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ABSTRACT. The bioaccumulation and retention capacities of some key local contaminants of the New Caledonia lagoon (Ag, As, Cd, Co, Cr, Cu, Mn, Ni and Zn) have been determined in the oyster *Isognomon isognomon* and the edible clam *Gafrarium tumidum* during transplantation experiments. In a first set of experiments, oysters and clams from a clean site were transplanted into contaminated sites. Uptake kinetics determined in the field indicated that for Cr and Cu in oysters and Co, Ni, and Zn in clams, concentrations in transplanted bivalves reached those of resident organisms after 100d, whereas for the other elements, it would require a longer time for transplanted bivalves to reach the same levels as in the resident populations (e.g., up to 3 years for Cd). However, the slow uptake rate for metals observed in the latter transplantation is rather related to low bioavailability of metals at the contaminated sites than to low bioaccumulation efficiency of the organisms. Indeed, results of a second transplantation experiment into two highly contaminated stations indicated a faster bioaccumulation of metals in both bivalves. Results of both transplantations point out that the clam *G. tumidum* is a more effective bioindicator of mining contamination than *I. isognomon*, since it is able to bioaccumulate the contaminants to a greater extent. However the very efficient metal retention capacity noted for most elements indicates that organisms originating from contaminated sites would not be suitable for monitoring areas of lower contamination. Hence, geographical origin of animals to be transplanted in a monitoring perspective should be carefully selected.

Keywords: Molluscs, Oyster, Clam, Bioaccumulation, Biomonitoring, Metals

I. Introduction

New Caledonia is a small South Pacific island whose main economic resources are derived from nickel exploitation. Among other, local mining activities result in large anthropogenic inputs of metals into the SW lagoon and thereby constitute a potential threat to the local coastal marine ecosystems (e.g., Bird et al. 1984) but it is only recently that relevant information was made available regarding levels of metal contamination and their possible impacts on the local marine ecosystems (e.g., Hédouin et al. 2009, Metian et al. 2008a). Therefore monitoring of environmental contamination originating from mining activities in the lagoon still needs some significant scientific inputs.

Among the common approaches used to study environmental contamination, the use of bivalve molluscs as bioindicator species has proved to be a valuable and informative technique (e.g., Mussel Watch, Goldberg et al. 1983). This approach has been particularly developed in temperate areas, whereas in sub-tropical and tropical areas the scarcity of available information makes the identification of species that could be used as suitable bioindicators difficult (e.g., Phillips 1991). However, the screening of metal concentrations in a variety of marine organisms from several parts of the SW lagoon of New Caledonia has identified the oyster *Isognomon isognomon*, the edible clam *Gafrarium tumidum*, and the alga *Lobophora variegata* as potential bioindicators (Hédouin et al. 2009). It has been shown recently that the alga *L. variegata* was an efficient bioindicator of metals in seawater in both controlled and *in situ* conditions (Hédouin et al. 2008, Metian et al. 2008b). In addition, recent experimental works on the oyster *I. isognomon* and the clam *G. tumidum* have indicated that these two species bioconcentrate and efficiently retain several elements when exposed via seawater, sediments or their food (Hédouin et al. 2010b). More

76 importantly, both bivalve species were shown to concentrate As, Cd, Co, Cr, Mn, Ni,
77 and Zn in direct proportion to their concentrations in seawater and food (Hédouin et
78 al. 2007, 2010b).

79 Although the former experiments were carried out under controlled conditions
80 simulating as closely as possible those in the natural environment, laboratory
81 experiments cannot reproduce exactly the conditions in the field. In this respect *in situ*
82 experiments offer a more ecologically-realistic approach, since they encompass all the
83 factors that actually occur in the field and may possibly interfere with or influence
84 bioaccumulation processes (e.g., Cain and Luoma 1985, Hédouin et al. 2008).

85 Active biomonitoring using transplantation of organisms from one site to another is a
86 very efficient way to follow the degree of contamination at various sites (e.g. Hédouin
87 et al. 2008). The main advantages over the traditional passive biomonitoring (viz.
88 monitoring of metal concentrations using resident natural populations) are that (1) the
89 sites to monitor may be chosen independently of the presence of natural populations
90 and (2) the influence of external and internal factors (e.g. seasonal variation, size or
91 age) susceptible to induce bias in data comparison is reduced (Phillips and Rainbow
92 1993).

93 The aim of the present field study was to determine the relevance of using the oyster
94 *I. isognomon* and the clam *G. tumidum* as bioindicator species of metal contamination
95 in tropical waters. Through two different field transplantation experiments, the ability
96 of both species to bioaccumulate and depurate 9 selected elements (Ag, As, Cd, Co,
97 Cr Cu, Mn, Ni and Zn) under natural conditions has been assessed as well as their
98 ability to inform about the contamination status of their surrounding environment. A
99 power analysis was also carried out to determine the sample size required to allow
100 differentiating among realistic field contamination levels.

II. Materials and methods

Between March and June 2005, two series of transplantation experiments were performed in New Caledonia using the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum*. Based on previous field results (Hédouin et al. 2009) sampling stations were selected according to their apparent degree of metal contamination. Maa Bay (subtidal station for oysters) and Ouano Beach (intertidal station for clams) were identified as clean stations with low element concentrations in bivalve tissues and sediments for all elements except As. In contrast, Boulari Bay (for oysters) and Grande Rade -GR_{Int}- (intertidal station for clams) were designated as highly contaminated stations (Fig. 1).

II.1. Experimental design

Since body size is well known to affect metal concentrations in marine invertebrates (e.g. Boyden 1977), only individuals with shell length longer than 70 mm for *I. isognomon* (Metian 2003) and shell width greater than 35 mm for *G. tumidum* (Hédouin et al. 2006) were considered in order to minimize size-related variability. Two types of transplantations were conducted. A first reciprocal transplantation aimed at assessing metal bioaccumulation and depuration processes in natural populations living in two contrasted environmental conditions (see Fig. 2). A second transplantation was conducted to test the ability of both selected species to inform about the contamination level of their surrounding environment in a heavily polluted area (unidirectional transplantation in Grande Rade; Fig. 2).

II.1.1. Experiment 1: Reciprocal transplantations

Eighty oysters and 80 clams were collected from the two selected clean stations, Maa Bay and Ouano Beach, respectively. A sub-sample of 10 organisms from each station

was used for determination of baseline concentrations of the 9 selected elements (Ag, As, Cd, Co, Cr Cu, Mn, Ni and Zn) at the beginning of the experiment. The remaining oysters and clams (n = 70 per species) were transplanted for 100 d to the heavily contaminated stations, Boulari Bay and Grande Rade, (GR_{Int}, intertidal station), respectively. The reciprocal transplantation was undertaken with another batch of 80 oysters and 80 clams collected in Boulari Bay and Grande Rade (GR_{Int}), respectively, and transplanted to the clean stations, Maa Bay (for oysters) or Ouano Beach (for clams).

Organisms (transplanted and control resident individuals) at each station were placed in plastic mesh cages (60 × 60 cm; 2-cm mesh size), which allowed free exchange of seawater. The plastic cages containing the oysters were placed at 5 m depth, which corresponds to their natural habitat; those with clams were fixed in an intertidal position and inserted within the sediments in order to reproduce to the best the living condition of the clams. In order to monitor possible natural variation in element concentrations at the different stations, resident organisms (n = 5 per species) and superficial sediments (top 3-cm layer) were sampled simultaneously with the transplanted organisms (n = 7) from clean and contaminated stations at different times. Oysters were collected by SCUBA diving and the clams by hand picking at low tide.

II.1.2. Experiment 2: Unidirectional transplantation in Grande Rade

Grande Rade is locally influenced by anthropogenic inputs from the 'Société Le Nickel' (SLN), a nickel processing plant. Two stations (GR_1 and GR_2) were chosen in Grande Rade for this experiment because they had different levels of metal contamination (Migon et al. 2007). GR_1 station is a highly polluted site due to its

proximity to the off-loading wharf of the SLN, whereas the second station GR_2, on the opposite side of the Rade just in front of the SLN factory, is less contaminated than GR_1 (Fig. 1).

The bivalves *I. isognomon* and *G. tumidum* (n = 140 per species) were collected from the clean stations Maa Bay and Ouano Beach, respectively. Twenty organisms were used for element analyses in order to establish the baseline concentrations of elements at day 0 of transplantation; the remaining organisms (n = 120 per species) were transplanted for 69 d into the two stations in Grande Rade (GR_1 and GR_2, n = 60 per station per species) and held in 60 × 60 cm plastic cages (2-cm mesh size) immerged at 5 m depth for both clams and oysters. Transplanted organisms (n = 30 per species) in GR_1 and GR_2, and resident organisms (n = 20) from the clean stations (Maa Bay for oysters and Ouano Beach for clams) were collected by SCUBA diving after 35 and 69 d. Sediment samples (top 3-cm layer) were collected simultaneously with organisms from the clean and transplantation sites.

II.1. Sampling preparation and analyses

Back to the laboratory, the bivalves were kept for 24 h in 30 l seawater from the same sampling station to allow depuration of gut contents and of particulate material present in the mantle cavity. Soft tissues were removed from the shells and were weighed (wet weight; wwt), dried at 60°C until constant weight, and weighed again (dry weight; dwt). They were then stored in acid-washed, hermetically sealed plastic containers until analysis.

Sediments were similarly stored in acid-washed, hermetically sealed plastic bags and frozen at -20°C. Sediments were then dried at 60°C for 5 d. In order to eliminate

heterogeneous materials (e.g., stones, fragment of corals), sediments were sieved (1-mm mesh size) prior to analysis.

Aliquots of the biological samples (300 to 500 mg dwt) and sediment samples (300 mg dwt) were digested using a 3:1 (v:v) nitric-hydrochloric acid mixture (65% suprapur HNO_3 and 30% suprapur HCl, Merck). Acid digestion of the samples was carried out overnight at room temperature. Samples were then mineralized using a CEM Corp. MARS 5 microwave oven (30 min with constantly increasing temperature up to 100°C for sediments and 115°C for biological material, then 15 min at these maximal temperatures). Each sample was subsequently diluted with milli-Q water according to the amount of sample digested (10 ml / 100 mg).

Elements were analyzed using a Varian Vista-Pro ICP-OES (As, Cr, Cu, Mn, Ni, and Zn) or a Varian ICP-MS Ultra Mass 700 (Ag, Cd and Co). Three control samples (two Certified Reference Materials - CRM - and one blank) treated and analyzed in the same way as the samples were included in each analytical batch. The CRM were dogfish liver DOLT-3 and lobster hepatopancreas TORT-2 (NRCC). The results for CRM indicated recoveries of the elements ranging from 81 % (Ni) to 113 % (Zn) (Table 1). The detection limits were 31.0 (As), 1.3 (Cr), 3.8 (Cu), 0.15 (Mn), 1.1 (Ni) and 2.4 (Zn) $\mu g\ g^{-1}$ dwt for ICP-OES and 0.1 (Ag), 0.15 (Cd) and 0.1 (Co) $\mu g\ g^{-1}$ dwt for ICP-MS. All element concentrations are given on a dry weight basis ($\mu g\ g^{-1}$ dwt).

II.2. Data treatment and statistical analyses

The uptake kinetics of the elements examined were described using either a simple linear regression model (eq. 1) or a saturation exponential model (eq. 2):

$$C_t = C_0 + k_u\, t \quad \text{(eq. 1)}$$

$$C_t = C_0 + C_1\,(1 - e^{-k_e\, t}) \quad \text{(eq. 2)}$$

where C_t and C_0 are the element concentrations in organisms at time t (d) and 0, respectively ($\mu g\ g^{-1}$); $C_1 + C_0$ is the concentrations at steady state (C_{ss}; $\mu g\ g^{-1}$); k_u is the uptake rate constant ($\mu g\ g^{-1}\ d^{-1}$) and k_e is the depuration rate constant (d^{-1}) (Whicker and Schultz 1982).

Depuration kinetics of elements was described by either a simple linear regression model (eq. 3) or a single-component exponential equation (eq. 4):

$$C_t = C_0 - k_e\, t \quad \text{(eq. 3)}$$

$$C_t = C_0\, e^{-k_e\, t} + A \quad \text{(eq. 4)}$$

where A is a constant ($\mu g\ g^{-1}$) .

Model constants and their statistics were estimated by iterative adjustment of the model and Hessian matrix computation using the nonlinear curve-fitting routines in the Statistica™ software 5.2.1.

Element concentrations of sediments and control organisms were plotted against time and fitted using simple linear regression. Statistical analyses of the data were performed using 1-way analysis of variance (ANOVA) followed by the multiple comparison test of Tukey (Zar 1996). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

A power analysis was performed using the whole set of data in order to assess the minimal sample size of organisms (oysters and clams) required to detect realistic (field-observed) differences in element concentration with statistical significance ($p < 0.05$) (Zar 1996).

III. Results

III.1. Experiment 1: Reciprocal transplantations

III.1.1. Sediments

Comparison of element concentrations in sediments from the two stations naturally inhabited by the oysters *I. isognomon* (Maa Bay and Boulari Bay) indicated that levels of As, Co, Cr, Mn and Ni in sediments collected from Boulari Bay were significantly higher ($p_{Tukey} \leq 0.0008$) than those collected from Maa Bay, whereas concentrations of Cu and Zn were significantly higher ($p_{Tukey} \leq 0.0002$) in Maa Bay compared to Boulari Bay (Table 2). No significant difference was observed between Cd concentrations in sediments from the two bays.

Element concentrations measured in sediments from the two stations naturally inhabited by the clams *G. tumidum* showed that concentrations of all elements in sediments collected in Grande Rade (GR_{Int}, contaminated station,) were significantly higher (p_{Tukey} always ≤ 0.0002) than those from Ouano Beach (clean station) (Table 2).

Element concentrations in sediments collected from the four stations at the different times showed no significant variation with time.

III.1.2. Oysters I. isognomon

At the beginning of the experiment, concentrations of all elements in oysters from Boulari Bay were significantly higher ($p_{Tukey} \leq 0.0002$, except for Zn: $p = 0.006$) than those collected from Maa Bay, except for As, Cd and Mn for which no significant difference was found.

Resident populations of *I. isognomon* from Maa Bay and Boulari Bay did not exhibit any significant variation in concentrations of any element during the experiment time course.

In oysters transplanted to the Boulari Bay station, the concentrations of Cr, Cu and Ni showed a significant linear increase (k_u: 0.054, 0.065 and 0.031 µg g^{-1} d^{-1}; $p < 0.003$; $R^2 = 0.14\text{-}0.24$) with time (Fig. 3). At the end of the experiment, Ni concentrations in oysters were significantly lower ($p_{Tukey} = 0.046$) than those in resident oysters from the Bay. No significant difference was found for Cr and Cu.

In oysters transplanted to the clean station (Maa Bay), only Ag, Co and Ni showed significant depuration. Ag and Co concentrations showed a significant linear decrease over time (k_e: 0.059 and 0.013 µg g^{-1} d^{-1}; $p < 0.03$; R^2: 0.08 and 0.10, respectively; Fig. 4). The depuration kinetics of Ni in oyster soft tissues was best fitted by an exponential model (k_e: 0.19 d^{-1}, $R^2 = 0.54$, $p < 0.0001$). The concentrations of Ag, Co and Ni in transplanted oysters at the end of the experiment were still significantly higher (p_{Tukey} always ≤ 0.0001) than those in resident oysters.

III.1.3. Clams G. tumidum

At the beginning of the transplantation experiment (day 0), concentrations of all elements in clams from Ouano Beach were significantly lower ($p_{Tukey} \leq 0.001$, except for Mn and Zn, $p \leq 0.02$) than those from Grande Rade (GR_{Int}). The only exceptions were As for which the highest concentration ($p_{Tukey} = 0.0003$) was measured in clams from Ouano Beach, and for Cd for which no significant difference was found between the clams of the two stations.

Control resident *G. tumidum* in Ouano Beach and Grande Rade showed no significant variation for any element along the duration of the experiments.

In clams transplanted to the contaminated station (GR_{Int}), the concentrations of Ag, Cd, Co, Cr, Cu and Zn displayed a significant linear increase (Ag, Cu and Zn k_u: 0.092, 0.105 and 0.21 µg g^{-1} d^{-1}, respectively; $p < 0.0001$; R^2: 0.26 - 0.83; Cd, Co and Cr k_u: 0.0014, 0.02 and 0.019 µg g^{-1} d^{-1}, respectively; $p < 0.02$; $R^2 \leq 0.12$) (Fig. 3). The uptake kinetics of Ni in clam soft tissues was best fitted by an exponential model ($R^2 = 0.65$, $p < 0.0001$) for which the estimated uptake rate constant, k_u, was 1.28 µg g^{-1} d^{-1}. The uptake rate of Ag, Cu, Ni and Zn was higher by one order of magnitude compared to that of the other elements (Fig. 3).

When clams from GR_{Int} were transplanted to the clean station, Ouano Beach, Ag and As concentrations displayed a significant linear increase (k_u: 0.078 and 0.541 µg g^{-1} d^{-1}; $p < 0.001$; R^2: 0.17 and 0.56, respectively) (Fig. 4). For the other elements, no significant depuration was observed.

When a significant increase/decrease in element concentration was observed, concentrations in transplanted organisms were compared to those of resident organisms. Statistical analyses indicated that at the end of the experiment, Ag, Cd, Cr and Cu concentrations in clams transplanted to GR_{Int} were significantly lower ($p_{Tukey} \leq 0.005$, except for Ag, $p = 0.047$) than in resident clams from GR_{Int} (up to 3.9 fold lower for Cd and Cr). No significant difference was found for Co, Ni and Zn concentrations between transplanted and resident clams.

At the end of the experiment, Ag concentrations in clams transplanted to Ouano Beach were significantly higher ($p_{Tukey} = 0.0001$) than those in resident clams at Ouano Beach, whereas for As, the opposite was observed ($p_{Tukey} = 0.0003$).

III.2. Experiment 2: Transplantation in Grande Rade

III.2.1. Sediments

Sediments collected from Ouano Beach, Maa Bay, GR_1 and GR_2 revealed that concentrations of all elements were significantly higher (1 to 3 orders of magnitude higher) in sediments from GR_1 (p_{Tukey} always ≤ 0.0002) compared to the other three stations, except for As that reached its highest concentration in GR_2 ($p_{Tukey} = 0.0002$) (Table 2).

III.2.2. Oysters I. isognomon

Element concentrations in resident oysters from Maa Bay showed no significant variation over the duration of experiment.

At the most contaminated station (i.e., GR_1), Co, Cr, Cu and Ni concentrations at 35 and 69 d were significantly higher than those at 0 d ($p_{Tukey} \leq 0.0006$ for Co, Cr, and Cu and $p = 0.005$ for Ni; Fig. 5). Among these four metals, only Ni concentrations after 69 d were significantly higher than those after 35 d of transplantation. Ag concentration after 69 d was significantly higher than those at 0 d and after 35 d ($p_{Tukey} = 0.03$), but no significant difference was found between concentrations at 0 d and after 35 d of transplantation. Concentrations of As, Cd, Mn and Zn exhibited no significant differences in the oysters at station GR_1 over the entire transplantation period.

At station GR_2, which displays a lower degree of contamination than GR_1 according to the element concentrations in sediments (Table 2), Ni concentrations after 35 and 69 d were significantly higher than those at 0 d and concentrations after 69 d were significantly higher than those after 35 d ($p_{Tukey} \leq 0.0001$). Concentrations of Cr and Cu after 35 and 69 d were significantly higher than those at 0 d ($p_{Tukey} \leq 0.0001$), but

311 no significant differences were found between 35 and 69 d. Ag concentrations after 69
312 d were significantly higher than those at 0 d (p_{Tukey} = 0.0002) and after 35 d (p_{Tukey} =
313 0.02), but no significant difference was found between concentrations at 0 d and after
314 35 d. No significant difference was found for the concentrations of As, Cd, Co, Mn
315 and Zn in oysters over the entire transplantation period in GR_2.

316 After 35 d, oysters transplanted into GR_1 displayed concentrations of Co, Cu and Ni
317 significantly higher than those at GR_2 ($p_{Tukey} \leq 0.0001$) whereas concentrations of Ag
318 and Zn in GR_1 oysters were significantly lower than those at GR_2 (p_{Tukey} = 0.02 and
319 0.048, respectively). After 69 d of transplantation, concentrations of Co, Cr, Cu, Mn
320 and Ni were significantly higher in oysters transplanted at station GR_1 than those at
321 GR_2 ($p_{Tukey} \leq 0.002$ for Co and Cu, and < 0.04 for Cr, Mn and Ni), whereas Ag
322 concentrations at GR_1 were significantly lower than those at GR_2 (p_{Tukey} = 0.009).

323 *III.2.3. Clams G. tumidum*

324 Element concentrations in resident clams from Ouano Beach showed no significant
325 difference over time.

326 At the most contaminated station (i.e., GR_1), Ag, Co and Ni concentrations after 35
327 and 69 d were significantly higher than those in clams measured at 0 d ($p_{Tukey} \leq$
328 0.0001 for Ni and $\leq$ 0.02 for Ag and Co) (Fig. 6) and concentrations after 69 d were
329 significantly higher than those after 35 d of transplantation. Concentrations of Cr and
330 Cu after 69 d were significantly higher than those at 0 and after 35 d ($p_{Tukey} \leq 0.0003$),
331 whereas no significant difference was found between the concentrations at 0 d and
332 after 35 d of transplantation. No significant difference was found between the
333 concentration of As, Mn and Zn after 35 and 69 d.

At the second station, GR_2, Ag and Ni concentrations after 35 and 69 d were significantly higher ($p_{Tukey} \leq 0.0005$) than those at 0 d, and concentrations after 69 d were significantly higher than those after 35 d ($p_{Tukey} = 0.0005$ and 0.03 respectively). Cr concentrations after 35 and 69 d were significantly higher ($p_{Tukey} \leq 0.0001$) than those at the beginning of the transplantation, but no significant differences were observed between 35 and 69 d. Cu and Mn concentrations after 69 d of transplantation were significantly higher ($p_{Tukey} = 0.039$ and 0.041) than those at the start of the experiment. No significant difference was found for Co and Zn concentrations at 0, and after 35 and 69 d. In contrast, As concentrations after 69 d were significantly lower than those at day 0 ($p_{Tukey} = 0.014$).

Element concentrations after 35 and 69 d of transplantation were compared between stations GR_1 and GR_2. Results indicated that after 35 d, Co, Cu and Ni concentrations in clams at GR_1 were significantly higher than those at GR_2 ($p_{Tukey} \leq 0.0002$, except for Cu: $p = 0.01$). For the other elements, no significant difference between GR_1 and GR_2 was found after 35 d. After 69 d of transplantation, the concentrations of Cd, Co, Cr, Cu and Ni in clams at GR_1 were significantly higher (p_{Tukey} always ≤ 0.0002) than those at GR_2.

III.3. Estimation of the minimum sample size required to detect a significant difference in concentrations

A power analysis was performed to determine the minimum sample size necessary to detect a significant difference ($\alpha = 0.05$) between concentrations of a given element in two batches of clams or oysters. The variability of the data was shown to be dependent upon the element, the species, the stations and the concentration levels. The highest variance was observed in the samples displaying the highest

concentrations, consequently, minimum and maximum variance of the transplanted batches were used to determine the range of minimal sample size necessary to detect given differences of concentrations with statistical significance. Considered differences of concentrations were selected to be representative of those that are actually encountered in the field (Table 3). Generally, a sample of size ≥ 50 organisms would be required to detect realistic differences in element concentrations, ranging from 0.5 (Cd) to 150 (As) $\mu g\ g^{-1}$ dwt.

IV. Discussion

This field study investigated the *in situ* accumulation and depuration of 9 selected elements in two tropical bivalves in order to validate their relevance as biomonitoring species. Element concentrations in resident control organisms from each site showed no significant variation with time during the transplantation time course, indicating that any increase (or decrease) of element concentrations in tissues of the transplanted individuals would actually reflect a higher (or a lower) metal contamination level at a given site, and should not be due to seasonal factors.

When the oysters and clams from the clean sites were transplanted into the contaminated sites (Experiment 1), the uptake of the selected elements displayed different trends (Figs 3 and 4). At the end of the transplantation period, concentrations observed in the organisms were either lower than or similar to those measured in resident populations of the contaminated site, or did not change compared to their initial levels.

Concentrations of Cr and Cu in oysters and Co, Ni and Zn in clams reached values similar to those measured in resident organisms. Similar findings have been previously reported for Cu and Zn in the soft tissues of the mussel *M. edulis*

transplanted to a temperate polluted bay (Roesijadi et al. 1984). However, since metal uptake displayed linear kinetics over the transplantation period, the concentrations of these elements would most probably have continued to increase if the duration of the experiment was longer. This hypothesis is supported by the observations made in the second transplantation experiment, in which clams transplanted to GR_1 and GR_2 displayed Co and Ni concentrations (up to 15.7 ± 4.8 and 140 ± 46 µg g^{-1} dwt, respectively) exceeding those of the resident clams from Grande Rade (7.2 ± 2.3 and 63.2 ± 13.5 µg g^{-1} dwt for Co and Ni, respectively).

In contrast, concentrations of Ni in transplanted oysters and, Ag, Cd, Cr, Cu in transplanted clams significantly increased during the transplantation period but did not reach the values measured in resident organisms. Taking into account the measured uptake rate constants of these elements in oysters and clams, it can be estimated that reaching the resident concentrations would require, for example, about 6 months for Ni in oysters and approximately 3 years for Cd in clams. Comparable results have been previously reported for the oysters *Crassostrea rhizophorae* (Wallner-Kersanach et al. 2000), the clam *Macoma balthica* (Cain and Luoma 1985) and the mussel *M. edulis* from Greenland (Riget et al. 1997). However, our results from the second transplantation (Experiment 2) indicated that when both species were transplanted to a more contaminated site (GR_1), accumulation of Ni in oysters and Cr in clams was faster than during the first transplantation experiment. Therefore, the slow uptake rate of Ni in oysters and Cr in clams observed in the latter transplantation is rather related to low bioavailability of these two metals at the contaminated site (Boulari Bay and GR_{Int} for oysters and clams, respectively) than to low bioaccumulation efficiency of the organisms.

In the case of Ag, As, Cd, Co, Mn and Zn in oysters and As and Mn in clams, concentrations did not show a significant increase during the transplantation from the clean site to the polluted one (Experiment 1). Even though similar observations were made for Cd and Zn concentrations in *Crenomytilus grayanus* after two months of transplantation (Shulkin et al. 2003), opposite trends have also been observed. For example, after 120 days of transplantation, a significant bioaccumulation of Cd and Zn was measured in tissues of oysters, clams and cockles (Baudrimont et al. 2005). Therefore, the lack of bioaccumulation of some elements in oysters and clams as observed in our study suggests that these elements were rather poorly bioavailable for the bivalves or that oysters and clams have efficient regulation mechanisms preventing these metals from being accumulated. In fact, when organisms were transplanted to GR_1 and GR_2 (Experiment 2), concentrations of Ag and Co in oysters and Mn in clams were actually efficiently bioaccumulated. In addition, in laboratory controlled conditions, metals including Co and Mn were efficient accumulated in oyster and clam tissues (Hédouin et al. 2010a). Therefore, these results support the low bioavailability hypothesis, at least for Ag and Co in Boulari Bay and Mn at Grande Rade GR_{Int}.

When organisms were transplanted to a clean station (Experiment 1), the concentrations of all elements in both bivalves were almost the same after 100 d of transplantation, except for Ag, Co and Ni in oysters, which showed a low but significant decrease with time. However, Ag, Co and Ni concentrations in oysters were far from reaching the concentrations measured in natural resident populations by the end of the experiment. Such incomplete metal elimination has been reported by several authors when organisms from polluted areas were transplanted to clean areas (e.g., Zn in the mussel *Mytilus edulis*, Roesijadi et al. 1984, Simpson 1979; Cd and Cu

in the oyster *Crassostrea gigas*, Geffard et al. 2002; Cr, Cu and Zn in the clam *Mercenaria mercenaria*, Behrens and Duedall 1981). The biological half life ($T_{b½}$) of these elements has been previously determined from radiotracer experiments in *I. isognomon* and *G. tumidum* (Hédouin et al. 2007, 2010b). Although, elements like Ag, Cd, Ni and Zn were very efficiently retained with $T_{b½} \geq 5$ months, the other elements displayed $T_{b½}$ ranging from 1 to 3 months in both bivalve species, independently of the uptake pathway tested (seawater, food or sediments). Comparison of the data indicates that, in the field, depuration processes would take longer for some metals than those previously estimated from laboratory experiments. This confirms that laboratory results cannot always be extrapolated directly to environmental situations, probably due to physiological adaptations of organisms living in contaminated conditions (e.g. sequestration mechanisms). Since oysters and clams showed very low depuration for most of the studied contaminants, bivalve tissues would be able to retain information of contamination events over very long periods of time. However, the subsequent drawbacks in a biomonitoring perspective are that (1) the element concentrations in transplanted organisms are not actually able to reflect the lower contamination levels occurring at a given location over a medium-scale time period (i.e., 3 months), and (2) the element concentrations in organisms collected from natural areas can reflect past contamination which is no longer occurring rather than actual contamination. These drawbacks arise from the fact that depuration is influenced by the past contamination history of the organisms. It was for example shown that Cu was more easily eliminated (30% after 30 d) by oysters temporarily transplanted into a metal-rich area for 60 d, than by resident oysters from the same metal-rich area (decrease limited to 9% after 30 d) (Wallner-Kersanach et al. 2000). This suggests that our specimens from the more contaminated area, which

456 were exposed to high metal concentrations possibly for their whole life, may have
457 developed more efficient sequestrating processes of metals to store them in their
458 tissues as non-toxic forms (e.g. in granules, Mason and Jenkins 1995). Such adaptive
459 mechanisms could occur in both studied species, and hence explain the efficient
460 retention observed in the field. Therefore, further studies should be focused on the
461 long-term depuration of elements in both bivalves from contaminated and clean sites,
462 in which bivalves would be previously exposed to contaminants in the field for 2-3
463 months before being transplanted into clean sites. Such experiments would
464 demonstrate whether the past contamination history of *I. isognomon* and *G. tumidum*
465 plays a role in the strong retention of elements observed in the field.

466 Interestingly, when clams from Grande Rade (GR_{Int}) were transplanted to Ouano
467 beach (Experiment 1), a significant bioaccumulation of As was observed in clam
468 tissues, although lower As concentration was reported in sediments from Ouano beach
469 (3.1 $\mu g\ g^{-1}$ dwt). High level of As in clam tissues from Ouano beach has been recently
470 reported (Hédouin et al. 2009), and the authors suggested that food was the main
471 pathway of As uptake in clams. Our transplantation experiment from Grande Rade
472 (GR_{Int}) to Ouano beach showed that As was highly bioavailable for clams in Ouano
473 beach. In addition, due to the low levels of As in sediments from Ouano beach, this
474 result supports the assumption that the high levels of As are most probably
475 bioaccumulated from the diet of the organisms (Sanders et al. 1989, Warnau et al.
476 2007, Hédouin et al. 2009). Since the clam *G. tumidum* is a seafood product in New
477 Caledonia and that its tissues showed high levels of As, the sources of As in Ouano
478 Beach and the potential toxicity of As for consumers should be further investigated.

479 In the second transplantation (Experiment 2), element concentrations in sediments
480 clearly indicated that GR_1 is the most contaminated site, reaching very high level of

Co, Cr, Mn, and Ni (up to 10,500 µg Ni g^{-1} dwt). These high concentrations in metals, and more specifically in Ni, concur with the very high concentration of Ni observed in the particulate phase within the water column (Migon et al. 2007). Ag, Co, Cr, Cu and Ni were efficiently accumulated in transplanted oysters and clams. In addition, results indicate that bioaccumulation was dependent on sampling location and species, and difference in the contamination level of the two stations was easier to observe when organisms were transplanted for a longer time (69 vs 35 d). For example, our results showed that the concentrations of 5 elements in bivalve tissues (Co, Cr, Cu, Mn and Ni in oysters and Cd, Co, Cr, Cu and Ni in clams) were significantly higher at GR_1 than at GR_2 after 69 d, whereas differences were significant only for 3 elements (Co, Cu and Ni) after 35 d.

In this second transplantation experiment, oysters and clams were transplanted to the same stations, hence exposed to the same environmental conditions. Their bioaccumulation capacities can thus be directly compared. Clams were more efficient than oysters in bioaccumulating the selected elements (e.g., concentrations measured after 69 d of transplantation increased by a factor 7 in oysters and by a factor 40 in clams). These findings were surprising considering previous results from laboratory radiotracer studies (e.g., Hédouin et al. 2010a) which indicated a more efficient bioconcentration capacity in oysters than in clams when exposed to dissolved elements (concentration factors were higher by several orders of magnitude). Such a difference between laboratory and *in situ* experiments strongly suggests that the seawater pathway is not the major route of accumulation driving global metal uptake in these organisms. Rather, ingestion of particulate materials would be the main pathway for metal uptake, an hypothesis that is supported by a previous study of Cd, Co and Zn bioaccumulation modeling in *I. isognomon* and *G. tumidum* (Hédouin

506 2006, 2010b). This may indeed explain the higher metal levels in *G. tumidum* which
507 lives buried in the sediment, and feeds mainly on organic (and metal)-rich particles at
508 the seawater-sediment interface.

509 Combining the results from transplantations 1 and 2 demonstrated the usefulness of
510 bioindicator species to assess the degree of contamination present in the marine
511 environment. Indeed, for some elements, the high levels of metals reported in
512 sediments were reflected in organism tissues (e.g. Cr, Ni) and a significant
513 bioaccumulation of these metals was observed in the tissues of the clams and the
514 oysters during the transplantation experiments. However, for some elements, the
515 metal bioaccumulation trends observed in clams and oysters were different from those
516 expected based on metal concentrations found in sediments at the different sites of
517 transplantation. For example, in sites characterized with low As concentrations in
518 sediments (Ouano beach), efficient bioaccumulation of As was observed in clam
519 tissues (Experiment 1), suggesting that other sources of As uptake are available for
520 organisms (e.g. food, see discussion above). In contrast, for Mn, although high levels
521 were measured in sediments, almost no bioaccumulation was observed in organism
522 tissues (Experiment 2). This clearly points out that only a fraction of the metals
523 present in the sediments is bioavailable for organisms. Mn bound in the lattice of
524 naturally occurring Mn-rich ores (e.g., laterite and garnierite) may be less available
525 for uptake by marine organisms compared to water-soluble forms. The different
526 patterns of metal bioaccumulation observed in clams and oysters during the two
527 transplantation experiments carried out in this work pointed out that the metal
528 contamination status cannot be based solely on metal analysis from the sediments and
529 this is the reason why the use of bioindicator species is an important asset to better
530 characterize the contamination status of a particular site. In addition, although it was

not performed in the present study, metal analysis in seawater is also a useful complementary information to those obtained from sediments and organisms. However accurate analysis of metals in seawater is uneasy and expensive, and is therefore generally not integrated in biomonitoring programmes. Nevertheless, nowadays the development of techniques such as the diffusive gradients in thin films (DGT) (e.g., Davison and Zhang 1994, Webb and Keough 2002) brings new insights to obtain time-integrated information on metal concentration in seawater. Ideally analysis of metals in sediments, seawater and organisms will be recommended for biomonitoring purposes since such combination enhances our understanding of the contamination status present in the marine environment, but also brings additional information for identifying the source of contamination.

In order to obtain accurate and reliable data in biomonitoring programmes, the determination of optimal sample size to be collected is of fundamental importance. In this context, the present study has investigated the minimum sample size required to detect a given difference in concentration. Results shown in Table 3 indicate that the detection of a 0.5 µg g^{-1} dwt difference in tissue concentrations in the highly contaminated organisms required the largest sample size. Relatively large variability in metal concentrations in organisms within a site has frequently been reported (e.g., Daskalakis 1996, Gordon et al. 1980). In the present study, the concentration variability was higher with increasing average concentration. Consequently, detecting small differences in concentration among organisms with higher metal concentrations will require an increase in sample size. Nevertheless, it is important to keep in mind that to be feasible, the sample size required in a biomonitoring programme should always remain realistic.

555 Compared to the actual metal concentration range measured in the New Caledonia
556 lagoon waters and sediments, the minimum difference in concentrations detectable
557 with sample sizes of 50-60 organisms would allow for an efficient differentiation
558 among sites naturally inhabited by the two targeted bivalves. For example, a
559 difference of 2 µg Ni g^{-1} dwt can be detected with a sample size of 7 oysters and 36
560 clams in a population showing low Ni levels (Table 3). However, 62 oysters and 30
561 clams would be necessary to detect differences of 30 and 8 µg g^{-1} dwt, respectively, in
562 a population characterized by high Ni concentrations (Table 3). A sample size of 50-
563 60 organisms was similarly recommended by other authors in order to facilitate the
564 detection of significant changes in concentrations (e.g. Gordon et al. 1980, Topping
565 1983). In current biomonitoring programmes, organisms collected (20 oysters and 30
566 mussels for the NOAA Mussel Watch, Beliaeff et al. 1998; 10 oysters and 50 mussels
567 for the French RNO, Claisse 1989) are pooled before analysis in order to reduce costs
568 of sample preparation and analysis. However, pooling leads to the loss of statistical
569 information on inter-individual variability, which is obviously an important issue to
570 assess significance of concentration differences among samples. These economic
571 constraints are obvious in the case of large national and international biomonitoring
572 programmes that assess the levels of numerous trace elements and organic
573 contaminants in many stations. However, in New Caledonia, which is mainly
574 impacted by mining activities, metal and metalloids are the contaminants of major
575 concern. Therefore, analytical costs would be reduced compared to biomonitoring
576 programmes that include the very expensive analysis of organic compounds. Hence,
577 in the specific context of the New Caledonia lagoon, it is highly recommended to
578 analyze individual samples in order to obtain information on inter-individual

variability that would provide scientifically-supported best practices in environmental management.

V. Conclusion

This study clearly indicates that the clam *G. tumidum* can be recommended for an active monitoring of contaminants in subtidal and intertidal stations of the New Caledonia lagoon on a spatiotemporal scale. Biomonitoring studies using transplanted organisms would be an efficient solution to survey environmental levels of key local metal contaminants in areas lacking resident bivalves. The advantage of using transplanted organisms (active biomonitoring) over sampling resident populations (passive biomonitoring) is that it allows selecting organisms of uniform initial element concentrations, of common origins and past history, and thus ensures comparable biological samples. However, if further studies confirm the observed very long element retention times in these organisms, organisms from sites displaying a low contamination will have to be used in order to prevent bias in element concentrations due to physiological adaptation of organisms (e.g. sequestration mechanisms).

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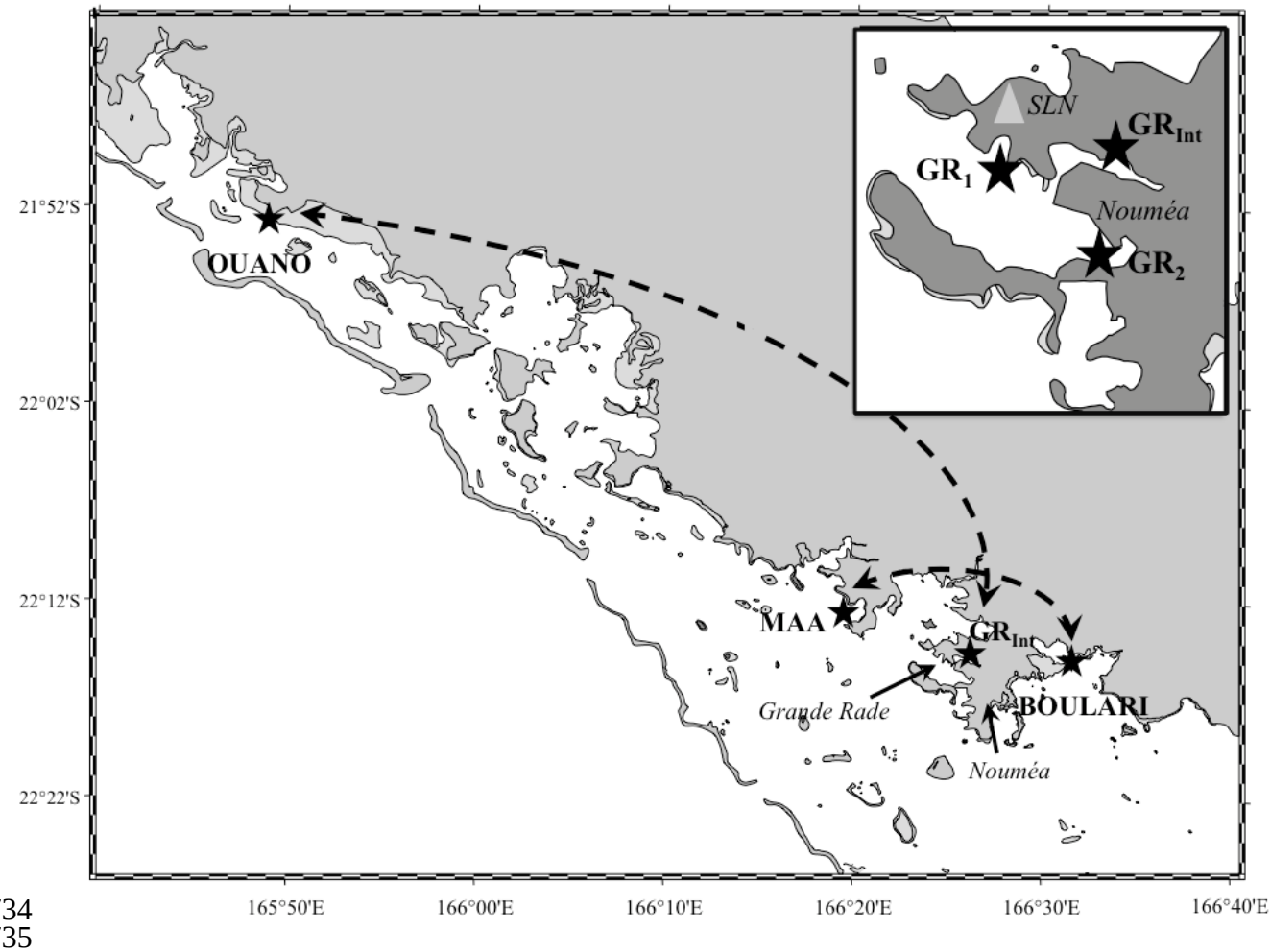
730 **Figure 1.** Map showing the stations selected for transplantation experiments.

731 OUANO: Ouano Beach; MAA: Maa Bay; BOULARI: Boulari Bay; GR_{Int}: Grande
732 Rade Interdital station; GR_1: Grande Rade subtidal site 1; GR_2: Grande Rade subtidal
733 site 2; SLN : « Société Le Nickel » Nickel ore processing plant.



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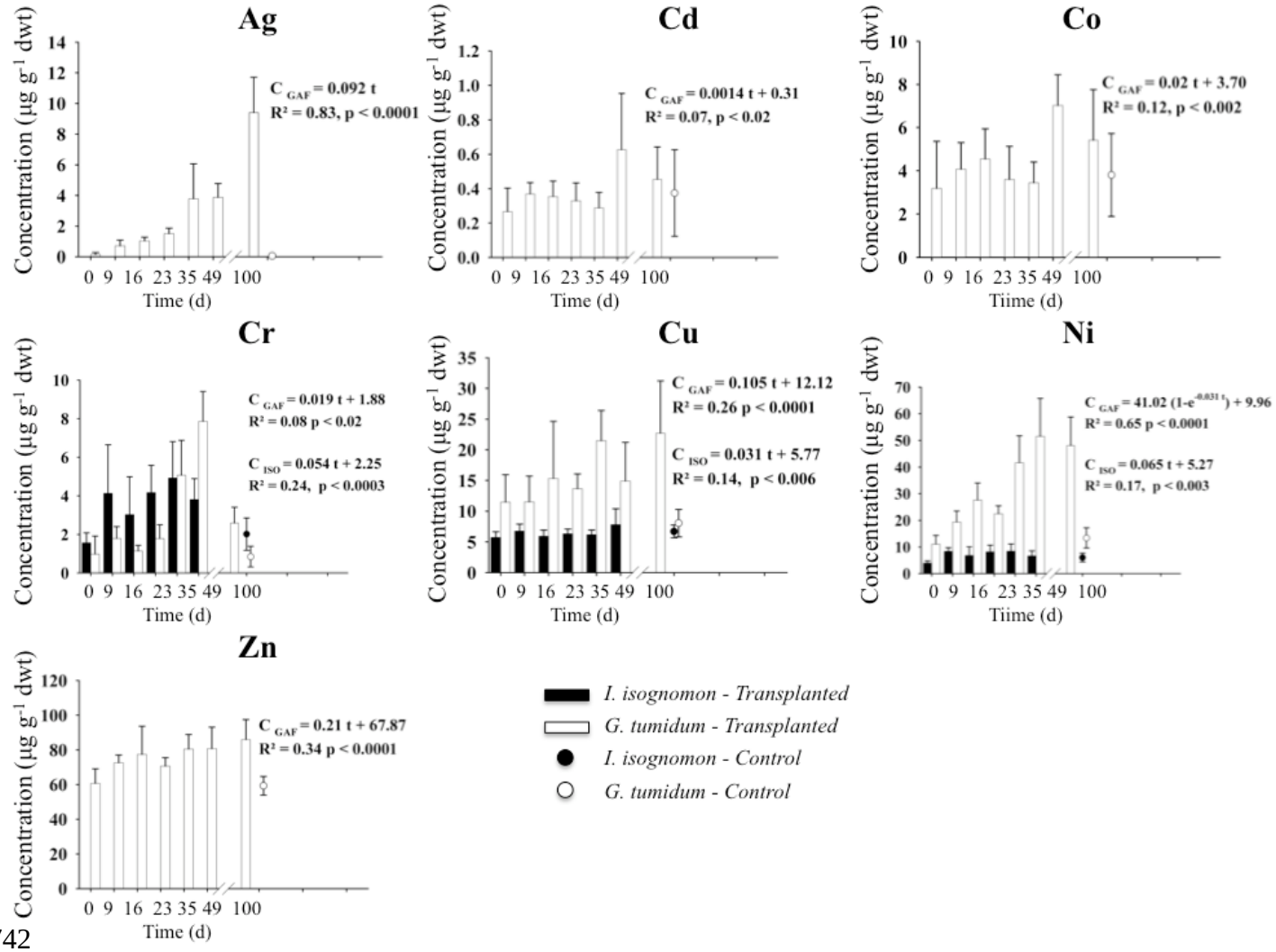
736 **Figure 2.** Element concentrations (mean ± SD; µg g^{-1} dwt; n = 7 for transplanted
737 organisms and n = 5 for control organisms) in oysters *Isognomon isognomon* and
738 clams *Gafrarium tumidum* transplanted from clean stations, Maa Bay (*I. isognomon*)
739 and Ouano Beach (*G. tumidum*), to the contaminated stations, Boulari Bay and
740 Grande Rade (GR_{Int}), respectively.

741 (only data showing a significant regression, $p < 0.05$, are presented)



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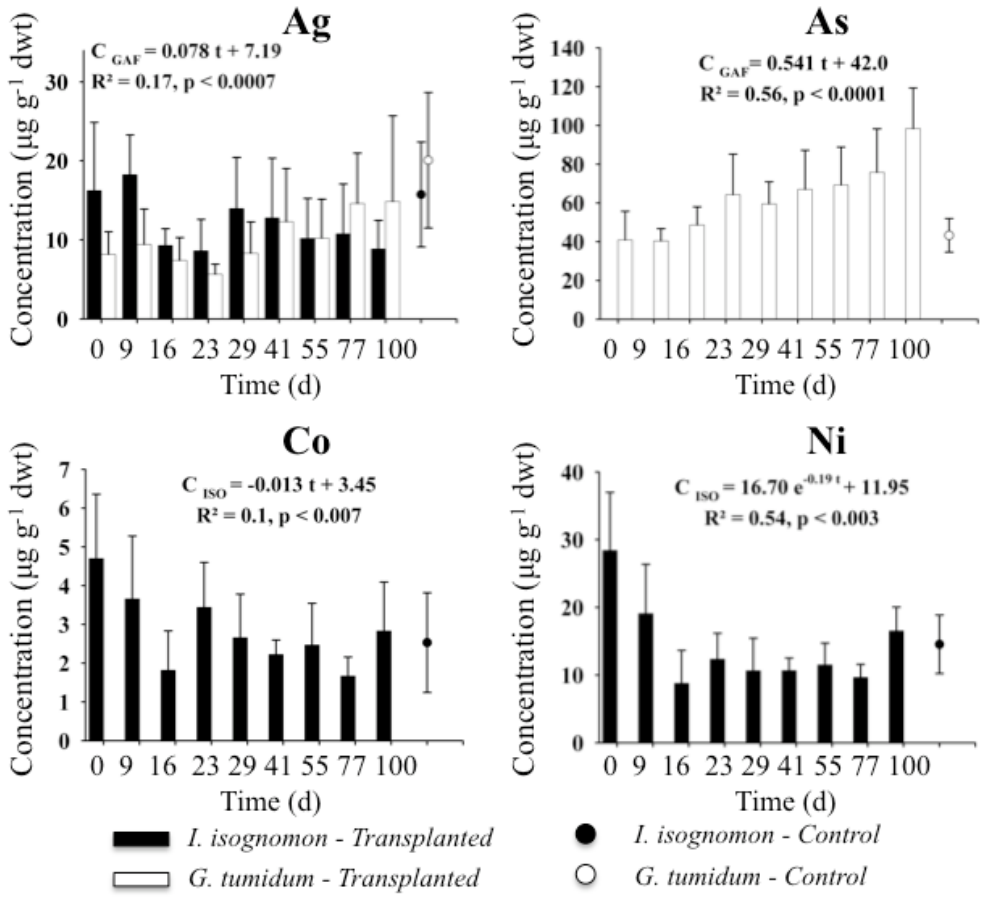
744 **Figure 3.** Element concentrations (mean ± SD; µg g^{-1} dwt; n = 7 for transplanted
745 organisms and n = 5 for control organisms) in oysters *Isognomon isognomon* and
746 clams *Gafrarium tumidum* transplanted from the contaminated stations, Boulari Bay
747 (*I. isognomon*) and Grande Rade (GR_{Int}, *G. tumidum*), to reference stations, Maa Bay
748 and Ouano Beach, respectively. (only data showing a significant regression, $p < 0.05$,
749 are presented)



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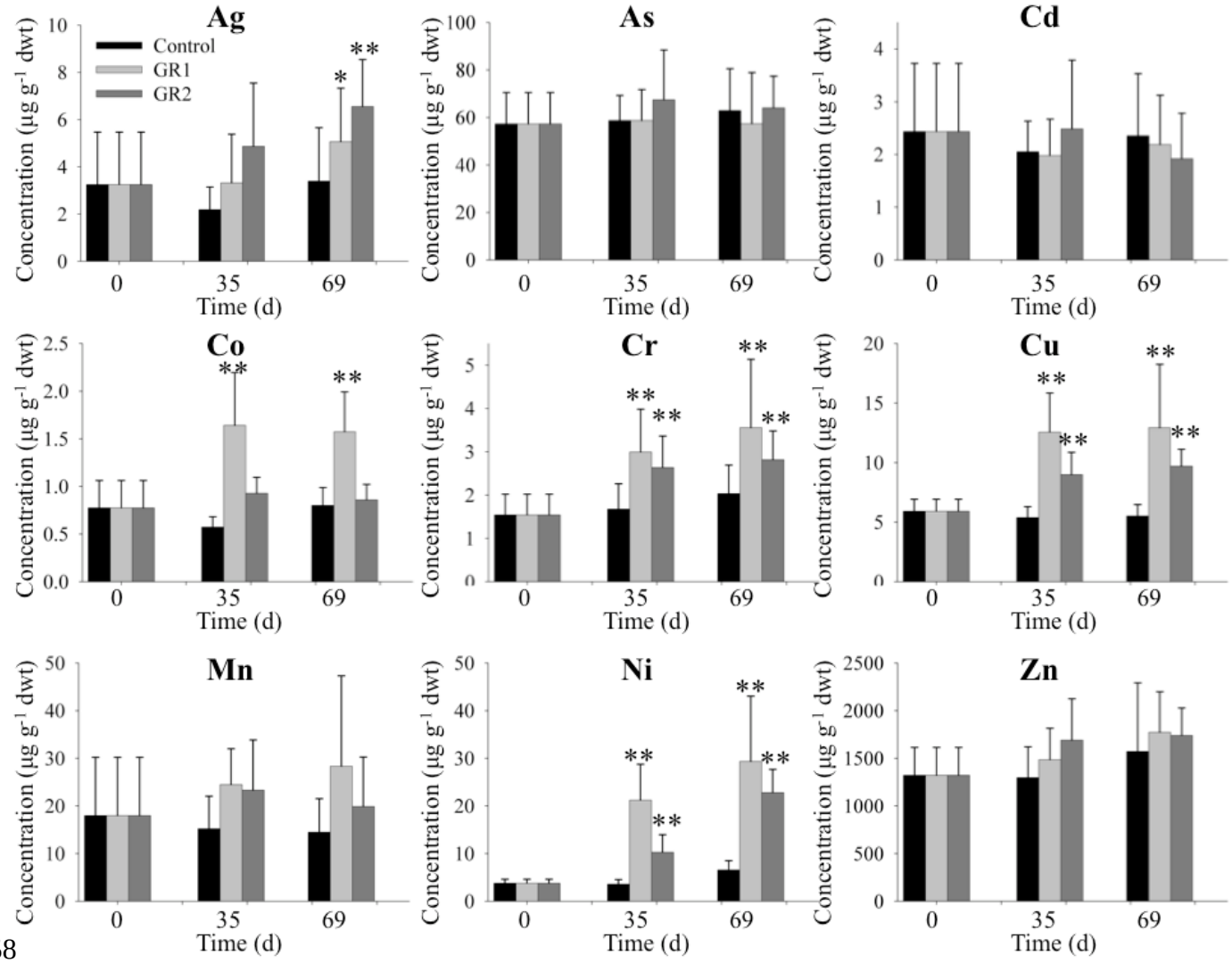
752 **Figure 4.** Element concentrations (mean ± SD; µg g^{-1} dwt; n = 30 for transplanted
753 organisms and n = 20 for control organisms) in oysters *Isognomon isognomon* from Maa
754 Bay transplanted into stations GR_1 and GR_2 in the Grande Rade.
755 (stars indicate that the concentration is significantly different from those in organisms
756 at 0 d; * $p < 0.05$, ** $p < 0.001$)
757



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760 **Figure 5.** Element concentrations (mean ± SD; µg g^{-1} dwt; n = 30 for transplanted
761 organisms and n = 20 for control organisms) in clams *Gafrarium tumidum* from
762 Ouano Beach transplanted into the stations GR_1 and GR_2 in the Grande Rade.
763 (stars indicate that the concentration is significantly different from those in organisms
764 at 0 d; * $p < 0.05$, ** $p < 0.001$)
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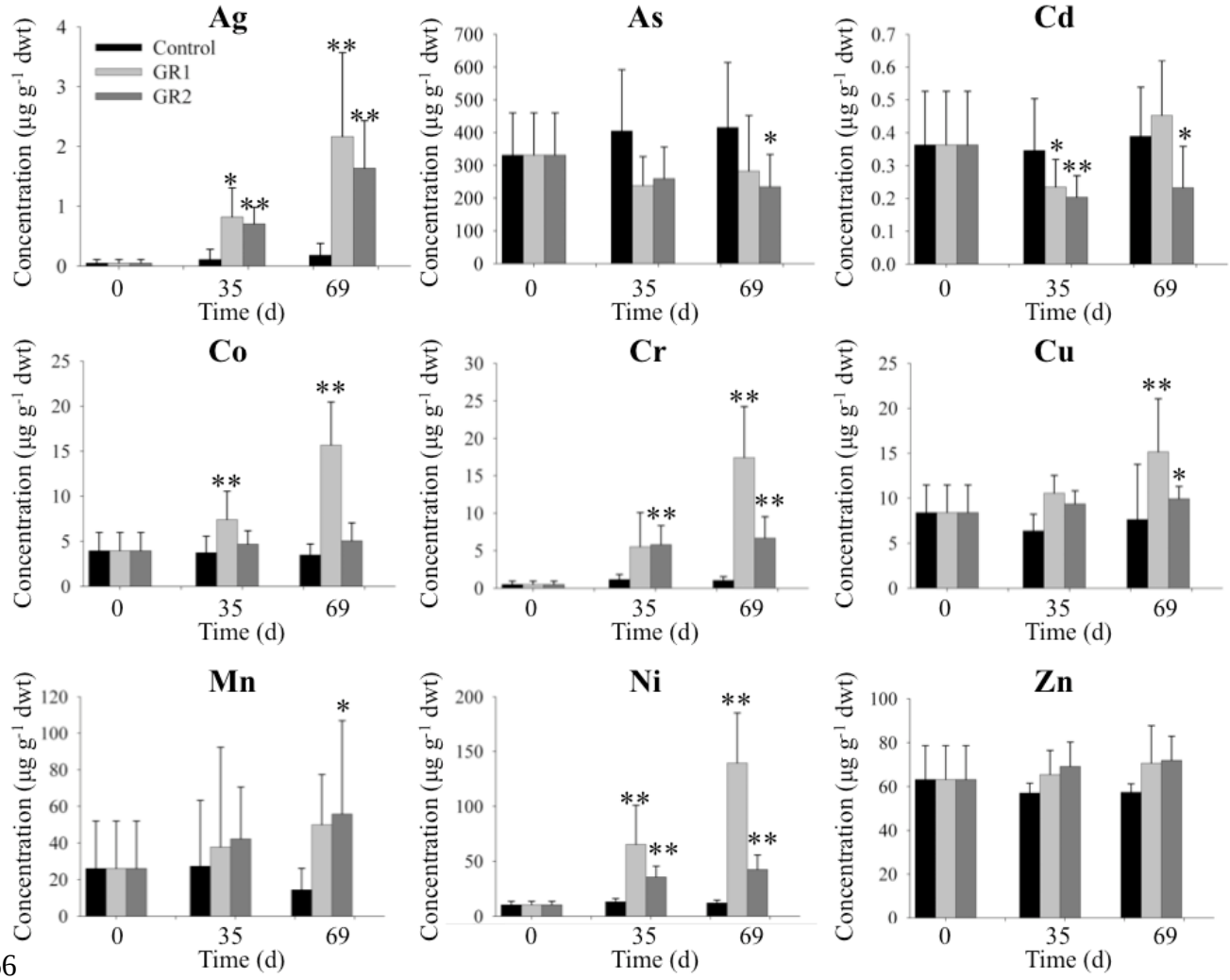

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Table 1. ICP-OES and ICP-MS analysis of certified reference materials: certified values and measured values (mean ± SD µg g^{-1} dwt)

		TORT-2			DOLT-3		
Element	**Method**	**Found** *Mean ± SD*	**Certified** *Mean ± SD*	**% Recovery**	**Found** *Mean ± SD*	**Certified** *Mean ± SD*	**% Recovery**
Ag	ICP-MS	No certified value			1.07 ± 0.092	1.20 ± 0.07	89.3
As	ICP-OES	22.28 ± 2.22	21.60 ± 1.80	103.2	9.45 ± 0.97	10.20 ± 0.50	92.7
Cd	ICP-MS	26.42 ± 3.75	26.70 ± 0.60	99.0	17.01 ± 3.12	19.40 ± 0.60	87.7
Co	ICP-MS	0.52 ± 0.089	0.51 ± 0.091	101.5	No certified value		
Cr	ICP-OES	0.66 ± 0.19	0.77 ± 0.15	85.3	No certified value		
Cu	ICP-OES	98.40 ± 11.17	106.0 ± 10.0	92.8	31.23 ± 2.40	31.20 ± 1.00	100.1
Mn	ICP-OES	12.46 ± 1.19	13.60 ± 1.20	91.6	No certified value		
Ni	ICP-OES	2.02 ± 0.35	2.50 ± 0.19	80.9	3.05 ± 0.76	2.72 ± 0.35	112.1
Zn	ICP-OES	187.6 ± 19.6	180.0 ± 6.0	104.2	97.67 ± 6.97	86.60 ± 2.40	112.8

774 **Table 2.** Element concentrations (mean ± SD; µg g^{-1} dwt, n = 3) in sediments collected in six sampling sites.

775 GR_{Int}: Grande Rade Interdital station; GR_1: Grande Rade subtidal site 1; **GR_2**: Grande Rade subtidal site 2

	Ouano beach	**Maa bay**	**Boulari bay**	**GR_{Int}**	**GR_1**	GR_2
Ag	0.019* ± 0.028	0.013* ± 0.014	0.06* ± 0.04	0.35* ± 0.13	0.17* ± 0.09	0.018* ± 0.015
As	3.1* ± 1.2	6.4* ± 0.3	16.7* ± 1.3	8.0* ± 1.2	7.0* ± 5.9	15.4 ± 0.5*
Cd	0.4 ± 0.2	1.0 ± 0.2	1.1 ± 0.3	2.5 ± 0.2	3.7 ± 1.2	0.8 ± 0.1
Co	0.8 ± 0.4	4.4 ± 2.3	15.4 ± 11.1	49.2 ± 5.2	366 ± 145	6.1 ± 0.9
Cr	7.8 ± 2.4	46.9 ± 4.0	71.5 ± 10.2	309 ± 39	1,290 ± 410	24.6 ± 2.9
Cu	1.4* ± 0.7	7.0 ± 0.5	0.9* ± 0.1	27.0 ± 3.6	9.6 ± 3.3	2.8* ± 0.4
Mn	44.7 ± 14.9	134 ± 6.7	545 ± 53.0	304 ± 15	1,600 ± 600	76.7 ± 8.1
Ni	5.6 ± 3.0	69.2 ± 5.6	101 ± 12.9	848 ± 78	10,500 ± 3,300	66.4 ± 15.8
Zn	3.5 ± 2.0	16.3 ± 1.3	7.1 ± 1.6	148 ± 11.0	73.3 ± 22.7	12.8 ± 1.8

776 ***: inferior to detection limit.**

Table 3. Minimal sample size of the oyster *Isognomon isognomon* and the clam *Gafrarium tumidum* necessary to detect with 90 % significance a difference ($p < 0.05$) of concentrations between two groups of organisms**.**

Observed range of element concentrations represents concentrations that have been measured in the two species resident from different stations along the New Caledonia coast; number between brackets represents concentrations that have been reached during transplantation experiments.

Element	Species	Observed Concentration range in tissues*	Difference (µg g^{-1} dwt)	*Sample size (number of individuals required)* *I. isognomon* Concentration		*G. tumidum* Concentration	
				Low	High	Low	High
Ag	Oyster	1.5 - 32.8	**1**	21	110	< 3	43
	Clam	0.02 - 33.1	**3**	**4**	**14**	**< 3**	**6**
			10	**< 3**	**< 3**	**< 3**	**< 3**
			30	**< 3**	**< 3**	**< 3**	**< 3**
As	Oyster	21.6 - 76.6	**10**	32	111	3,713	8,260
	Clam	37.4 - 441	**20**	**9**	**29**	921	2,065
			40	**4**	**8**	231	517
			80	**< 3**	**< 3**	59	130
			150	**< 3**	**< 3**	**18**	**38**
			350	**< 3**	**< 3**	**5**	**8**
Cd	Oyster	1.2 - 2.5	**0.2**	220	894	4	160
	Clam	0.17 - 1.8	**0.5**	36	144	**< 3**	**27**
			1	**10**	**37**	**< 3**	**8**
			2	**4**	**10**	**< 3**	**4**
Co	Oyster	0.5 - 2.5	**0.2**	8	170	780	> 10,000
	Clam	1.1 - 7.2 (15.7)	**0.5**	**< 3**	**28**	126	1,945
			1	**< 3**	**8**	32	487
			2	**< 3**	**< 3**	9	122
			5	**< 3**	**< 3**	**< 3**	**21**
			10	**< 3**	**< 3**	**< 3**	**6**
Cr	Oyster	1.6 – 9.0	**1**	**9**	**54**	7	993
	Clam	1.1 - 10.5 (17.4)	**2**	**4**	**15**	< 3	248
			4	**< 3**	**5**	**< 3**	**63**
			8	**< 3**	**< 3**	**< 3**	**17**
			15	**< 3**	**< 3**	**< 3**	**8**
Cu	Oyster	3.1 - 17.3	**2**	6	153	15	184
	Clam	5.6 - 88.2	**4**	**< 3**	**39**	**5**	**47**
			8	**< 3**	**11**	**< 3**	**12**
			15	**< 3**	**4**	**< 3**	**5**
			30	**< 3**	**< 3**	**< 3**	**< 3**
			60	**< 3**	**< 3**	**< 3**	**< 3**
Mn	Oyster	17.0 - 34.7	**2**	260	1,938	727	8,590
	Clam	5.5 - 187.4	**4**	66	485	183	2,148
			8	18	122	47	538
			15	**6**	**36**	14	154
			30	**< 3**	**10**	**5**	**40**
			60	**< 3**	**4**	**< 3**	**11**
			120	**< 3**	**< 3**	**< 3**	**4**
Ni	Oyster	2.2 - 16.0 (32.4)	**2**	7	963	36	6,505
	Clam	8.1 - 63.2 (140)	**4**	< 3	242	10	1,627
			8	**< 3**	**62**	4	410
			15	**< 3**	**19**	< 3	117
			30	**< 3**	**5**	**< 3**	**30**
			60	**< 3**	**< 3**	**< 3**	**9**
			120	**< 3**	**< 3**	**< 3**	**4**
Zn	Oyster	1700 - 13,820	**5**	>10,000	>10,000	14	248
	Clam	55.6 - 154	**10**	722	4,180	**5**	**63**
			50	182	1,045	**< 3**	**4**
			100	**9**	**43**	**< 3**	**< 3**
			500	**4**	**12**	**< 3**	**< 3**
			10,000	**< 3**	**< 3**	**< 3**	**< 3**

* from Breau 2003, Hédouin et al. 2008a, Present study