

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

4.1. Seawater pathway

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

Previous experimental investigations of Ni bioaccumulation in marine organisms generally considered exposure concentrations (e.g. up to 80 mg Ni l^{-1}, Friedrich and Fillice, 1976) that were far above (several orders of magnitude) the natural background ones. The concentrations tested in the present study (up to 1400 ng added Ni l^{-1}) were selected in order to include the entire concentration range which can be measured in the coastal waters of the New Caledonian lagoon (Fernandez et al., 2002). Results showed that the bioconcentration of Ni in the clam *G. tumidum* and in both oysters *M. regula* and *I. isognomon* was directly proportional to the Ni concentration in seawater virtually over the entire range of Ni concentrations tested. Similar observations were made for other elements accumulated by *I. isognomon* and *G. tumidum* such as Co, Cr, Mn also present in Ni ores (Hédouin, 2006). The capacity of Ni bioconcentration reported here in clams and oysters (CF$_{14d}$ ranging from 70 to 280 ml g^{-1} dry wt) was quite lower than that determined in comparable experimental conditions for some metals such as Ag and Zn, which reached CF values up to 200 000 ml g^{-1} dry wt (Hédouin, 2006). However, the observed CFs are in the range of those reported in previous studies related to Ni in bivalves (4 ml g^{-1} dry wt in the clam *Prototheca staminea*, Hardy and Roesijadi, 1982; from 10 to 607 ml g^{-1} dry wt in the mussel *Mytilus edulis*, Friedrich and Fillice, 1976; from 156 to 336 ml g^{-1} wet wt in

A- SEAWATER

A-1. Clams
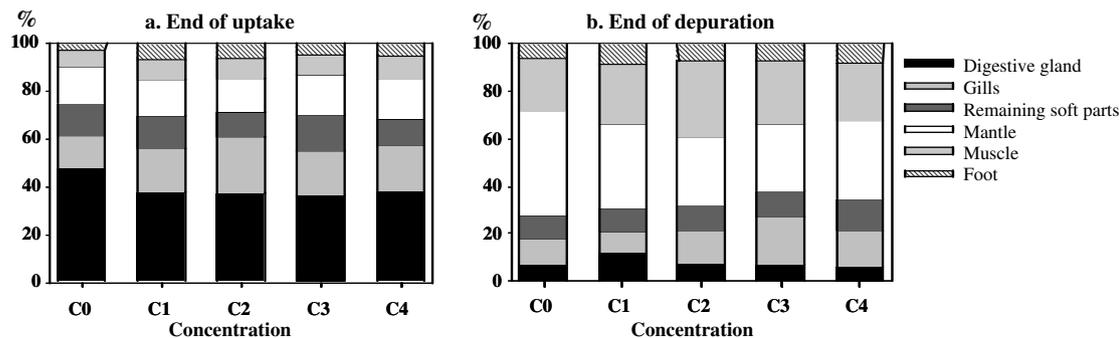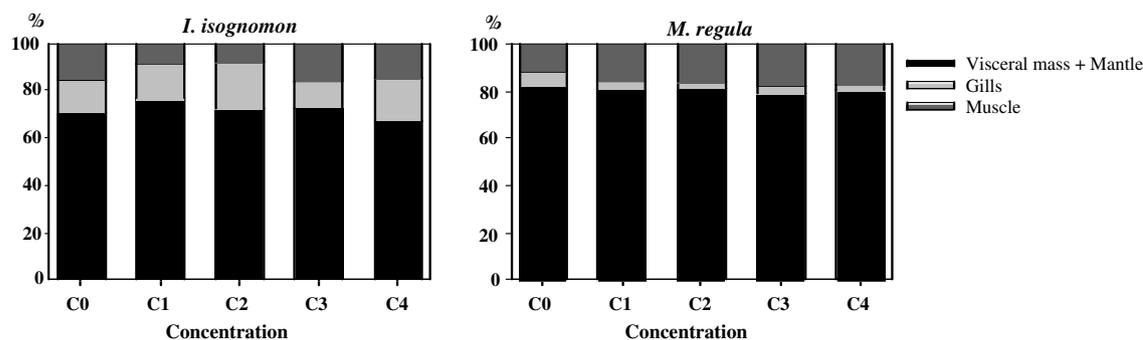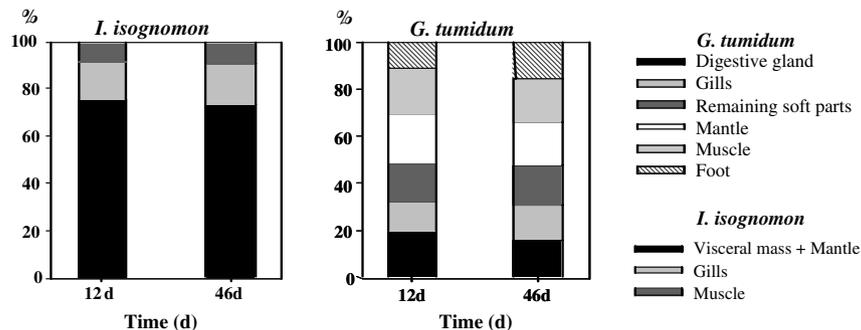
Fig. 2. Distribution of ^{63}Ni (mean%) among the body compartments of clams and oysters. (A) Body distribution ($n = 5$) in the seawater experiments in (A-1) the clam *Gafrarium tumidum* after a 14-d exposure to 5 different dissolved Ni concentrations (end of uptake) and a subsequent 32-d depuration 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}Ni-labelled *Isochrysis galbana* cells.

Crassostrea virginica and *M. edulis*, Zaroogian and Johnson, 1984).

Results indicated that, in relative units, loss kinetics of Ni from the soft parts of *G. tumidum* and *M. regula* were also independent on the Ni concentrations to which the organisms were previously exposed. These observations are in agreement with those of Zaroogian and Johnson (1984) who reported that the loss rate of Ni was similar in oysters exposed to two different Ni treatments (5000 and 10 000 ng Ni l^{-1}). In addition, our study showed that Ni was efficiently retained in both bivalve species, with biological half-lives not significantly different from infinity. These values should of course be considered with caution

due to the relatively short duration of the experiment (32d). Nevertheless, they clearly indicate that both clams and oysters would be able to preserve information regarding their contamination history over a very long timescale (probably several months).

The very close resemblance of the two oyster species *I. isognomon* and *M. regula*, both in their appearance and ecophysiology (Yonge, 1968) was also reflected in their bioaccumulation and depuration capacities for Co, Cr, Zn and, to a lesser extent, Cd (Hédouin, 2006). Similarly, the present work indicated that bioconcentration of dissolved Ni was quite similar in both species on a whole-body basis, although a slight decrease in CF was observed in *I. isognomon* at the

Table 3
Assimilation efficiency (AE,%), loss rate constant (k_{el}, d^{-1}) and biological half life ($T_{b1/2l}$, d) of ^{63}Ni in whole soft parts of the clam *Gafrarium tumidum* and the oyster *Isognomon isognomon*, after a 2-h feeding on radiolabelled *Isochrysis galbana* cells

Species	AE ± ASE	k_e ± ASE	$T_{b1/2}$ ± ASE	R^2
G. tumidum	61.2 ± 4.5[b]	0.019 ± 0.04[b]	35 ± 7	0.49
I. isognomon	17.1 ± 6.9[a]	0.0001 ± 0.015[*]	n.s.i.	0.59

Significance of the estimated parameters.

n.s.i.: $T_{b1/2}$ not significantly different from the infinity.

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

[*] $p > 0.05$.

[a] $p < 0.05$.

[b] $p < 0.0001$.

highest Ni concentration tested. However, examination of CF values at the organ level indicated that gills of *I. isognomon* concentrate Ni much more efficiently (up to one order of magnitude) than those of *M. regula*. This suggests that although these two species are very closely related in many aspects at a macroscopic scale, mechanisms controlling Ni uptake could be quite different in *I. isognomon* and *M. regula*. Furthermore, previous studies have observed that gills are generally a major site of Ni intake in bivalves (e.g. Hardy and Roesijadi, 1982; Wilson, 1983), indicating that this dissimilarity between these two oysters deserves further investigation.

4.2. Food pathway

Although it is now well documented that assimilation of metals ingested with food plays an important role in their bioaccumulation in marine organisms (e.g. Wang and Fisher, 1999b), very few studies have been devoted to trophic transfer of Ni. Kumblad et al. (2005) have shown that assimilation efficiency (AE) of sediment-associated ^{63}Ni (II) ranged from 43% to 49% in the clam *Macoma balthica*, the amphipod *Monoporeia affinis* and the priapulid *Halicryptus spinulosus*. In the present study, the estimated AE of ^{63}Ni was much higher in the clam *G. tumidum* (61%) than in the oyster *I. isognomon* (17%). Such a low assimilation in *I. isognomon* compared to *G. tumidum* as well as to the species studied by Kumblad et al. (2005) could be related to the differences in feeding physiology (e.g. digestion efficiency) of these different organisms (Lee, 1991; Mayer et al., 2001). However, in a recent study on other metals, AEs reaching values up to 77% were found for Ag in *I. isognomon* (Hédouin, 2006), suggesting that low AE is not a physiological characteristic of this species. Rather, the low assimilation of Ni in *I. isognomon* is likely related to the oyster behaviour towards Ni and/or how it is able to deal with the way Ni is bound to algal cells.

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

4.3. Conclusions

Within a range of dissolved Ni concentrations that cover the natural range occurring in New Caledonia seawaters, (1) Ni bioconcentration was directly proportional to the Ni concentration in seawater for *G. tumidum, M. regula* and, to a lesser extent, *I. isognomon*, and (2) the retention efficiency of Ni in clams and oysters was independent on the total Ni concentration previously accumulated by the organisms. In addition, clams and oysters were shown to efficiently assimilate Ni ingested with their food (in particular in clams) and retain it very efficiently (in particular in oysters). These characteristics suggest that these New Caledonian bivalves could be used to detect Ni contamination in their surrounding environment and to preserve this information over long periods of time. In addition, the clams and oysters displayed different bioaccumulation behaviour for Ni, especially when exposed via the food. Both groups would thus be worth further characterization with regard to their values as bioindicators for Ni contamination. Indeed, both bivalve groups could provide complementary ecotoxicological information as they interact differently with their environment.

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