



HAL
open science

Comparison in waterborne Cu, Ni and Pb bioaccumulation kinetics between different gammarid species and populations: Natural variability and influence of metal exposure history

Nastassia Urien, Antoine Farfarana, Emmanuelle Uher, Lise C. Fechner, Arnaud Chaumot, Olivier Geffard, Jérémie D. Lebrun

► To cite this version:

Nastassia Urien, Antoine Farfarana, Emmanuelle Uher, Lise C. Fechner, Arnaud Chaumot, et al.. Comparison in waterborne Cu, Ni and Pb bioaccumulation kinetics between different gammarid species and populations: Natural variability and influence of metal exposure history. *Aquatic Toxicology*, 2017, 193, pp.245-255. 10.1016/j.aquatox.2017.10.016 . hal-01695094

HAL Id: hal-01695094

<https://hal.science/hal-01695094>

Submitted on 29 Jan 2018

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Comparison in waterborne Cu, Ni and Pb bioaccumulation kinetics between different gammarid species and populations: natural variability and influence of metal exposure history

N. Urien^{a,1}, A. Farfarana^a, E. Uher^a, L.C. Fechner^{a,2}, A. Chaumot^b, O. Geffard^b, J.D. Lebrun^{a,c}

Affiliations:

^a Irstea, UR HBAN – Artemhys, 1 rue Pierre-Gilles de Gennes, CS 10030, F-92761 Antony Cedex, France

^b Irstea, UR MALY, laboratoire d'écotoxicologie, centre de Lyon-Villeurbanne, F-69625 Villeurbanne, France

^c FIRE FR-3020, 4 place Jussieu, 75005 Paris, France

¹ present address: Institut national de la recherche scientifique, Centre Eau Terre et Environnement (INRS-ETE), 490 de la Couronne, Québec (Québec) G1K 9A9, Canada

² present address: Ifremer, 155 rue J. J. Rousseau, 92138 Issy-les Moulineaux, France

Key words (5 max):

Amphipods; Trace metals; Life history; Kinetic parameters; Inter-population variability

Abstract

Kinetic parameters (uptake from solution and elimination rate constants) of Cu, Ni and Pb bioaccumulation were determined from two *Gammarus pulex* and three *Gammarus fossarum* wild populations collected from reference sites throughout France in order to assess the inter-species and the natural inter-population variability of metal bioaccumulation kinetics in that sentinel organism. For that, each population was independently exposed for seven days to either 2.5 $\mu\text{g}\cdot\text{L}^{-1}$ Cu (39.3 nM), 40 $\mu\text{g}\cdot\text{L}^{-1}$ Ni (681 nM) or 10 $\mu\text{g}\cdot\text{L}^{-1}$ Pb (48.3 nM) in laboratory controlled conditions, and then placed in unexposed microcosms for a 7-day depuration period. In the same way, the possible influence of metal exposure history on subsequent metal bioaccumulation kinetics was addressed by collecting wild gammarids from three populations inhabiting stations contaminated either by Cd, Pb or both Pb and Ni (named pre-exposed thereafter). In these pre-exposed organisms, assessment of any changes in metal bioaccumulation kinetics was achieved by comparison with the natural variability of kinetic parameters defined from reference populations. Results showed that in all studied populations (reference and pre-exposed), no significant Cu bioaccumulation was observed at the exposure concentration of 2.5 $\mu\text{g}\cdot\text{L}^{-1}$. Concerning the reference populations, no significant differences in Ni and Pb bioaccumulation kinetics between the two species (*G. pulex* and *G. fossarum*) was observed allowing us to consider all the five reference populations to determine the inter-population natural variability, which was found to be relatively low (kinetic parameters determined for each population remained within a factor of 2 of the minimum and maximum values). Organisms from the population exhibiting a Pb exposure history presented reduced Ni uptake and elimination rate constants, whereas no influence on Ni kinetic parameters was observed in organisms from the population exhibiting an exposure history to both Ni and Pb. Furthermore Pb bioaccumulation kinetics were unaffected whatever the condition of pre-exposure in natural environment. Finally, these results highlight the complexity of confounding factors, such as metal exposure history, that influence metal bioaccumulation processes and showed that pre-exposure to one metal can cause changes in the bioaccumulation kinetics of other metals. These results also address the question of the underlying mechanisms developed by organisms to cope with metal contamination.

1. Introduction

Trace metals are natural components of the Earth's crust, but human activities have largely contributed to their release in freshwaters into chemical forms that are more mobile and eventually more available for biota (Thévenot et al., 2007). Even at low concentrations, diffuse and chronic metallic exposure may result in metal accumulation and toxicity in aquatic species (Couture and Kumar, 2003; Gismondi et al., 2017). Exposed populations may undergo changes for instance in physiological mechanisms limiting metal toxicity, which may eventually lead to tolerant populations (Luoma, 1977). Among those changes, the most commonly reported in the literature is the induction of metal detoxification mechanisms, for example, by increasing the synthesis of molecules designed to sequester metals either in or out of the cells and prevent them from exerting their toxic effects such as metallothioneins (MT) or metal-rich granules (MRG) (Rainbow and Luoma, 2011; Roesijadi, 1992; Vijver et al., 2004). Pre-exposition to metals may also affect subsequent metal bioaccumulation kinetics as a result of reduction of metal uptake (from the soluble phase or the diet) and/or increase of metal excretion/elimination (Mason and Jenkins, 1995; Postma et al., 1996; Wang and Rainbow, 2005). Nevertheless, changes in bioaccumulation kinetics following metal pre-exposure is complex and most underlying mechanisms are poorly understood (Mouneyrac et al., 2003; Rainbow et al., 1999; Rainbow and Luoma, 2011).

The amphipods from the *Gammarus* genus, used as test organisms in the present study, are largely distributed in rivers and streams of Europe, where they are often present in high density in various habitats (Barnard and Barnard, 1983). Gammarids have been studied for many years both in laboratory and field conditions for ecotoxicological purposes, and are known to be accumulators of various metals at environmental exposure levels (Besse et al., 2013; Fialkowski and Rainbow, 2006; Lebrun et al., 2015, 2014, Urien et al., 2016, 2015). For these reasons, gammarids constitute relevant candidates for biomonitoring metal contamination in freshwaters (Besse et al., 2012).

Biodynamic modelling has become an established tool for describing trace metal bioaccumulation processes in various aquatic organisms including gammarids (Cresswell et al., 2014; Hadji et al., 2016; Lebrun et al., 2011; Ponton and Hare, 2010; Urien et al., 2015). Briefly, the biodynamic model assumes that bioaccumulation is the result of a balance between the metal uptake rate, from aqueous and dietary routes, and loss rate (Luoma and Rainbow, 2005). Biodynamic model of bioaccumulation is characterized by kinetic parameters (namely the uptake (k_m) and

elimination (k_{out}) rate constants, and the assimilation efficiency (AE)) which can be determined under laboratory conditions. By comparing kinetic parameters determined for different populations, we assume that the biodynamic model had the potential to highlight changes in bioaccumulation processes among populations (Rainbow et al., 2009, 2003).

Khan et al. (2011) explored differences in metal (Cu and Zn) uptake rates in a population of *Gammarus pulex* historically impacted with Cu and Zn and in a transplanted naive population. The authors reported a lower uptake of Cu and Zn in the historically impacted population, suggesting adaptation likely leading to higher tolerance. In another study, Schaller et al. (2011) concluded that *G. pulex* could be adapted to high levels of metals by reducing their uptake. However, studies evaluating the influence of metal exposure history on metal bioaccumulation kinetics usually use a single reference population. Roughly, we can count as many reference populations as existing studies assessing metal bioaccumulation kinetics. Generally, those reference populations come from various hydrosystems characterized by contrasting ambient physicochemical parameters likely to affect animal physiological functions and potentially their bioaccumulation abilities. Interference of physiological differences among reference populations may also confound bioaccumulation interpretation. Blackmore and Wang (2003a) investigated metal (Cd, Cr, Se and Zn) bioaccumulation kinetics in coastal green mussels (*Perna viridi*) collected from two sites with contrasting salinity levels and acclimated to different levels of salinity in the laboratory. The authors reported that mussels from the high salinity site accumulated metals faster than mussels from the low salinity site. In another study of marine bivalves, Blackmore and Wang (2003b) observed that Cd and Zn uptake (dissolved and particulate) and clearance rates varied little over large geographical distances and climatic zones (subarctic and temperate). In the ragworm, *Nereis diversicolor*, Kalman et al. (2010) reported limited inter-population variability in Ag, Cd and Zn bioaccumulation parameters in two populations from different climatic zones in Europe (England and Spain). Therefore, natural variability of bioaccumulation kinetics in freshwater invertebrates deserves to be investigated.

In the present study, two objectives were defined: (i) to confirm the lack of species differences and quantify the natural inter-population variability of waterborne Cu, Ni and Pb bioaccumulation kinetics in populations of gammarids (*G. pulex* and *G. fossarum*) coming from sites exempt of metal contamination (reference populations), distributed over a large geographical distance in France and exhibiting different ambient physicochemical parameters, and (ii) to assess the

influence of metal field-exposure history on the bioaccumulation kinetics of Cu, Ni and Pb in gammarid populations with different metal contamination history profiles. The natural inter-population variability of kinetic constants obtained from (i) was used to assess (ii).

For that, populations of *Gammarus pulex* and *G. fossarum* coming from five reference sites and three sites exhibiting metallic contamination, were collected for subsequent exposure in the laboratory. In the laboratory, kinetic parameters (uptake and elimination rate constants) of Cu, Ni and Pb were determined for each metal/population combination. Based on the similar accumulation pattern reported in the literature between the two species (Lebrun et al., 2015) and the fact that those species are closely related (Pacaud, 1945; Roux, 1970), we assumed that no substantial differences in bioaccumulation kinetic constants will be observed. Nevertheless, before seeking inter-population differences, the lack of inter-specific differences between *G. pulex* and *G. fossarum* among reference populations had to be confirmed. Only then could kinetic parameters be determined for the reference populations and used to assess natural inter-population variability of metal bioaccumulation kinetic. Finally, we investigated if kinetic parameters determined in the three different contaminated populations fell into natural variability or not. The choice to focus on the three elements, Cu, Ni and Pb lies in the fact that they are representative of an anthropic-related contamination, targeted by European and national regulations and have already been studied in our previous studies (Urien et al., 2016, 2015).

2. Materials and methods

2.1. Sampling sites

Five wild populations of gammarids considered as not contaminated with trace metals from their environment (referred to reference populations thereafter) and three populations subjected to metal contamination (referred as pre-exposed populations thereafter) were collected in the field. Among the reference populations, three were collected from the Seine watershed (North of France), named GUE, MENE and NEAU, and two were collected from the Rhône watershed (South-East of France), named TOUR and BACU (Figure 1, open symbols). Two pre-exposed populations were collected from the Seine watershed, named GAL and BIE, whereas the third pre-exposed population was collected from the Rhône watershed and named ARDI thereafter (Figure 1, full symbols).

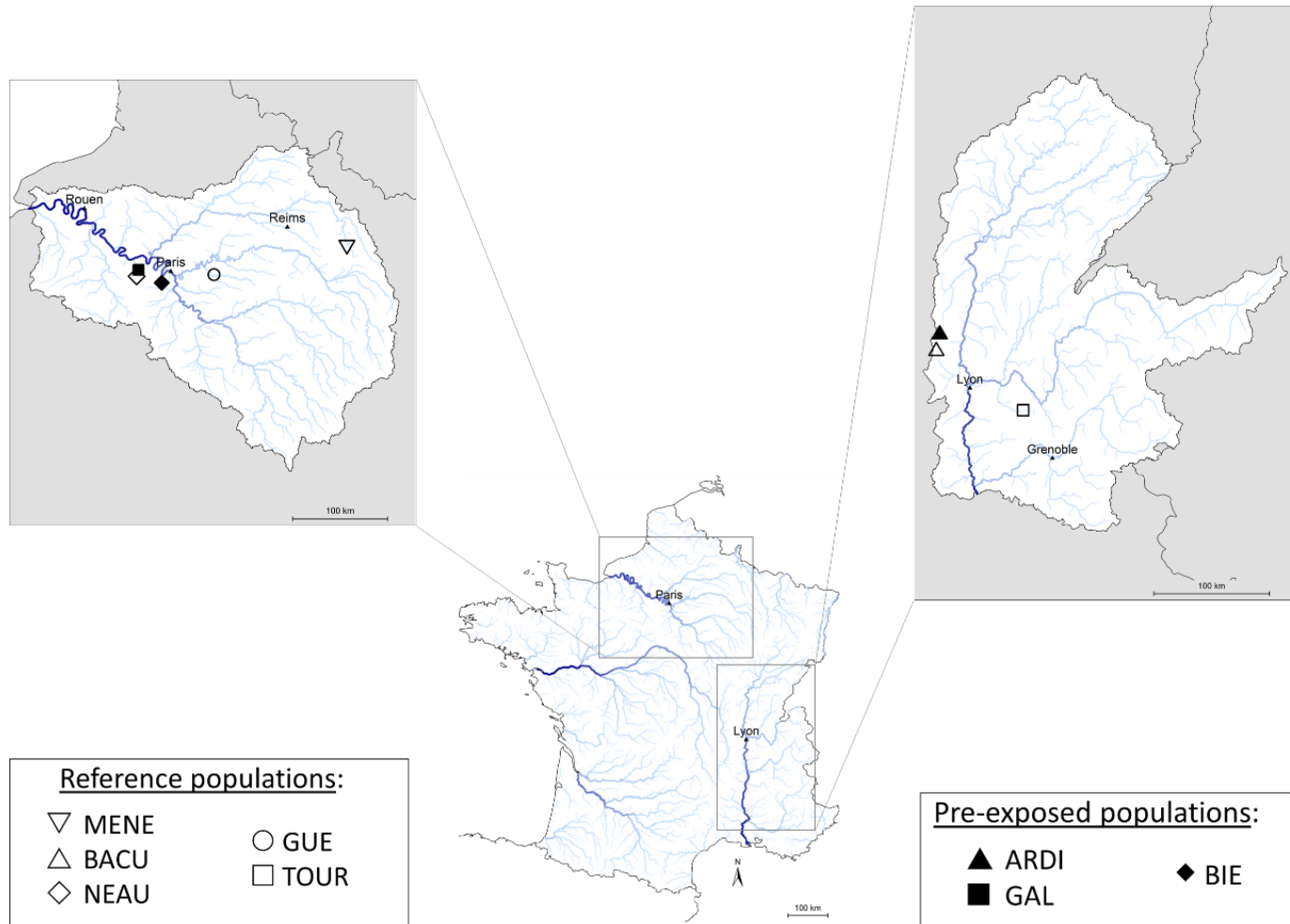


Figure 1: Localization of the five sites from which reference populations of gammarids (open symbols) and the three sites from which pre-exposed populations (full symbols) were sampled for subsequent exposure to Cu, Ni and Pb in the laboratory.

ARDI, BACU, GUE and TOUR gammarids were identified as *G. fossarum*, whereas BIE, GAL, MENE and NEAU gammarids were identified as *G. pulex*. Note that, *G. pulex* and *G. fossarum* are the most commonly found species in French rivers, and the choice to collect populations from those two species was driven by the decision to be as representative as possible of the variability found in environmental conditions.

As in the study of Vigneron et al. (2015), the “reference” or “pre-exposed” status of each selected population was determined based on an “active biomonitoring approach” based on a caging methodology as previously described in our studies (Besse et al., 2013; Ciliberti et al., 2017; Urien et al., 2016). Using *in situ* caging methodology consists of transplanting for 7 days size-calibrated male gammarids from a single source population (Bourbe river, France) acclimated for 3 weeks in the laboratory, at the site under study. Bioaccumulated metal concentrations in the transplanted gammarids are then compared to threshold values of bioavailable contamination proposed for French rivers for Ni and Pb (Ciliberti et al., 2017) and at a regional scale (Rhône-Alpes) for Cu (Besse et al., 2013). For each element, concentrations above this threshold value is considered as a sign of a significant bioavailable contamination of the study site, whereas concentrations under the threshold value is considered as reflecting a site conforming to the background level of concentrations. Bioaccumulated metal concentrations in the transplanted gammarids are presented in Table 1.

Regarding the pre-exposed populations, they were characterized by different metallic pressures: GAL is located downstream from a military shooting range and in a peri-urban area where gammarids are contaminated with Pb; BIE is located in an urban zone and receives domestic effluents and gammarids are contaminated with Pb and to a lesser extent with Ni ; ARDI population of gammarids is located in a rural area and is known to be naturally contaminated with Cd due to the geochemical background (Vigneron et al., 2015).

Table 1: Metal concentrations in transplanted *G. fossarum* at each studied site for Cd, Cu, Ni and Pb (Urien et al., 2016). All values are expressed in $\mu\text{g.g}^{-1}$ dry weight and bold data means that a metallic contamination of gammarids was highlighted using the active biomonitoring approach of Ciliberti et al. (2017). Mean values are given with their standard deviation when the number of replicates was equal to three ($n = 3$). If not, it means that only one replicate was available.

	Cu	Ni	Pb	Cd
Reference sites				
BACU	52 ± 1.0	< LoQ ± NA	0.64 ± 0.2	0.30 ± 0.06
GUE	68 ± 1.7	0.55 ± 0.06	0.24 ± 0.1	0.15 ± 0.02
MENE	67	0.63	0.28	0.20
NEAU	72	0.67	0.32	0.20
TOUR	55 ± 5.0	< LoQ ± NA	0.31 ± 0.1	0.18 ± 0.04
Contaminated sites				
ARDI	59 ± 2.3	1.05 ± 0.25	0.65 ± 0.11	1.43 ± 0.10
BIE	56 ± 1.6	1.20 ± 0.29	4.23 ± 1.12	0.13 ± 0.02
GAL	62 ± 9.7	< LoQ ± NA	6.65 ± 1.80	0.08 ± 0.03

2.2. Collection and maintenance of gammarids in the laboratory

Adult gammarids from reference populations were collected in July 2013 for subsequent exposures to Cu, Ni and Pb in the laboratory, whereas gammarids from pre-exposed populations were collected in January 2015. Due to logistic constraints, all populations were not collected at the same time, but gammarids from GUE (a reference population) were collected twice in July 2013 and January 2015 so as to check for any influence of the sampling period on metal bioaccumulation kinetics.

During the sampling procedure, gammarids were sieved (between mesh-size of 2 and 2.5 mm) to collect only adult individuals (length of about 1 cm for a width between 2 and 2.5 mm), transported to the laboratory, and then acclimatized for 7 days at 14°C in aerated mineral water (Volvic® Ca²⁺ 11.5, Na⁺ 11.6, Mg²⁺ 8.0, K⁺ 6.2, Cl⁻ 13.5, SO₄²⁻ 8.1 mg.L⁻¹ and pH 7) with a 10:14-h light:dark photoperiod. Volvic® mineral water was chosen because of its low mineral content

which decreases the risk of metal precipitation during exposures. Organisms were fed *ad libitum* with hornbeam leaves (*Carpinus betulus*) directly collected at their respective sampling site.

2.3. Physicochemical parameters of the water column at the reference sites

At each site where reference populations were collected, pH and conductivity in the water column were measured. Raw water samples, filtered through a 0.45-mm PES (polyethersulfone) filter (Millipore), were also collected to measure the concentrations of major ions by chromatographic analysis [Dionex DX120, column IonPac CS16 Dionex No 057573 (4x250 mm)]. The physicochemical parameters are presented in Table 2.

Table 2: Physicochemical parameters measured in the water column at each reference site.

	TOUR	NEAU	BACU	MENE	GUE_2013	GUE_2015
Ca ²⁺	115	123	8	130	105	87
Na ⁺	7	30	7	8	16	13
Mg ²⁺	3	15	2	2	8	7
Cl ⁻ (mg/L)	16	58	7	29	27	30
K ⁺	2	6.6	1.3	1.5	2	1.2
HCO ₃ ⁻	310	279	< 30	274	229	NA
SO ₄ ²⁻	14	71	8	32	31	44
DOC	53	6	6	2.3	68	NA
Conductivity (µS/cm)	560	760	90	600	550	656
Temperature (°C)	14	11	14	10	13	5
pH	8.3	8.1	8.4	7.7	7.5	8

2.4. Laboratory experiments

2.4.1. Metal accumulation and depuration kinetics

Aquatic microcosms consisting of plastic beakers were filled with 500 mL Volvic® mineral water and independently spiked with CuSO₄·5H₂O, NiSO₄·6H₂O or Pb(NO₃)₂ (Sigma-Aldrich), so as to obtain final nominal concentrations of 2.5 µg L⁻¹ (39.3 nM), 10 µg L⁻¹ (48.3 nM) and 40 µg L⁻¹ (681 nM) of Cu, Pb and Ni, respectively. Exposure concentrations were chosen so as to be close to either the Environmental Quality Standard (EQS) of the European Water Framework Directive

(Directive 2008/105/EC, 2008) or Predicted No Effect Concentration (PNEC) values from the literature (Bisson et al., 2005). Each population (reference and pre-exposed) was independently exposed to each metal and controls were also included, where gammarids from each site were not exposed to metals. Each condition was performed in triplicate, i.e., three microcosms per condition and per population (Figure 2).

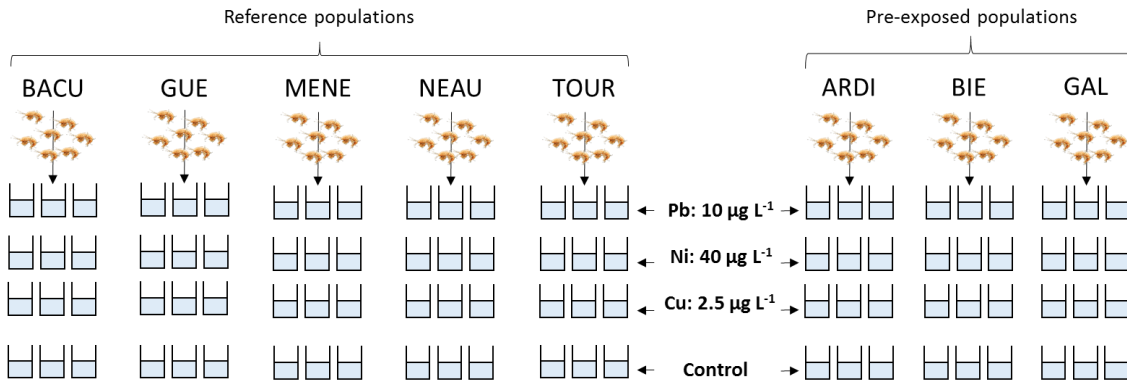


Figure 2: Experimental design of the laboratory work. Collected gammarids from each site were exposed to metal in the laboratory for 7 days followed by a 7-day depuration period. Each experimental condition was performed in triplicate and for each metal/population combination a control was added (where gammarids were not exposed to metals).

So as to ensure a correct final metal exposure, each beaker was pre-equilibrated for 48h with the final metallic solution to saturate the potential adsorption sites on beaker walls before performing the exposure. Then, fifty 24h-starved gammarids from each population were introduced to each beaker for metallic exposure and left without food for 7 days which were followed by a 7-day depuration period. During the exposure phase, water was renewed every day to ensure a constant exposure and oxygenation. During the depuration period, water was daily renewed with uncontaminated Volvic® mineral water and hornbeam leaves from each sampling site were added to feed the gammarids. A pool of five gammarids per beaker was sampled on days 0, 1, 2, 4 and 7 for the exposure phase, and on days 8 (except for the Pb experiment), 9, 11 and 14 for the depuration phase. Each time, gammarids were rinsed in a solution of 2 and then 0.5 mM EDTA (ethylene-diamine tetra-acetic acid, Sigma-Aldrich) right after sampling, and then rinsed twice

with ultrapure water to remove metal potentially adsorbed on their cuticle (Lebrun et al., 2011) and then stored at -20°C until metal analysis. At each sampling time, dead gammarids were counted and removed, and the renewed water was sampled and acidified with HNO_3 (65% Suprapur, Merck, Darmstadt, Germany) at 1% v/v to check dissolved metal concentrations.

2.4.2. Metal analyses in water and gammarids

Exposure concentrations in beakers were checked using a graphite furnace atomic absorption spectrophotometer (AAS; SpectrAA 220Z with Zeeman background correction, Varian, Pao Alto, California, USA). The quality of the analysis was checked by analyzing two certified reference materials every 20 samples (natural waters: SPS-SW1, Spectrapure standard A,S Oslo, Norway, and EP-L-2, EnviroMAT, SCP Science, Villebon-sur-Yvette, France).

For analysis, gammarids were freeze-dried (Christ Alpha 2-4 LD plus), weighed, and digested at 95°C (Digiprep Jr, SCP Science, Villebon-sur-Yvette, France) with HNO_3 (65% suprapur, Merck, Darmstadt, Germany), H_2O_2 (suprapur, Merck), and diluted with ultrapure water. Cu, Ni and Pb concentration analyses in gammarids were also performed by AAS. In the case of Pb, analyses were performed by standard addition to limit matrix effects. The quality of the whole analysis process of gammarids including digestion was checked by analyzing the two reference materials cited above every 20 samples as well as a certified reference material of biological tissue which had undergone the same digestion procedure as gammarids (CMR: Mussel Tissue, European Reference Material ERM[®]-CE278, Sigma-Aldrich). The mean concentration of metals of each certified reference material was consistently within the certified 95% confidence limit range for all the studied metals. Metal concentrations measured in gammarids were expressed in $\mu\text{g}\cdot\text{g}^{-1}$ dw (dry weight).

2.5. Bioaccumulation modelling and kinetic parameters determination

2.5.1. Theory

According to a first-order kinetic model (Landrum et al., 1992), net waterborne metal bioaccumulation can be described by a simplified biodynamic model by the following equation:

$$Ca(t) = C_w \times k_{in} / k_{out} \times (1 - \exp^{(k_{out} \times t)}) \quad \text{Eq. (1)}$$

where C_a is the net amount of metal accumulated by the organism ($\mu\text{g}\cdot\text{g}^{-1}\text{ dw}$), t is the time, C_w is the concentration of dissolved metal in the water ($\mu\text{g}\cdot\text{L}^{-1}$), k_{in} is the uptake rate constant ($\text{L}\cdot\text{g}^{-1}\cdot\text{d}^{-1}$) and k_{out} is the elimination rate constant (d^{-1}).

The net bioaccumulation is obtained by subtracting the metal concentrations measured in the organisms at the beginning of the exposure (basal concentrations) from the total metal concentrations measured after exposure. Basal concentrations are often close to $0\ \mu\text{g}\cdot\text{g}^{-1}$ and can be neglected for non-essential metals such as Pb, but can be particularly high for essential metals such as Cu (Besse et al., 2013; Urien et al., 2016). Indeed, for essential metals, the basal concentration is assumed to represent the metal incorporated in essential biochemical components (proteins) and necessary to ensure physiological functions in the organisms.

At the end of the exposure phase, organisms were introduced into beakers containing un-spiked Volvic® mineral water for the depuration phase; C_w was, therefore, considered as null. Under these conditions, metal elimination is described as follows:

$$C_a(t) = C_{a(0)} \times \exp(-k_{out} \times t) \quad \text{Eq. (2)}$$

where $C_{a(0)}$ is the net concentration of metal in organisms at the beginning of the depuration phase ($\mu\text{g}\cdot\text{g}^{-1}$).

Note that, in the present study, the elimination rate constant is more likely to reflect a real efflux rate constant, on the assumption that any uptake during the depuration phase in un-spiked water is negligible in comparison to the measured elimination rate.

2.5.2. Fitting to bioaccumulation data and statistical analyses

To determine the kinetic parameters, k_{in} and k_{out} , a kinetic model was fitted by nonlinear least square regression to the net bioaccumulation data simultaneously for both the exposure and the depuration phases together, since Eq. (1) and Eq. (2) share the parameter k_{out} . Kinetic parameters (k_{in} and k_{out}) were determined using the R software (R Core Team, 2015) and the 'nlstools' package (Baty et al., 2014). They are given with their respective 95% confidence intervals and are considered significantly different when 95% confidence intervals do not overlap.

Nonparametric Kruskal-Wallis tests were used to compare metal concentrations in gammarids ($n = 3$) during the experiment (exposed versus control gammarids).

2.6. Natural inter-population variability of bioaccumulation kinetic parameters

To quantify natural variability range of kinetic parameters among reference populations, we chose to use the calculation proposed by Barret et al. (2015), that enclosed 95 % of data, as a classic confidence interval range, and taking also into account the number of populations used as follows:

$$\bar{y} \pm t_{\frac{\alpha}{2}, n-1} \times \text{standard deviation} \times \sqrt{1 + 1/n}$$

where \bar{y} is the mean value of the kinetic parameters, $t_{\frac{\alpha}{2}, n-1}$ is the $(1-\alpha/2)$ fractile of a t-distribution with $n - 1$ degrees of freedom (equal to 2.78 for $\alpha = 0.05$), and n is the number of observations.

3. Results

3.1. Reference populations of gammarids

During the exposure phase, metal concentrations measured in the water of microcosms were constant and close to the nominal concentrations (i.e., mean concentrations ($\pm sd$): 2.3 (± 0.1) $\mu\text{g.L}^{-1}$ for Cu, 10.6 (± 0.6) $\mu\text{g.L}^{-1}$ for Pb and 45 (± 1.3) $\mu\text{g.L}^{-1}$ for Ni, $n = 12$).

3.1.1. Bioaccumulation kinetics

Cu concentrations measured in gammarids from the reference populations ranged from 60 to 100 $\mu\text{g.g}^{-1}$ dw and did not vary over time. Moreover, no changes in Cu contents in exposed gammarids were observed compared to the control group, as well as no differences between species (MENE and NEAU versus TOUR, BACU and GUE) ($p > 0.05$, Figure 3). As a consequence, no model could be fitted to the data and bioaccumulation kinetic constants could not be estimated. Interestingly, note that although there is no increase in Cu body burden in Cu-exposed gammarids relative to the controls, it appears to be a common trend in all groups to slightly increase from days 1 to 3, and to decrease thereafter. Although no clear explanation can be drawn, it could be suggested that the introduction of gammarids from the acclimation tank to the beakers for metallic exposure induced a stress leading to hyperventilation and a short increase of Cu incorporation in order to transport more oxygen, afterwards regulated.

Ni was significantly accumulated by gammarids during the 7-day exposure phase, and similar uptake and elimination patterns were observed among all the reference populations, independently of the species: Ni body concentrations sharply increased the first 48 hours of exposure, followed by a progressive slow down to finally tend towards steady-state concentrations (mean concentration (\pm *sd*) measured at day 7 ($\mu\text{g}\cdot\text{g}^{-1}$ dw) for TOUR: 13. (\pm 0.9); NEAU: 10.9 (\pm 2.4), BACU: 17.2 (\pm 4.1), MENE: 13.3 (\pm 6.1) and GUE: 10.4 (\pm 1.3)). During the depuration phase, accumulated metal concentrations fell exponentially and, after 7 days, gammarids recovered their basal Ni concentrations (Figure 3). In the control group, Ni concentrations remained stable and very low ($< 0.5 \mu\text{g}\cdot\text{g}^{-1}$ dw, data not shown).

As observed for Ni, Pb was significantly accumulated by gammarids during the 7-day exposure phase, and followed a similar accumulation and elimination pattern among populations i.e. a sharp increase during the first 48 hours of exposure, followed by a progressive slow down to finally tend towards steady-state concentrations (mean concentration (\pm *sd*) measured at day 7 ($\mu\text{g}\cdot\text{g}^{-1}$ dw) for TOUR: 16.8 (\pm 2.9); NEAU: 13.9 (\pm 6.7), BACU: 22.4 (\pm 1.7), MENE: 22.8 (\pm 1.9) and GUE: 23.7 (\pm 5.0)). During the depuration phase, accumulated metal concentrations decreased exponentially, and, unlike what was observed for Ni, basal Pb concentrations in gammarids were not fully recovered after 7 days (Figure 3). In the control group, Pb concentrations remained stable and very low ($< 0.5 \mu\text{g}\cdot\text{g}^{-1}$ dw, data not shown). As for Ni, no marked difference in the pattern of accumulation or elimination of Pb was observed between *G. pulex* and *G. fossurum*.

Regarding the results for Ni and Pb, a first-order kinetic model was fitted to the bioaccumulation data for each population so as to estimate for these two metals and for each reference population the kinetic parameters, k_{in} and k_{out} .

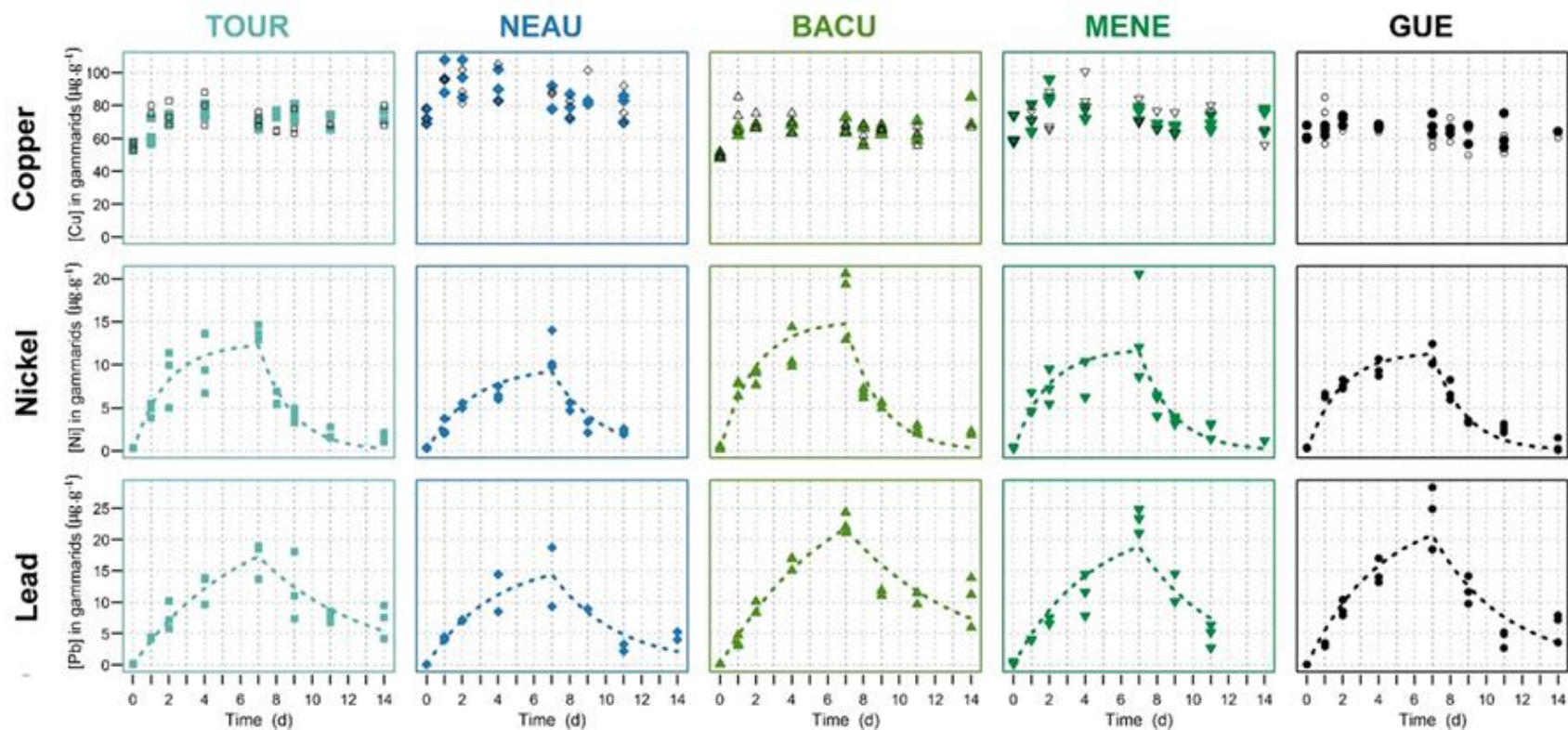


Figure 3: Bioaccumulation kinetics of Cu, Ni and Pb in the five reference populations of gammarids (TOUR, NEAU, BACU, MENE and GUE). For Ni and Pb, full points represent the net accumulation of metals in gammarids (without basal concentrations). For Cu, full points represent the total Cu concentrations measured in exposed gammarids, whereas the empty points account for Cu concentrations measured in the control group of gammarids (unexposed gammarids) (for visual convenience, basal Cu concentration measured in the control group of gammarids was not subtracted to total Cu concentrations as the results would be too close to zero). Each point represents a pool of 5 gammarids. The dotted lines represent the best model fit on the bioaccumulation data.

3.1.2. Natural inter-population variability of bioaccumulation kinetics

Among the *G. pulex* populations, MENE and NEAU, no significant differences in the k_{in} values of Ni nor in the k_{out} values were observed (Figure 4). The same conclusion was drawn concerning Pb (Figure 4). Concerning the *G. fossarum* populations (TOUR, BACU and GUE), results from the estimated bioaccumulation kinetic parameters, k_{in} and k_{out} , showed no differences among populations for Ni as well as for Pb (Figure 4). Comparison of the bioaccumulation kinetic constants of Ni and Pb between the two species showed overall no substantial difference, confirming the assumption of a lack of interspecies differences.

The k_{in} values of Ni estimated in the five reference populations of gammarids ranged from 0.09 L.g⁻¹.d⁻¹ to 0.18 L.g⁻¹.d⁻¹. The k_{in} value estimated for NEAU was significantly lower than the k_{in} values estimated for TOUR and BACU (0.09 [0.06-0.11] versus 0.15 [0.12-0.19] and 0.18 [0.14-0.23], respectively (mean [95% confidence interval])), but was not different from the values estimated for MENE and GUE. The k_{out} values ranged from 0.41 to 0.57 d⁻¹ and no significant difference among populations was observed (Figure 4).

The k_{in} values of Pb ranged from 0.41 to 0.56 L.g⁻¹.d⁻¹ and no significant difference among reference populations was observed. Regarding k_{out} , the values ranged from 0.15 to 0.27 d⁻¹ and no significant difference among populations was observed (Figure 4).

Again, as assumed earlier, these results confirmed that there is no difference in Ni and Pb bioaccumulation kinetics between the two species *G. pulex* and *G. fossarum*.

Thus, for Ni and Pb, a range of kinetic parameters (k_{in} and k_{out}) that we assume to enclose the natural inter-population variability of bioaccumulation kinetics in the studied *G. pulex* and *G. fossarum* populations was proposed (see Materials and Methods 2.6 for calculation). All the values are presented in Table 3.

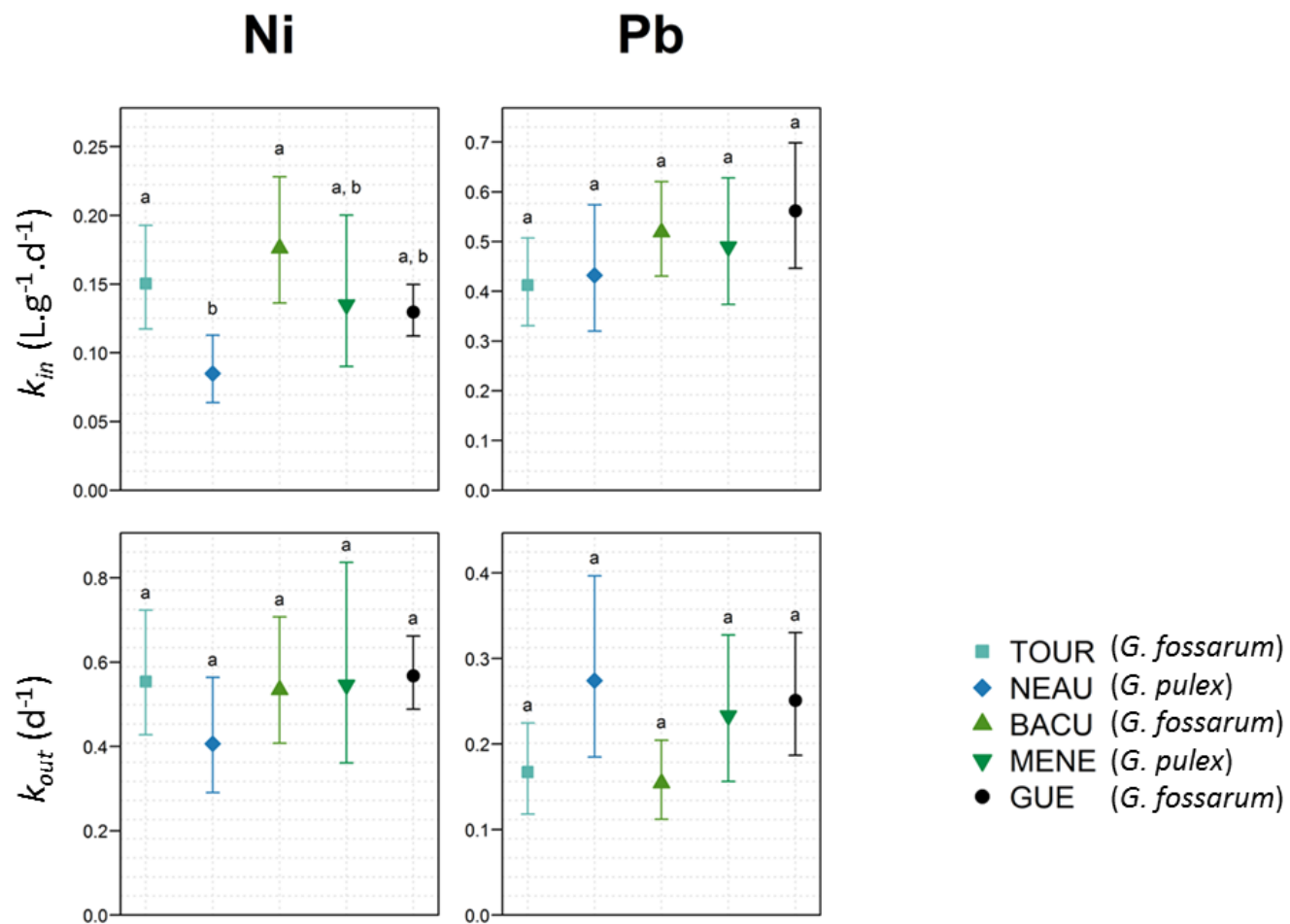


Figure 4: Uptake rate constants (k_{in}) and elimination rate constants (k_{out}) determined in gammarids from TOUR, NEAU, BACU, MENE and GUE, and their 95% confidence intervals for Ni and Pb. In each box, points sharing a common letter are not significantly different.

Table 3: Lower and upper limits of natural inter-population variability of Ni and Pb uptake and elimination rate constants (enclosed 95 % of data and taking into account the number of populations used). Uptake rate constant, k_{in} , is expressed in $L.g^{-1}.d^{-1}$ whereas the elimination constant, k_{out} , is expressed in d^{-1} .

		Lower value	Upper value
Ni	k_{in}	0.04	0.24
	k_{out}	0.33	0.72
Pb	k_{in}	0.20	0.67
	k_{out}	0.05	0.35

3.2. Gammarids pre-exposed to metals in their environment

As for the reference populations, for pre-exposed gammarids, exposure concentrations were checked in each microcosm during the exposure phase and were in compliance with the nominal concentrations (mean concentrations ($\pm sd$): 2.5 (± 0.1) $\mu g.L^{-1}$ for Cu, 9.8 (± 0.1) $\mu g.L^{-1}$ for Pb and 43.9 (± 0.4) $\mu g.L^{-1}$ for Ni, n= 12).

3.2.1. Bioaccumulation kinetics

As observed for the reference populations, pre-exposed gammarids subsequently exposed to Cu for 7 days in the laboratory did not significantly accumulate Cu over time and Cu body concentrations remained similar to the concentration observed in the control group (ARDI: 55.6 (± 12) $\mu g.g^{-1}$ dw (control) versus 57.4 (± 12) $\mu g.g^{-1}$ dw (exposed), BIE: 100 (± 13) $\mu g.g^{-1}$ dw (control) versus 91.1 (± 9) $\mu g.g^{-1}$ dw (exposed) and GAL: 84.8 (± 13) $\mu g.g^{-1}$ dw (control) versus 78.8 (± 10) $\mu g.g^{-1}$ dw (exposed), $p > 0.05$, mean concentrations ($\pm sd$)).

Uptake and elimination pattern of Ni observed in pre-exposed gammarids were relatively similar to what was observed in reference populations (Ni concentrations increased linearly for the first 2 days of exposure, before a progressive slow down to finally tend towards a steady-state at day 7. During the depuration periods, Ni concentrations fell exponentially over time). Note however that net accumulation of Ni at the peak was approximatively twice lower for BIE and ARDI as for the reference populations (about 6-8 $\mu g.g^{-1}$ dw for BIE and ARDI, and about 10-15 $\mu g.g^{-1}$ dw for the reference populations), and even lower for GAL (about 3 $\mu g.g^{-1}$ dw) (Figure 5).

Concerning Pb, bioaccumulation kinetics in pre-exposed gammarids followed the pattern observed for the reference populations, and Pb body concentrations at the peak were slightly higher than those observed for the reference populations but remained in the same order of magnitude (roughly around 20 to 30 $\mu\text{g}\cdot\text{g}^{-1}$ dw) (Figure 5).

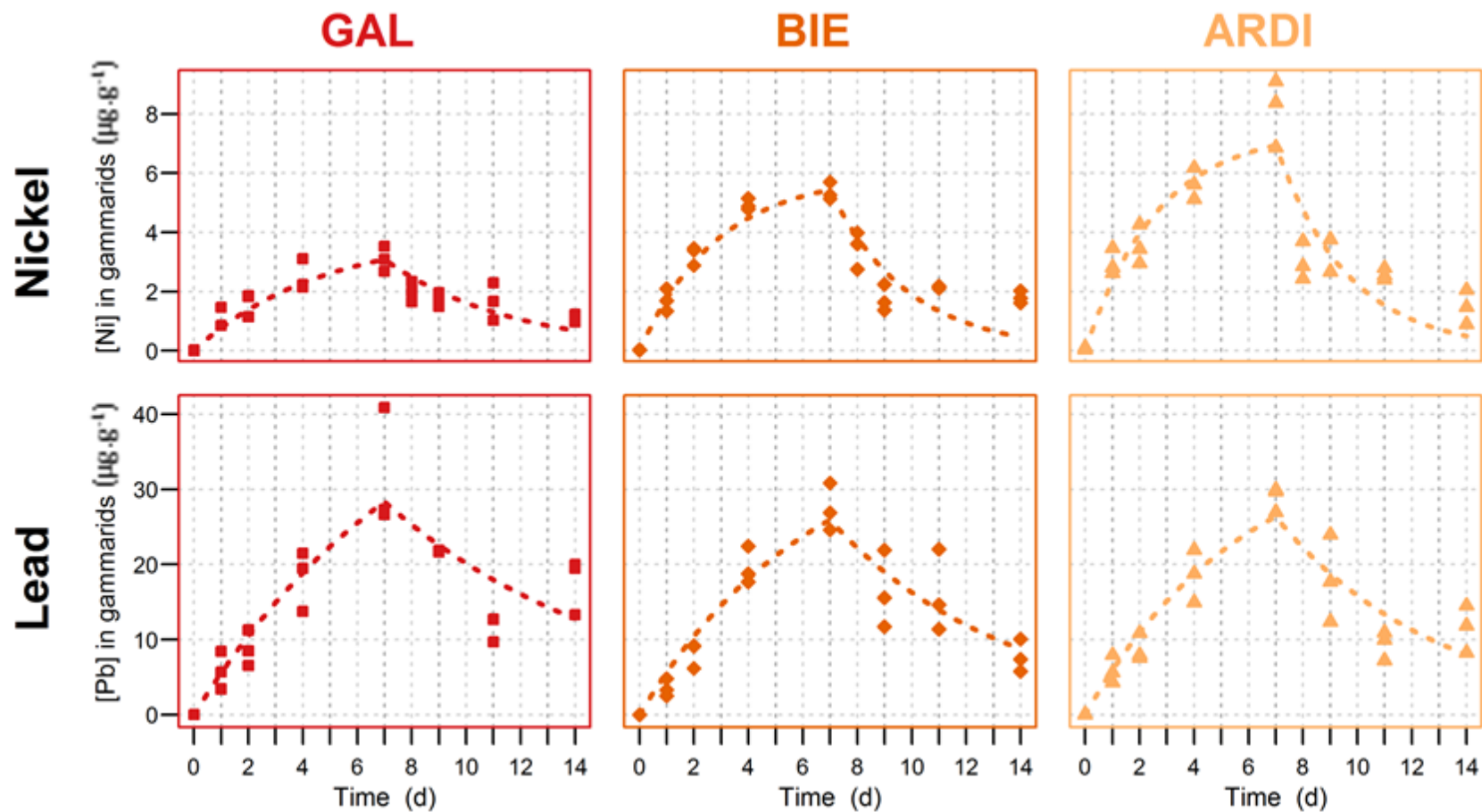


Figure 5: Bioaccumulation kinetics of Ni and Pb in the three pre-exposed populations of gammarids (GAL, BIE and ARDI). Full points represent the net accumulation of metals in gammarids (without basal concentrations). Each point represent a pool of 5 gammarids. The dotted lines represent the best model fit on the bioaccumulation data.

3.2.2. Comparison of kinetic parameters from pre-exposed and reference populations using the estimated natural inter-population variability

As mentioned above, gammarids pre-exposed to metals in their environment and gammarids from reference populations were sampled at two different periods for subsequent exposure in the laboratory. To ensure comparable results, the influence of the sampling period on kinetic parameters was tested by collecting gammarids from GUE at the two sampling periods. Results showed that for both metals, kinetic parameters were not significantly different between the two sampling periods and were within the natural variability range (k_{in} Pb: 0.56 [0.45-0.70] and 0.70 [0.57-0.85]; k_{out} Pb: 0.25 [0.19-0.33] and 0.22 [0.17-0.29]. k_{in} Ni: 0.13 [0.10-0.15] and 0.07 [0.05-0.10]; k_{out} Ni: 0.57 [0.49-0.66] and 0.39 [0.26-0.56], for GUE collected in July 2013 and January 2015, respectively. Kinetic parameters are given with their 95% confidence intervals). Thus, in the following, we assume that kinetic parameters determined for the three pre-exposed populations are directly comparable to natural inter-population variability.

Figure 6 represents the kinetic parameters, k_{in} and k_{out} , calculated for Ni and Pb for each pre-exposed population by fitting a first-order kinetic model to the bioaccumulation data. We examined if k_{in} and k_{out} for GAL, BIE and ARDI were within the natural variability range of kinetic parameters proposed earlier (for reference populations) or not (dotted lines in Figure 6). Regarding Pb, we observed that both kinetic parameters, k_{in} and k_{out} , estimated in the three pre-exposed populations fell into the natural variability range and moreover, were not significantly different among pre-exposed populations (no overlapping of 95% confidence intervals). Note, however, that the k_{in} values were close to the upper limit of the natural inter-population variability range. By contrast, for Ni, both k_{in} and k_{out} estimated for GAL were significantly below the lower bound of natural variability range. Note that for GAL, the accumulation peak of Ni after 7 days of exposure was substantially lower than for BIE and ARDI (Figure 5). Concerning BIE and ARDI populations, although their k_{in} and k_{out} fell into the natural variability range proposed in the present study, both kinetic parameters were found to be in the lower limit of the natural inter-population variability range (Figure 6).

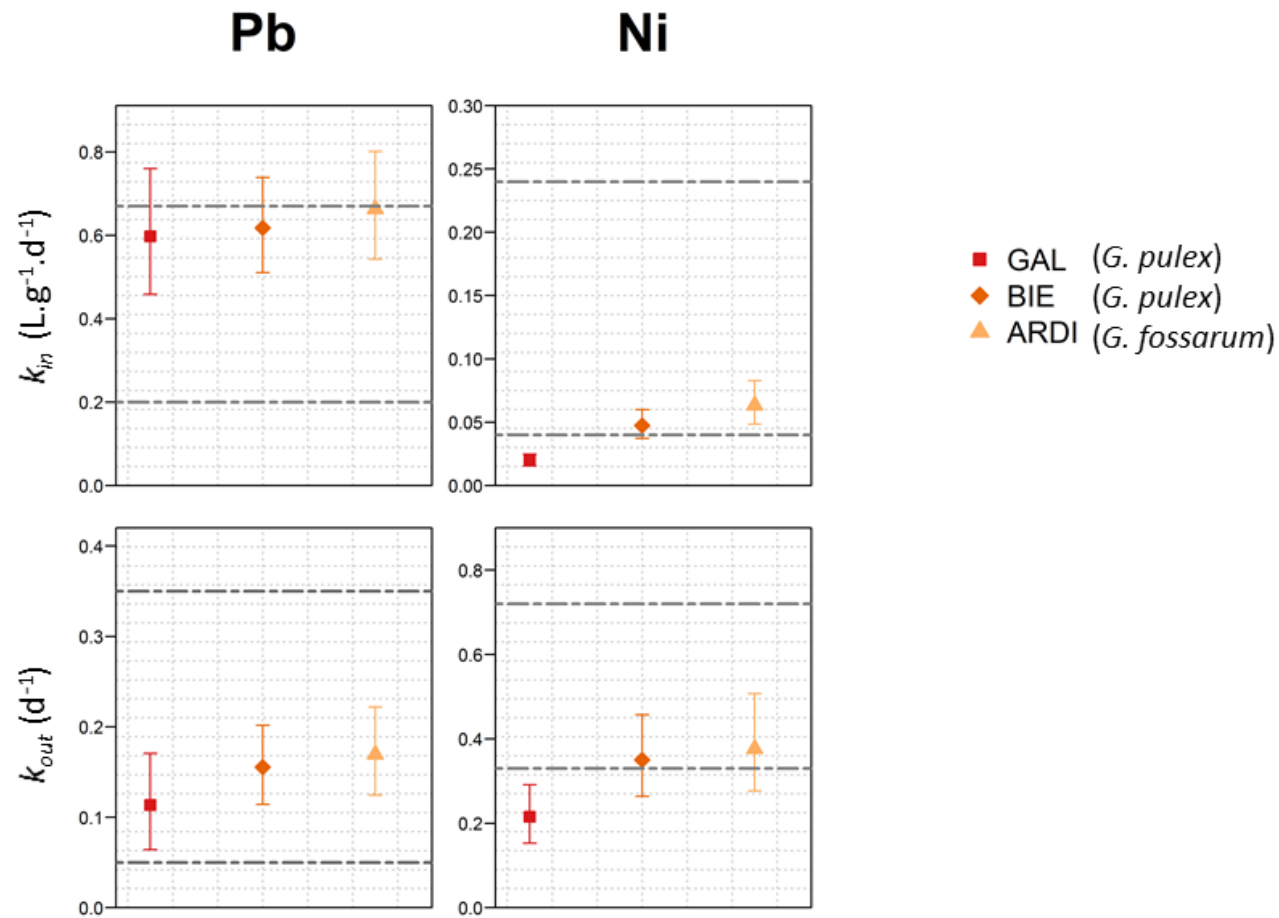


Figure 6: Uptake rate constants (k_{in}) and elimination rate constants (k_{out}) determined in pre-exposed gammarids from GAL, BIE and ARDI and their 95% confidence intervals for Ni and Pb. Dotted line represents the lower and upper limit values of natural inter-population variability of kinetic parameters for reference populations.

4. Discussion

Unlike Ni and Pb, Cu was not significantly accumulated by gammarids over time at the exposure concentration used and consequently no kinetic parameters could be determined. By comparison, in another gammarid study assessing bioaccumulation kinetics in similar controlled conditions using *G. pulex*, the authors reported that organisms significantly accumulated waterborne Cu at the concentration $1 \mu\text{g}\cdot\text{L}^{-1}$ and beyond (Lebrun et al., 2012). Our results rather suggest that gammarids were able to regulate their internal Cu concentrations at the exposure concentration tested in the present study (about $2.3 \mu\text{g}/\text{L}$), which is in agreement with the essential character of Cu for biota since it is involved in many biological functions. The internal regulation of Cu by other aquatic organisms is also well documented (Atli and Canli, 2011; Rainbow, 2007). In the laboratory and with another amphipod, *Hyaella azteca*, Borgmann and Norwood (1995) reported that organisms could control body Cu concentrations during exposure to elevated Cu exposure ($50 \mu\text{g}\cdot\text{L}^{-1}$). In field conditions, the regulation abilities of gammarids regarding Cu contamination was previously observed with indigenous *G. pulex* from the Seine watershed (France) (Lebrun et al., 2014), and with *G. fossarum* caged over 140 sites all around France (Besse et al., 2013; Urien et al., 2016). Finally, our results suggest that amphipods from the *G. pulex* and *G. fossarum* species are not appropriate to monitor Cu contamination because bioaccumulated Cu levels do not reflect Cu exposure.

Regarding Ni and Pb, both metals were significantly accumulated over time in gammarids collected from reference sites, showing their ability to effectively accumulate Ni and Pb at levels that can be encountered in the environment. These results are in accordance with some previous studies assessing metal bioaccumulation in *G. pulex* and *G. fossarum* at environmental concentrations in the laboratory and *in situ* (Dedourge-Geffard et al., 2009; Geffard et al., 2010; Lebrun et al., 2011; Urien et al., 2016, 2015). Ni and Pb bioaccumulation capacities were also reported in other invertebrate taxa, such as *Hyaella azteca*, *Chaoborus* or *Lymnaea stagnalis* (Besser et al., 2005; Borgmann et al., 2001; Niyogi et al., 2014; Ponton and Hare, 2010). For both Ni and Pb, the uptake and elimination rate constants among the reference populations in our study varied within a factor of two, suggesting a reasonably low natural variability of Ni and Pb bioaccumulation abilities among different reference populations found over a large geographical distance (about 500 km) and relatively contrasted ambient physicochemical parameters. In addition, the kinetic parameters were not significantly different between the two species meaning

that metal bioaccumulation kinetics were independent from the gammarid species selected in this study (*G. pulex* versus *G. fossarum*). This result is in accordance with Lebrun et al. (2015), who observed no significant difference in metal concentrations measured in two populations of *G. pulex* and *G. fossarum* transplanted for seven days along a poly-metallic contamination gradient in the same river (Seine River).

After seven days of depuration, gammarids tended to depurate Ni faster than Pb. This result also appears through the elimination constant rates determined for Ni and Pb (about 0.5 d⁻¹ for Ni versus 0.2 d⁻¹ for Pb). The higher elimination capacities of Ni observed in gammarids compared to other metals (Zn, Cd) were previously reported in other studies (Lebrun et al., 2011; Pellet et al., 2009; Xu and Pascoe, 1993). It could be hypothesized that this difference could be partly due to the different subcellular metal partitioning observed in gammarids for Ni and Pb; Ni has been shown to be distributed both in cytosolic and insoluble fractions whereas Pb is mainly found to be sequestered in insoluble granules with low turnover (Geffard et al., 2010).

Recently, studies have shown that an active biomonitoring approach, based on encaged gammarids, could offer promising lines of inquiry to monitor bioavailable contamination in freshwaters (Besse et al., 2013; Lebrun et al., 2015; Urien et al., 2016). More precisely, such an approach consists in transplanting size-calibrated gammarids from a single source population, considered as a reference population (Besse et al., 2013), at the sites of interest. This approach has the advantage to allow the assessment of the bioavailable contamination levels of the transplantation sites at large scale. Transplantation sites can thus be compared between them with accuracy. Moreover rivers devoid of indigenous gammarids can be monitored. Thus, from a biomonitoring point of view, the relatively constant bioaccumulation parameters of Ni and Pb observed among the five reference populations studied stress the relevance of this active approach and suggest that different reference gammarid populations could be used as a source organisms for active biomonitoring. Similarly, a recent study of Prygiel et al. (2016), assessing the effects of metal-polluted sediment re-suspension on *G. fossarum* using an active approach, showed that the two reference *G. fossarum* populations used for caging did not exhibit strong differences in metal bioaccumulation.

The comparison of kinetic parameters determined in three populations that were differently pre-exposed to metals in their environment to the natural variability range of Ni and Pb bioaccumulation parameters for reference populations (see 3.1.2 and Table 3) showed that metal

exposure history encountered in the present study did not influence subsequent Pb bioaccumulation kinetics. In the isopod *Asellus meridianus*, Brown et al. (1977) observed a low decrease of the Pb uptake rates from solution in Pb-tolerant organisms for an exposure to 500 µg/L of Pb, which is not environmentally realistic. In the present study, field exposure to Pb was potentially not marked enough to induce such observable changes in organism's uptake behaviour. On the contrary to Brown et al. (1977), Pb uptake rate constants rather tended to be close to the upper limit of natural variability which means that Pb uptake could be rather increased.

Pre-exposure to Cd (ARDI) did not influence Pb bioaccumulation kinetic in gammarids. It is now thought that both Pb and Cd preferentially enter the cells via Ca^{2+} channels, but also that Cd can probably enter via facilitated transport such as Zn carrier proteins (Macdonald et al., 2002; Qiu et al., 2005; Wang and Fisher, 1999). However, these results also shed light on the fact that gammarids could internally cope with these two metals in different ways involving different mechanisms. Indeed, Pb is known to be sequestered in the insoluble fraction, incorporated in metal-rich insoluble granules, whereas Cd tends to be sequestered by metallothioneins (MT) or metallothionein-like protein (MTLP) found in the soluble fraction (Geffard et al., 2010). Therefore, differences in binding and storage likely resulted in the relative independent bioaccumulation of Cd and Pb in gammarids.

Interestingly, our results showed lower Ni uptake and elimination rate constants for the population pre-exposed to Pb (lower k_{in} and k_{out} in GAL population compared to the natural variability range proposed in the present study). Our results, thus, showed that a pre-exposure to one metal (here, Pb) may affect the bioaccumulation kinetics of another metal (Ni in this case). Earlier, McGeer et al. (2007) observed that rainbow trout (*Oncorhynchus mykiss*) pre-exposed to a chronic sublethal Cu concentration in the laboratory showed cross-acclimation to Cd by modifying Cd uptake and tissue distribution. Previous studies on marine invertebrates have also reported the effect of pre-exposure to Cd, Cu or Zn on the uptake rate of other metals such as Ag (see review of Wang and Rainbow, 2005). Our results suggest that physiological changes in gammarids pre-exposed to Pb may have occurred, conferring therefore an ability to limit Ni uptake rate and also its elimination rate. A reduced number of binding sites on the cell membranes was previously suggested by Brown et al. (1977) to explain reduced Pb bioaccumulation in a metal-tolerant isopod, *Asellus meridianus*, but to date the cause of this

reduced Ni uptake rate in gammarids remains unknown. Previous studies have investigated the mechanisms of toxicity and uptake of Ni and Pb in other freshwater invertebrates such as the Cladoceran, *Daphnia magna* or pulmonate snail, *Lymnaea stagnalis* (Brix et al., 2012; Niyogi et al., 2014; Pane et al., 2003), but to date, very scarce information on *Gammarus* is available and more investigations are needed as metal uptake can be species-specific. In addition and counter intuitively, no difference on subsequent Ni bioaccumulation has been observed with BIE population, even though this population was pre-exposed to both Ni and Pb in their environment. This result underlines the fact that metal bioaccumulation kinetics is complex and that underlying mechanisms of bioaccumulation in *Gammarus* deserve to be more deeply investigated.

Recently, two studies have determined kinetic parameters (k_{in} and k_{out}) of Ni and Pb in a reference *G. pulex* population exposed to dissolved Ni and Pb in similar laboratory conditions as in the present study (Lebrun et al., 2011; Urien et al., 2015). By comparison with the natural inter-population variability proposed in our study, we observed that the kinetic parameters for both Ni and Pb were out of the natural variability (k_{in} above the upper limit for Ni, and k_{in} and k_{out} under the lower limit for Pb). To date, we can only speculate on the causes leading to this difference and further investigations are needed, but these results may indicate two different points: first, the exposure life history of the *G. pulex* population in those two previous studies may have not been well identified and resulted in changes in physiological mechanisms in gammarids leading to changes in metal bioaccumulation kinetics. This highlights the importance to well characterize the population of interest, especially because inter-population differences may have critical implication for bioaccumulation interpretation. Second, we are aware that the proposed range of natural inter-population variability of Ni and Pb kinetic parameters is based on five populations and it is plausible that other populations not impacted by metals may be out of the range because of other environmental factors. Nevertheless, our study has the merit to be a first step towards considering the natural variability of metal bioaccumulation in freshwater invertebrates and these results could be seen as a first database to be completed in the future.

The biodynamic model is nowadays increasingly used to describe, predict and understand metal bioaccumulation in aquatic organisms, among which *Gammarus*, as this genus has gained important interest as a biomonitor of freshwater quality in Europe (Besse et al., 2012; Urien et al., 2016, 2015). Most of the time, calibration of such models is based on one reference population leading to the determination of a single set of kinetic parameters linked to the studied population.

As a perspective of the present work, we propose a set of kinetic parameters of Ni and Pb (k_{in} and k_{out}) combining all the parameters determined for each of the five reference population as generalized parameters. For that, a single model was fitted on the whole dataset (of reference populations) for Ni and Pb, as showed in Figure 7.

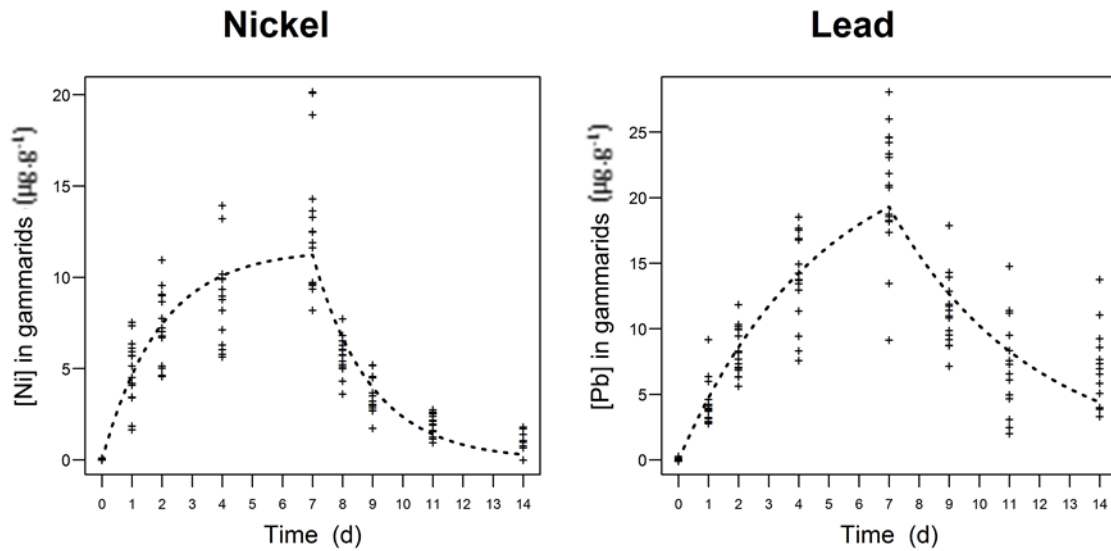


Figure 7: Best model fits (dotted lines) on the whole bioaccumulation dataset for Ni and Pb in the reference populations (TOUR, NEAU, BACU, MENE and GUE). Each point represents the concentration of metal measured in 5 gammarids.

Kinetic parameters and 95% confidence intervals determined for Ni were $k_{in} = 0.13 \text{ L.g}^{-1}.\text{d}^{-1}$ [0.12 – 0.15] and $k_{out} = 0.52 \text{ d}^{-1}$ [0.45 – 0.60] and parameters established for Pb were $k_{in} = 0.52 \text{ L.g}^{-1}.\text{d}^{-1}$ [0.47 – 0.57] and $k_{out} = 0.21 \text{ d}^{-1}$ [0.18 – 0.24]. In this way, we think that for further studies, those parameters could be applied in a generic biodynamic model of metal bioaccumulation more representative of the populations encountered across large geographical distances in France.

5. Conclusion

In conclusion, the present study shed light on the relatively low inter-population variability of Ni and Pb bioaccumulation kinetic parameters among wild gammarids from various reference sites located over a large geographical area in France. The determination of a natural inter-population variability range of bioaccumulation kinetic parameters to detect changes in bioaccumulation in gammarids pre-exposed to metal was encouraging. These results also highlight that pre-exposure to one metal can affect bioaccumulation kinetic of another metal. Finally this work raises questions about the underlying mechanisms leading to such changes in bioaccumulation processes in gammarids a response to chronic metal exposure in their environment.

Acknowledgment

This study was supported by funding from a grant from French National Research Agency (ANR-2012-ADAP-003) as a part of the SequAdapt project. This work benefited also from the support from the two programs: ANR CESA program GAMMA 021 02 and Blanc program Multistress 0004-03. The authors would like to thank Irène Friedel for her technical assistance in the laboratory, Adeline François and Hervé Quéau for their involvement in the field. We wish to thank the anonymous reviewers for their helpful comments on a previous draft of this paper.

References

- Atli, G., Canli, M., 2011. Essential metal (Cu, Zn) exposures alter the activity of ATPases in gill, kidney and muscle of tilapia *Oreochromis niloticus*. *Ecotoxicology* 20, 1861–1869.
- Barnard, J.L., Barnard, C.M., 1983. *Freshwater Amphipoda of the world*.
- Barrett, T.J., Hille, K.A., Sharpe, R.L., Harris, K.M., Machtans, H.M., Chapman, P.M., 2015. Quantifying natural variability as a method to detect environmental change: Definitions of the normal range for a single observation and the mean of m observations. *Environ. Toxicol. Chem.* 34, 1185–1195. <https://doi.org/10.1002/etc.2915>
- Baty, F., Ritz, C., Charles, S., Brutsche, M., Flandrois, J.P., Delignette-Muller, M.-L., 2014. A Toolbox for Nonlinear Regression in R: The Package nlstools. *Revis. J. Stat. Softw.*
- Besse, J.-P., Coquery, M., Lopes, C., Chaumot, A., Budzinski, H., Labadie, P., Geffard, O., 2013. Caged *Gammarus fossarum* (Crustacea) as a robust tool for the characterization of bioavailable contamination levels in continental waters: Towards the determination of threshold values. *Water Res.* 47, 650–660. <https://doi.org/10.1016/j.watres.2012.10.024>
- Besse, J.-P., Geffard, O., Coquery, M., 2012. Relevance and applicability of active biomonitoring in continental waters under the Water Framework Directive. *TrAC Trends Anal. Chem.* 36, 113–127. <https://doi.org/10.1016/j.trac.2012.04.004>
- Besser, J.M., Brumbaugh, W.G., Brunson, E.L., Ingersoll, C.G., 2005. Acute and chronic toxicity of lead in water and diet to the amphipod *Hyaella azteca*. *Environ. Toxicol. Chem. SETAC* 24, 1807–1815. <https://doi.org/10.1897/04-480R.1>
- Bisson, M., Houeix, N., Gay, G., Jolibois, B., Lacroix, G., Lefèvre, J.P., Magaud, H., Morin, A., Tissot, S., 2005. Fiche de données toxicologiques et environnementales des substances chimiques—Cuivre et ses dérivés. INERIS.
- Blackmore, G., Wang, W.-X., 2003a. Inter-population differences in Cd, Cr, Se, and Zn accumulation by the green mussel *Perna viridis* acclimated at different salinities. *Aquat. Toxicol.* 62, 205–218. [https://doi.org/10.1016/S0166-445X\(02\)00083-8](https://doi.org/10.1016/S0166-445X(02)00083-8)
- Blackmore, G., Wang, W.-X., 2003b. Comparison of metal accumulation in mussels at different local and global scales. *Environ. Toxicol. Chem.* 22, 388–395. <https://doi.org/10.1002/etc.5620220221>
- Borgmann, U., Néron, R., Norwood, W.P., 2001. Quantification of bioavailable nickel in sediments and toxic thresholds to *Hyaella azteca*. *Environ. Pollut.* 111, 189–198. [https://doi.org/10.1016/S0269-7491\(00\)00076-2](https://doi.org/10.1016/S0269-7491(00)00076-2)
- Borgmann, U., Norwood, W.P., 1995. Kinetics of excess (above background) copper and zinc in *Hyaella azteca* and their relationship to chronic toxicity. *Can. J. Fish. Aquat. Sci.* 52, 864–874.
- Brix, K.V., Esbaugh, A.J., Munley, K.M., Grosell, M., 2012. Investigations into the mechanism of lead toxicity to the freshwater pulmonate snail, *Lymnaea stagnalis*. *Aquat. Toxicol.* 106–107, 147–156. <https://doi.org/10.1016/j.aquatox.2011.11.007>
- Brown, B.E., 1977. Uptake of copper and lead by a metaltolerant isopod *Asellus meridianus* Rac. *Freshw. Biol.* 7, 235–244. <https://doi.org/10.1111/j.1365-2427.1977.tb01671.x>
- Ciliberti, A., Chaumot, A., Recoura-Massaquant, R., Chandesris, A., François, A., Coquery, M., Ferréol, M., Geffard, O., 2017. Caged *Gammarus* as biomonitors identifying thresholds of toxic metal bioavailability that affect gammarid densities at the French national scale. *Water Res.* 118, 131–140. <https://doi.org/10.1016/j.watres.2017.04.031>
- Couture, P., Kumar, R.P., 2003. Impairment of metabolic capacities in copper and cadmium contaminated wild yellow perch (*Perca flavescens*). *Aquat. Toxicol.* 64, 107–120.

- Cresswell, T., Simpson, S.L., Smith, R.E.W., Nugegoda, D., Mazumder, D., Twining, J., 2014. Bioaccumulation and retention kinetics of cadmium in the freshwater decapod *Macrobrachium australiense*. *Aquat. Toxicol.* 148, 174–183. <https://doi.org/10.1016/j.aquatox.2014.01.006>
- Dedourge-Geffard, O., Palais, F., Biagiante-Risbourg, S., Geffard, O., Geffard, A., 2009. Effects of metals on feeding rate and digestive enzymes in *Gammarus fossarum*: An in situ experiment. *Chemosphere* 77, 1569–1576. <https://doi.org/10.1016/j.chemosphere.2009.09.042>
- Directive 2008/105/EC, 2008. Directive 2008/105/EC of the European parliament and of the council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council, Official Journal of European Communities.
- Fialkowski, W., Rainbow, P.S., 2006. The discriminatory power of two biomonitors of trace metal bioavailabilities in freshwater streams. *Water Res.* 40, 1805–1810.
- Geffard, A., Sartelet, H., Garric, J., Biagiante-Risbourg, S., Delahaut, L., Geffard, O., 2010. Subcellular compartmentalization of cadmium, nickel, and lead in *Gammarus fossarum*: Comparison of methods. *Chemosphere* 78, 822–829. <https://doi.org/10.1016/j.chemosphere.2009.11.051>
- Gismondi, E., Thomé, J.-P., Urien, N., Uher, E., Baiwir, D., Mazzucchelli, G., De Pauw, E., Fechner, L.C., Lebrun, J.D., 2017. Ecotoxicoproteomic assessment of the functional alterations caused by chronic metallic exposures in gammarids. *Environ. Pollut.* 225, 428–438. <https://doi.org/10.1016/j.envpol.2017.03.006>
- Hadji, R., Urien, N., Uher, E., Fechner, L.C., Lebrun, J.D., 2016. Contribution of aqueous and dietary uptakes to lead (Pb) bioaccumulation in *Gammarus pulex*: From multipathway modeling to in situ validation. *Ecotoxicol. Environ. Saf.* 129, 257–263. <https://doi.org/10.1016/j.ecoenv.2016.03.033>
- Kalman, J., Smith, B.D., Riba, I., Blasco, J., Rainbow, P.S., 2010. Biodynamic modelling of the accumulation of Ag, Cd and Zn by the deposit-feeding polychaete *Nereis diversicolor*: Inter-population variability and a generalised predictive model. *Mar. Environ. Res.* 69, 363–373. <https://doi.org/10.1016/j.marenvres.2010.01.001>
- Khan, F.R., Irving, J.R., Bury, N.R., Hogstrand, C., 2011. Differential tolerance of two *Gammarus pulex* populations transplanted from different metallogenic regions to a polymetal gradient. *Aquat. Toxicol.* 102, 95–103. <https://doi.org/10.1016/j.aquatox.2011.01.001>
- Landrum, P.F., Lydy, M.J., Lee, H., 1992. Toxicokinetics in aquatic systems: Model comparisons and use in hazard assessment. *Environ. Toxicol. Chem.* 11, 1709–1725. <https://doi.org/10.1002/etc.5620111205>
- Lebrun, J.D., Geffard, O., Urien, N., François, A., Uher, E., Fechner, L.C., 2015. Seasonal variability and inter-species comparison of metal bioaccumulation in caged gammarids under urban diffuse contamination gradient: Implications for biomonitoring investigations. *Sci. Total Environ.* 511, 501–508. <https://doi.org/10.1016/j.scitotenv.2014.12.078>
- Lebrun, J.D., Perret, M., Geffard, A., Gourlay-Francé, C., 2012. Modelling copper bioaccumulation in *Gammarus pulex* and alterations of digestive metabolism. *Ecotoxicology* 21, 2022–2030.
- Lebrun, J.D., Perret, M., Uher, E., Tusseau-Vuillemin, M.-H., Gourlay-Francé, C., 2011. Waterborne nickel bioaccumulation in *Gammarus pulex*: Comparison of mechanistic models and influence of water cationic composition. *Aquat. Toxicol.* 104, 161–167. <https://doi.org/10.1016/j.aquatox.2011.04.011>
- Lebrun, J.D., Uher, E., Tusseau-Vuillemin, M.-H., Gourlay-Francé, C., 2014. Essential metal contents in indigenous gammarids related to exposure levels at the river basin scale: Metal-

- dependent models of bioaccumulation and geochemical correlations. *Sci. Total Environ.* 466, 100–108.
- Luoma, S.N., 1977. Detection trace contaminant effect in aquatic ecosystems. *J. Fish. Res. Board Can.* 436–439.
- Luoma, S.N., Rainbow, P.S., 2005. Why is metal bioaccumulation so variable? Biodynamics as a unifying principle. *Environ. Sci. Technol.* 39, 1921–1931.
- Macdonald, A., Silk, L., Schwartz, M., Playle, R.C., 2002. A lead–gill binding model to predict acute lead toxicity to rainbow trout (*Oncorhynchus mykiss*). *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* 133, 227–242. [https://doi.org/10.1016/S1532-0456\(02\)00107-2](https://doi.org/10.1016/S1532-0456(02)00107-2)
- Mason, A.Z., Jenkins, K.D., 1995. Metal detoxification in aquatic organisms, in: Tessier, A., Turner, D., van Leeuwen, H.P. (Eds.), *Metal Speciation and Bioavailability in Aquatic Systems*, IUPAC Series on Analytical and Physical Chemistry of Environmental Systems. J. Wiley & Sons, Chichester, UK, pp. 479–608.
- McGeer, J.C., Nadella, S., Alsop, D.H., Hollis, L., Taylor, L.N., McDonald, D.G., Wood, C.M., 2007. Influence of acclimation and cross-acclimation of metals on acute Cd toxicity and Cd uptake and distribution in rainbow trout (*Oncorhynchus mykiss*). *Aquat. Toxicol.* 84, 190–197. <https://doi.org/10.1016/j.aquatox.2007.03.023>
- Mouneyrac, C., Mastain, O., Amiard, J.C., Amiard-Triquet, C., Beaunier, P., Jeantet, A.-Y., Smith, B.D., Rainbow, P.S., 2003. Trace-metal detoxification and tolerance of the estuarine worm *Hediste diversicolor* chronically exposed in their environment. *Mar. Biol.* 143, 731–744. <https://doi.org/10.1007/s00227-003-1124-6>
- Niyogi, S., Brix, K.V., Grosell, M., 2014. Effects of chronic waterborne nickel exposure on growth, ion homeostasis, acid-base balance, and nickel uptake in freshwater pulmonate snail, *Lymnaea stagnalis*. *Aquat. Toxicol.* <https://doi.org/10.1016/j.aquatox.2014.02.012>
- Pacaud, A., 1945. Les Amphipodes de la faune nutritive des eaux douces francaises. *Bull. Fr. Piscic.* 105–120.
- Pane, E.F., Smith, C., McGeer, J.C., Wood, C.M., 2003. Mechanisms of Acute and Chronic Waterborne Nickel Toxicity in the Freshwater Cladoceran, *Daphnia magna*. *Environ. Sci. Technol.* 37, 4382–4389. <https://doi.org/10.1021/es034317l>
- Pellet, B., Geffard, O., Lacour, C., Kermoal, T., Gourlay-Francé, C., Tusseau-Vuillemin, M.-H., 2009. A model predicting waterborne cadmium bioaccumulation in *Gammarus pulex*: the effects of dissolved organic ligands, calcium, and temperature. *Environ. Toxicol. Chem.* 28, 2434–2442.
- Ponton, D.E., Hare, L., 2010. Nickel dynamics in the lakewater metal biomonitor *Chaoborus*. *Aquat. Toxicol.* 96, 37–43. <https://doi.org/10.1016/j.aquatox.2009.09.011>
- Postma, J.F., VanNugteren, P., De Jong, M.B.B., 1996. Increased cadmium excretion in metal-adapted populations of the midge *Chironomus riparius* (diptera). *Environ. Toxicol. Chem.* 15, 332–339.
- Prygiel, E., Billon, G., François, A., Dumoulin, D., Chaumot, A., Geffard, O., Criquet, J., Prygiel, J., 2016. Active biomonitoring for assessing effects of metal polluted sediment resuspension on gammarid amphipods during fluvial traffic. *Environ. Pollut.* 218, 129–139. <https://doi.org/10.1016/j.envpol.2016.08.010>
- Qiu, J.-W., Xie, Z.-C., Wang, W.-X., 2005. Effects of calcium on the uptake and elimination of cadmium and zinc in Asiatic clams. *Arch. Environ. Contam. Toxicol.* 48, 278–287.
- R Core Team, 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rainbow, P.S., 2007. Trace metal bioaccumulation: models, metabolic availability and toxicity. *Environ. Int.* 33, 576–582. <https://doi.org/10.1016/j.envint.2006.05.007>

- Rainbow, P.S., AmiardTriquet, C., Amiard, J.C., Smith, B.D., Best, S.L., Nassiri, Y., Langston, W.J., 1999. Trace metal uptake rates in crustaceans (amphipods and crabs) from coastal sites in NW Europe differentially enriched with trace metals. *Mar. Ecol. Prog. Ser.* 183, 189–203. <https://doi.org/10.3354/meps183189>
- Rainbow, P.S., Blackmore, G., Wang, W., 2003. Effects of previous field-exposure history on the uptake of trace metals from water and food by the barnacle *Balanus amphitrite*. *Mar. Ecol. Prog. Ser.* 259, 201–213. <https://doi.org/10.3354/meps259201>
- Rainbow, P.S., Luoma, S.N., 2011. Biodynamic parameters of the accumulation of toxic metals, detoxification and the acquisition of metal tolerance. *Toler. Environ. Contam.* 127–151.
- Rainbow, P.S., Smith, B.D., Luoma, S.N., 2009. Differences in trace metal bioaccumulation kinetics among populations of the polychaete *Nereis diversicolor* from metal-contaminated estuaries. *Mar. Ecol. Prog. Ser.* 376, 173–184.
- Roesijadi, G., 1992. Metallothioneins in metal regulation and toxicity in aquatic animals. *Aquat. Toxicol.* 22, 81–113. [https://doi.org/10.1016/0166-445X\(92\)90026-J](https://doi.org/10.1016/0166-445X(92)90026-J)
- Roux, A.L., 1970. Le cycle de reproduction de deux espèces étroitement parentes de crustacés amphipodes : *Gammarus pulex* et *G. fossarum*. *Ann. Limnol. - Int. J. Limnol.* 6, 27–49. <https://doi.org/10.1051/limn/1970011>
- Schaller, J., Brackhage, C., Dudel, E.G., 2011. Invertebrates minimize accumulation of metals and metalloids in contaminated environments. *Water. Air. Soil Pollut.* 218, 227–233.
- Thévenot, D.R., Moilleron, R., Lestel, L., Gromaire, M.-C., Rocher, V., Cambier, P., Bonté, P., Colin, J.-L., de Pontevès, C., Meybeck, M., 2007. Critical budget of metal sources and pathways in the Seine River basin (1994–2003) for Cd, Cr, Cu, Hg, Ni, Pb and Zn. *Sci. Total Environ.* 375, 180–203.
- Urien, N., Lebrun, J.D., Fechner, L.C., Uher, E., François, A., Quéau, H., Coquery, M., Chaumot, A., Geffard, O., 2016. Environmental relevance of laboratory-derived kinetic models to predict trace metal bioaccumulation in gammarids: Field experimentation at a large spatial scale (France). *Water Res.* 95, 330–339. <https://doi.org/10.1016/j.watres.2016.03.023>
- Urien, N., Uher, E., Billoir, E., Geffard, O., Fechner, L.C., Lebrun, J.D., 2015. A biodynamic model predicting waterborne lead bioaccumulation in *Gammarus pulex*: Influence of water chemistry and in situ validation. *Environ. Pollut.* 203, 22–30. <https://doi.org/10.1016/j.envpol.2015.03.045>
- Vigneron, A., Geffard, O., Coquery, M., François, A., Quéau, H., Chaumot, A., 2015. Evolution of cadmium tolerance and associated costs in a *Gammarus fossarum* population inhabiting a low-level contaminated stream. *Ecotoxicology* 24, 1239–1249. <https://doi.org/10.1007/s10646-015-1491-z>
- Vijver, M.G., van Gestel, C.A., Lanno, R.P., van Straalen, N.M., Peijnenburg, W.J., 2004. Internal metal sequestration and its ecotoxicological relevance: a review. *Environ. Sci. Technol.* 38, 4705–4712.
- Wang, W.-X., Fisher, N.S., 1999. Effects of calcium and metabolic inhibitors on trace element uptake in two marine bivalves. *J. Exp. Mar. Biol. Ecol.* 236, 149–164. [https://doi.org/10.1016/S0022-0981\(98\)00195-6](https://doi.org/10.1016/S0022-0981(98)00195-6)
- Wang, W.-X., Rainbow, P.S., 2005. Influence of metal exposure history on trace metal uptake and accumulation by marine invertebrates. *Ecotoxicol. Environ. Saf.* 61, 145–159.
- Xu, Q., Pascoe, D., 1993. The bioconcentration of zinc by *Gammarus pulex* (L.) and the application of a kinetic model to determine bioconcentration factors. *Water Res.* 27, 1683–1688. [https://doi.org/10.1016/0043-1354\(93\)90132-2](https://doi.org/10.1016/0043-1354(93)90132-2)