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A biodynamic model predicting waterborne lead bioaccumulation in *Gammarus pulex*: Influence of water chemistry and in situ validation

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Abstract

Metals bioaccumulated in aquatic organisms are considered to be a good indicator of bioavailable metal contamination levels in freshwaters. However, bioaccumulation depends on the metal, the species, and the water chemistry that influences metal bioavailability. In the laboratory, a kinetic model was used to describe waterborne Pb bioaccumulated in *Gammarus pulex*. Uptake and elimination rate constants were successfully determined and the effect of Ca²⁺ on Pb uptake was integrated into the model. Thereafter, accumulated Pb concentrations in organisms were predicted with the model and compared with those measured in native populations from the Seine watershed (France). The predictions had a good agreement with the bioaccumulation levels observed in native gammarids and particularly when the effect of calcium was considered. To conclude, kinetic parameters experimentally derived for Pb in *G. pulex* are applicable in environmental conditions. Moreover, the consideration of the water's chemistry is crucial for a reliable interpretation of bioaccumulation.

1. Introduction

Trace metals naturally occur in the environment but human activities have largely contributed to their release in continental waters. Among trace metals, lead (Pb) is rightly considered within the European Water Framework Directive (WFD) as a priority substance (Directive, 2000/60/EC). Indeed, this metal is one of the most important trace metal contaminants occurring in the aquatic environment as a consequence of both natural and anthropogenic processes, i.e. mining, coal burning, cement manufacturing, and traffic (Kutlu et al., 2002; WHO, 1995). Moreover, Pb is nonessential for biological processes and may cause damage to aquatic organisms even at very low concentrations. For example, studies reported reproductive effects in rainbow trout and *Daphnia magna* or behavioural disorders in fish and snails after chronic exposure to Pb (Berglin et al., 1985; Holcombe et al., 1976; Pyatt et al., 2002; Ruby et al., 2000).

Chemical analyses of dissolved metal concentrations are recommended by the WFD to monitor metal contamination in river water. Nevertheless, this approach gives a pointwise picture of the metal contamination in the environment and does not give information on bioavailable metals, which are the fraction of metals able to interact with organisms and potentially exert toxicity. Moreover, bioavailability is strongly affected by the geochemistry conditions. As a consequence, chemical analyses of environmental compartments, such as the water, sediment, are not sufficient for diagnosing the impact of metallic contamination on aquatic biota. Alternatively, the quantification of metal concentrations accumulated in aquatic organisms gives an integrated measure of their exposure and includes the effect of water chemistry on dissolved metal bioavailability. Thus,

bioaccumulation of metals in aquatic species has been successfully used to assess spatial and temporal trends of bioavailable metals in continental waters (Baudrimont et al., 1999; Besse et al., 2012; Chevreuil et al., 1996).

Among the aquatic organisms predisposed to monitor trends of bioavailable metal contamination in the freshwaters, the amphipods of the genus *Gammarus* arouse interest as they are widespread and ubiquitous in rivers and streams of Europe, where they are often present in high density. Gammarids are also of ecological relevance as they actively contribute to the leaf litter breakdown and represent a good source of food for predators like birds, fish, amphibians (Forrow and Maltby, 2000; Graça et al., 2001; Welton, 1979). Moreover, gammarids are known to be net accumulators of various metals, essential or not, and are therefore considered as promising species to monitor metal bioavailability in freshwaters (Felten et al., 2008; Fialkowski and Rainbow, 2006; Lebrun et al., 2012, 2014).

The link between metal contamination in the water and bioaccumulation in organisms is not straightforward. Today, biodynamic modelling is a common approach used to describe metal bioaccumulation in aquatic organisms (Bourgeault et al., 2010; Casado-Martinez et al., 2009; Cresswell et al., 2014; Kalman et al., 2010; Lebrun et al., 2012; Luoma and Rainbow, 2005). The biodynamic model assumes that bioaccumulation is the result of a balance between the uptake rates, from aqueous and dietary routes, and loss rates (Luoma and Rainbow, 2005). While it is likely to be significant, the relative importance of the diet to total metal uptake is still to be determined for many freshwater invertebrates under specific exposure conditions, including the case of Pb accumulation by *Gammarus pulex*. It is still relevant, therefore, to investigate Pb accumulation by this amphipod after uptake from solution alone, in order to assess the significance of this waterborne source to total lead accumulation. During waterborne exposures, metal fluxes depend on kinetic parameters, the uptake and the elimination rate constants, which are specific to the metal/organism couple and which can be determined under laboratory conditions. Nonetheless, studies about kinetics of Pb uptake are limited unlike Cd, Zn, Cu or Ni, which have been widely investigated in freshwater invertebrates, including *Gammarus* species (Lebrun et al., 2012, 2011; Pellet et al., 2009; Xu and Pascoe, 1993). Indeed, in freshwater macroinvertebrates, bioaccumulation kinetic parameters of Pb have been determined for *D. magna* (Komjarova and Blust, 2009) or the amphipod *Hyaella azteca* (MacLean et al., 1996). To our knowledge no kinetic models have been developed for Pb in gammarids (Lebrun et al., 2011, 2012; Pellet et al., 2009, 2014). Furthermore, there is a relative lack of information on the modelling of Pb bioaccumulation by freshwater invertebrates, especially because of the lack of suitable radioisotopes for Pb, and because of limited access to suitable alternative stable isotope measurement facilities.

Water chemistry has often been pointed out as influencing the bioavailability of metals. In the literature, it is well accepted that the main soluble metal forms suggested as bioavailable are free ions (Di Toro et al., 2001; Morel, 1983). Indeed, metallic ions can directly interact with the biological binding sites of aquatic organisms (Niyogi and Wood, 2004). Major ions present in the water, such as calcium (Ca^{2+}), magnesium (Mg^{2+}) or sodium (Na^+), have the potential to interact with free metal ions on biological surfaces and consequently modulate metal bioavailability. For example, different studies have reported the decrease of Pb accumulation with increasing Ca^{2+} in many species including crustaceans (Besser et al., 2005; Komjarova and Blust, 2009), fish (Grosell et al., 2006; Macdonald et al., 2002) or algae (Slaveykova and Wilkinson, 2002). Such an effect of Ca^{2+} on Cd and Ni uptake has also been reported in various aquatic species (Croteau and Luoma, 2007; Keithly et al., 2004; Kienle et al., 2009; Tan and Wang, 2008) including *G. pulex* (Lebrun et al., 2011; Pellet et al., 2009). Taking into account water's cationic composition could be a way to improve metal bioaccumulation modelling and to better interpret bioaccumulation in terms of bioavailable contamination (Bourgeault et al., 2010; Pellet et al., 2009).

The aim of this study was to calibrate, under laboratory conditions, a kinetic model predicting waterborne metal bioaccumulation in *G. pulex*. The influence of water's cationic composition on Pb uptake was also investigated. Finally, the question of the applicability of the model to estimate bioaccumulation of Pb *in situ* was addressed. To this end, calibrated *G. pulex* were independently exposed to different Pb concentrations in controlled conditions (from 1 mg L^{-1} to 100 mg L^{-1}). The concentration of Pb in organisms was measured over time for the determination of kinetic parameters. Then, gammarids were exposed to Pb in media exhibiting different concentrations of Ca^{2+} , Na^+ , or Mg^{2+} in order to evaluate their influence on Pb uptake. Finally,

predictions made by the model taking into account the effect of calcium or not were compared to the contamination levels of native *G. pulex* collected at the Seine watershed (France).

2. Materials and methods

2.1. Experimental design

2.1.1. Collection and maintenance of *G. pulex*

Adult *G. pulex* were collected in February 2012 in a river (Ru de l'Etang, Doue, France) where the dissolved concentrations for several metals were overall below world average values in uncontaminated rivers (Gaillardet et al., 2003) and below the Environmental Quality Standard (EQS) recommended by the WFD for metals belonging to the list of priority substances (Directive, 2013/ 39/EC). 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 acclimatised 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. Organisms were fed ad libitum with hornbeam leaves (*Carpinus betulus*) from the same sampling site.

2.1.2. Kinetics of bioaccumulation

Aquatic microcosms created in plastic beakers were filled with 500 mL of Volvic® mineral water and spiked with Pb(NO₃)₂ so as to obtain final Pb exposure concentrations of 1, 2.5, 5, 10, 25, 50, and 100 mg L⁻¹. The range of Pb concentrations chosen for the exposures was made to be approximately between EQS/10 and EQS*10 i.e. from 1 to 100 mg L⁻¹ (EQS = 7.2 mg L⁻¹ (Directive, 2008/105/EC)). Each condition was performed in triplicates of microcosms, which were pre-equilibrated for 48 h to saturate the potential adsorption sites before performing the experiment in order to ensure a constant metal exposure. Then, fifty 24-h-starved *G. pulex* were introduced in each beaker without food for 5 days of exposure followed by a 7-day depuration period. During the exposure phase, each medium was renewed every day to ensure a constant exposure and a correct oxygenation rate. During the depuration period, each beaker was renewed only with uncontaminated Volvic® mineral water and gammarids could feed ad libitum on hornbeam leaves. A pool of five gammarids per beaker was sampled on day 0, 0.5, 1, 2, and 5 for the exposure phase and on day 7, 9, and 12 for the depuration phase. Each time, gammarids were rinsed in a solution of 2 and then 0.5 mM EDTA, and then rinsed twice with ultrapure water to remove metal potentially adsorbed on their cuticle (Lebrun et al., 2011) and stored at -20 °C. At the same time, dead gammarids were counted and removed. At each sampling time, renewed water was sampled and the water contamination level was checked for Pb using a graphite furnace atomic absorption spectrophotometer (AAS; SpectrAA 220Z Varian with Zeeman background correction).

For analysis, gammarids were freeze-dried (Christ Alpha 2-4 LD plus), weighed, and digested at 95 °C (Digiprep Jr, SCP Science) with HNO₃ (65% suprapur, Merck), H₂O₂ (suprapur, Merck), and finally diluted with ultrapure water. Pb analysis in gammarids was performed by AAS in standard addition to limit matrix effects. Calibration was performed after every three samples. The quality of the analysis of waters has been checked by analysing two natural water certified reference materials every 20 samples (SPS-SW1, Spectrapure standard AS Oslo and EP-L-2CR, EnviroMAT). The quality of the whole analysis process of gammarids including digestion has been checked by analysing the two natural water certified reference materials cited above every 20 samples, and a certified reference material of biological tissue (CMR: Mussel Tissue ERM-CE278). The mean of every certified material was consistently within the certified 95% confidence limit range for Pb.

In this study, every unit related to the mass of gammarids is expressed in dry weight.

The background Pb concentration in control organisms was determined to be $0.64 \pm 0.23 \mu\text{g g}^{-1}$ (n = 12). This value was subtracted from all subsequent tissue Pb measurements to determine the net accumulated Pb from the exposure.

2.1.3. Influence of major ions on Pb uptake

To assess the influence of major cations on metal uptake, gammarids were exposed for 2 days to 10 mg L⁻¹ of Pb in Volvic® microcosms (plastic beakers of 500 mL) containing 30, 60, and 100 mg L⁻¹ of Na⁺ or Mg²⁺ or 25, 50, 75, 100, and 150 mg L⁻¹ of Ca²⁺ obtained by adding solutions of NaNO₃, Mg(NO₃)₂·6H₂O or CaCl₂ to the

starting Volvic® medium. The ranges of cation concentrations are of environmental relevance since they have been chosen in accordance with the ranges found in the Seine watershed from the Agence de l'Eau de Seine-Normandie public database (<http://www.eau-seine-normandie.fr/>). Each condition was performed in triplicate and all microcosms were pre-equilibrated for 48 h before the experiment. Then, ten 24-h-starved *G. pulex* were introduced in each beaker without food for 2 days of exposure. Each medium was renewed on day 1 to ensure a constant exposure. After 2 days, a pool of five gammarids per beaker was sampled, rinsed, digested, and analysed for Pb by AAS as described in Section 2.1.2. Cationic concentrations in media were determined via chromatographic analysis [Dionex DX120, column IonPac CS16 Dionex N°057573 (4 x 250 mm)] and Pb exposure was analysed by AAS. An exposure concentration equal to 10 mg L⁻¹ of Pb was chosen because it was the most appropriate concentration to observe a significant accumulation after 2 days of exposure and to stay in agreement with the EQS defined by the WDF before 2013 (EQS = 7.2 mg L⁻¹ (Directive, 2008/105/EC)).

2.2. Field data

Sampling of native *G. pulex* from 21 sites from the Seine watershed was performed, among which 14 were sampled in 2008, three in 2009, two in 2012, and two in 2013 (Fig. 1). Collection sites are characterised by various anthropogenic pressures and contrasted water chemistry (Lebrun et al., 2014; Tusseau-Vuillemin et al., 2007). At each site, 50 adult gammarids were collected (for a resulting length of 1 cm, as described in 2.1.1), transported to the laboratory in plastic bottles containing river water, and identified according to a reference taxonomic determination. Then, collected gammarids were distributed in triplicates of 10 individuals in 50-mL polypropylene tubes (SCP Science), treated as described in Section 2.1.2, and analysed by inductively coupled plasma mass spectrometry (ICP-MS, X series 2, Thermo Fisher Scientific) to determine their Pb content in tissues.

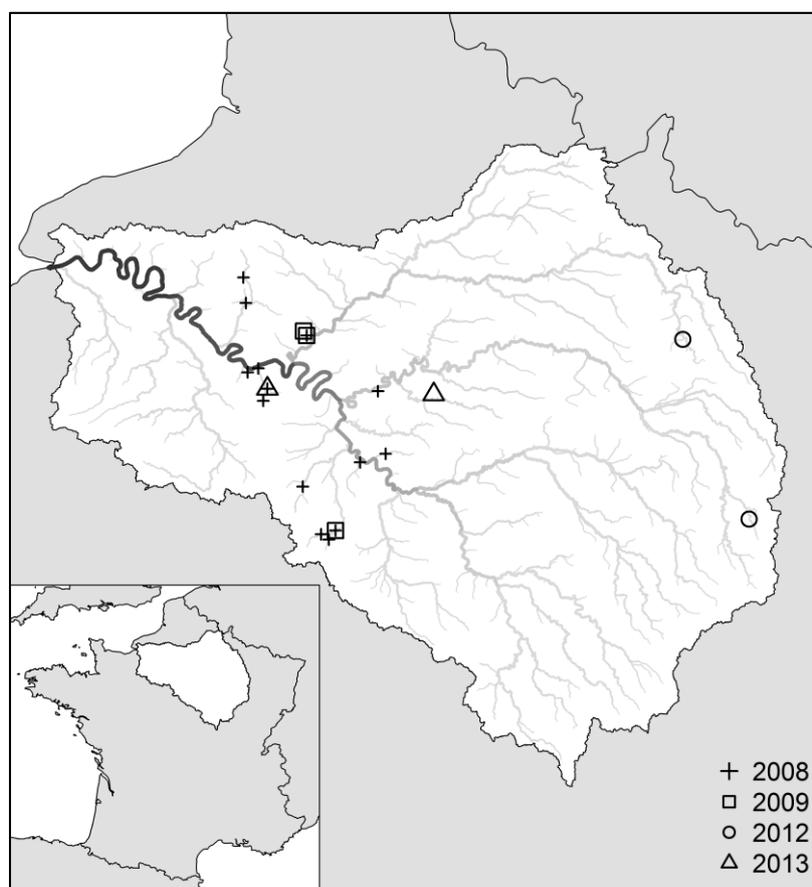


Fig. 1. Twenty one sites at the Seine watershed in France where native gammarids were collected for Pb contents analyses. Sampling consisted in collecting three replicates of five calibrated adult individuals (n = 3). Each symbol corresponds to a sampling year.

River water was sampled, filtered through a 0.45-mm PES syringe filter (Millipore), and then acidified with HNO₃ *sp* at 1% v/v for dissolved Pb concentrations analysis (ICP-MS). Physicochemical characteristics of river water were taken from the Agence de l'Eau de Seine-Normandie public database for sites sampled in 2008 and 2009 (<http://www.eau-seine-normandie.fr/>). For the other sites, 1 mL of raw water was sampled and analysed by chromatographic analysis [Dionex DX120, column IonPac CS16 Dionex N°057573 (4 x 250 mm)] for Ca²⁺, Na⁺ and Mg²⁺ concentrations determination. The digestion method and analysis of the organisms were successfully validated by the reference material (CMR Mussel Tissue ERM-CE278) and the analysis of the media waters was validated with natural water certified reference material (NIST 1640). Reference materials results were within the certified 95% confidence limit range for Pb.

2.3. Kinetic modelling and parameter estimation

2.3.1. Theory and assumptions

In the present study, we only focused on Pb uptake and accumulation from solution, ignoring any trophic uptake of Pb by the amphipods. Since no significant growth of organisms has been observed during the 12-d of experiment, metal bioaccumulation from solution can be expressed according to the first-order kinetic model as follows:

$$\frac{dC_{metal}}{dt} = k_{in} \cdot C_w - k_{out} \cdot C_{metal} \quad (1)$$

where C_{metal} is the metal concentration accumulated in organisms ($mg\ g^{-1}$), C_w is the dissolved concentration of Pb in the column water ($mg\ L^{-1}$), and k_{in} and k_{out} the kinetic parameters that are, respectively, the uptake rate constant ($L\ g^{-1}\ d^{-1}$) and the elimination rate constant (d^{-1}). These kinetic parameters are specific to each metal/organism couple and they are generally assumed to be independent of C_w (Luoma and Rainbow, 2005). Moreover, C_w is assumed to be constant during the exposure.

The integration of Eq. (1) characterises metal accumulation as follows:

$$C_{metal} = \frac{k_{in} \cdot C_w}{k_{out}} (1 - e^{-k_{out}t}) \quad (2)$$

Under the assumption of steady-state achievement at the end of the exposure, metal bioaccumulation from the solution ($t \rightarrow \infty$) is expressed as follows:

$$C_{ss} = \frac{k_{in} \cdot C_w}{k_{out}} \quad (3)$$

with C_{ss} , the concentration of metal in organisms in the steady state ($mg\ g^{-1}$).

At the end of the exposure phase, organisms are introduced for the depuration phase into uncontaminated microcosms where C_w is null. In these conditions, Eq. (1) is reduced to:

$$\frac{dC_{metal}}{dt} = -k_{out} \cdot C_{metal} \quad (1)$$

The integration of Eq. (4) characterises metal elimination as follows:

$$C_{metal} = C_{metal}(0) \cdot e^{-k_{out} \cdot t} \quad (2)$$

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

2.3.2. Fitting to laboratory data

The kinetic model was fitted to Pb concentrations in organisms for both the exposure and the depuration phases together, since Eq. (2) (exposure) and Eq. (5) (depuration) share the parameter k_{out} . All the Pb exposure conditions (1, 2.5, 5, 10, 25, 50, and 100 $\mu\text{g L}^{-1}$) were considered simultaneously, and to check the independence of k_{in} and k_{out} from C_w , two models were built: one model where kinetic parameters k_{in} and k_{out} were concentration-specific (model #1, $2 \times 7 = 14$ parameters in total), and one simpler model where k_{in} and k_{out} were concentration-independent, i.e., kinetic parameters are common to all exposure conditions (model #2, two parameters in total). Model #1 and model #2 were compared based on their AIC (Akaike (1981) information criterion), which provides a means for model selection by the trade-off between the goodness of fit and the complexity of the model. This enabled us to determine whether kinetic parameters are concentration-specific (model #1) or can be considered as concentration-independent (model #2). Models were fitted to the data by nonlinear least square regression. Data were scaled (divided by the exposure concentration) to ensure a constant variance of data across exposure concentrations.

2.3.3. Modelling the influence of major ions on Pb uptake

According to Bourgeault et al. (2010), the effects of ions on metal uptake can be explicitly taken into account with the model. This requires the use of a competition model in which the uptake rate constant is related to the concentrations of the ions considered and the affinity constants of the ions for their biological binding sites and is expressed as follows:

$$k_{in} = k_{in \max} / \left(1 + \sum_i \frac{C_i}{K_{iC_i}} \right) \quad (3)$$

where $k_{in \max}$ is the uptake rate constant of the metal in the absence of the considered ions in water, C_i is the concentrations of ions in the media water, and K_{iC_i} is the affinity constant that represents the ion concentration required to divide by two the metal uptake rate constant. The weaker the affinity constant, the stronger the competition effect.

The fit by nonlinear regression of Eq. (6) on the k_{in} dataset given by varying ionic concentrations in the water enables us to estimate the parameters $k_{in \max}$ and K_{iC_i} . Then, considering Eq. (3) and Eq. (6), we obtain:

$$C_{ss} = \frac{k_{in \max} / \left(1 + \sum_i \frac{C_i}{K_{iC_i}} \right)}{k_{out}} \cdot C_w \quad (4)$$

2.3.4. Estimations toward in situ conditions

In this study, the suitability of the model to estimate bioaccumulation of Pb in gammarids under realistic environmental conditions was investigated. To this end, bioaccumulation was predicted with (1) the bioaccumulation model expressed by Eq. (3) using the experimentally determined k_{in} and k_{out} and the dissolved Pb concentrations (C_w) measured in situ and (2) the bioaccumulation model integrating additionally the effect of cations (Eq. (7)) using the experimentally determined $k_{in \max}$, K_{iC_i} and k_{out} and the dissolved Pb concentrations (C_w) and ion concentrations (C_i) measured in situ. Then predictions were compared with bioaccumulations observed in collected native gammarids. The match of the predictions with the observations was calculated using an indicator of goodness of fit, the scaled root mean square error:

$$SRMSE = \frac{100}{\overline{observations}} \times \sqrt{\frac{1}{n} \times \sum_{i=1}^n (estimation_i - observation_i)^2} \quad (5)$$

where $observation_i$ = observed internal Pb concentrations at site i , $estimation_i$ = modelled Pb contents in gammarids at site i , n = total number of observations, and $\overline{observations}$ = mean of all the observed Pb contents in gammarids. The lower the SRMSE, the better the model estimation.

2.3.5. Statistical analysis

Nonlinear regressions and AIC calculations were performed using R (R Core Team, 2012) and the 'nlstools' package (Baty et al., Accepted). Estimates of parameters k_{in} , $k_{in\ max}$, Ki_{Ca} and k_{out} are given with a confidence interval of 95%. Nonparametric Kruskal-Wallis tests were used to compare Pb concentrations after 5 days in exposed versus control gammarids as well as Pb concentrations after 2 days in gammarids exposed to Pb with versus without Ca^{2+} , Na^{+} or Mg^{2+} . The background level of Pb in gammarids at the beginning of the exposure phase is given as mean \pm standard deviation.

3. Results and discussion

3.1. Kinetic parameters

After five days of experiment, exposed gammarids significantly accumulated Pb compared to the control gammarids (Kruskal-Wallis, $p < 0.05$). The accumulation and depuration kinetics obtained for each group of exposed gammarids are depicted in Fig. 2. Pb accumulation increased linearly in the first 24 h and started to slow down from the second day, finally tending towards the achievement of a steady-state until the end of the exposure phase. These results are in agreement with the efficient metal accumulation capacities of *G. pulex* already observed for Cd, Ni, Cu, and Zn (Lebrun et al., 2011, 2012; Pellet et al., 2009; Vellinger et al., 2012; Xu and Pascoe, 1993). Dedourge-Geffard et al. (2009) and Besse et al. (2013) also reported the ability of another closely related species, *Gammarus fossarum*, to accumulate metals during *in situ* experiments. Thus, the genus *Gammarus* is currently proposed as a promising candidate for monitoring water quality (Besse et al., 2013; Clason et al., 2004; Fialkowski et al., 2003; Xu and Pascoe, 1994).

During the depuration phase, Pb was exponentially excreted leading to body concentrations in gammarids reaching the background level within 9 days, which showed that excretion mechanisms are favoured rather than storage strategies.

The kinetic model was fitted to Pb concentrations in organisms for both exposure and depuration phases together in order to estimate the kinetic parameters k_{in} and k_{out} . The results of the AIC score calculation showed that the AIC score for model #1 was greater than the AIC score for model #2, which confirms that kinetic parameters were concentration-independent leading to the following global kinetic parameters:

$$k_{in} = 1.01 \text{ L g}^{-1} \text{ d}^{-1} [0.88; 1.14] \text{ and } k_{out} = 0.33 \text{ d}^{-1} [0.28; 0.38].$$

The uptake rate constant for Pb (k_{in}) was similar to the uptake rate constants reported in *H. azteca* and *D. magna*, equal to $1.5 \text{ L g}^{-1} \text{ d}^{-1}$ and $1.2 \text{ L g}^{-1} \text{ d}^{-1}$ respectively (Komjarova and Blust, 2008; MacLean et al., 1996). The k_{in} value was also in the same order of magnitude as the k_{in} value reported in *G. pulex* for Cd ($0.46 \text{ L g}^{-1} \text{ d}^{-1}$) by Pellet et al. (2009), which suggests that Pb and Cd have similar affinity to biological binding sites. This analogy may be justified by the implication of similar biological pathways as reported by Macdonald et al. (2002).

The elimination rate constant of Pb determined in *G. pulex* was close to the one established in another freshwater amphipod *H. azteca*, with a value of 0.52 d^{-1} (MacLean et al., 1996), suggesting a similar Pb excretion capacities. Moreover, *G. pulex* seems to have similar abilities to excrete Pb and Ni since their k_{out} values are in the same order of magnitude, respectively 0.33 and 0.13 d^{-1} (Lebrun et al., 2011). On the contrary, the elimination rate constant of Pb was about 10 times higher than the one of Cd of 0.032 d^{-1} (Pellet et al., 2009), which suggests that different excretion mechanisms occurred.

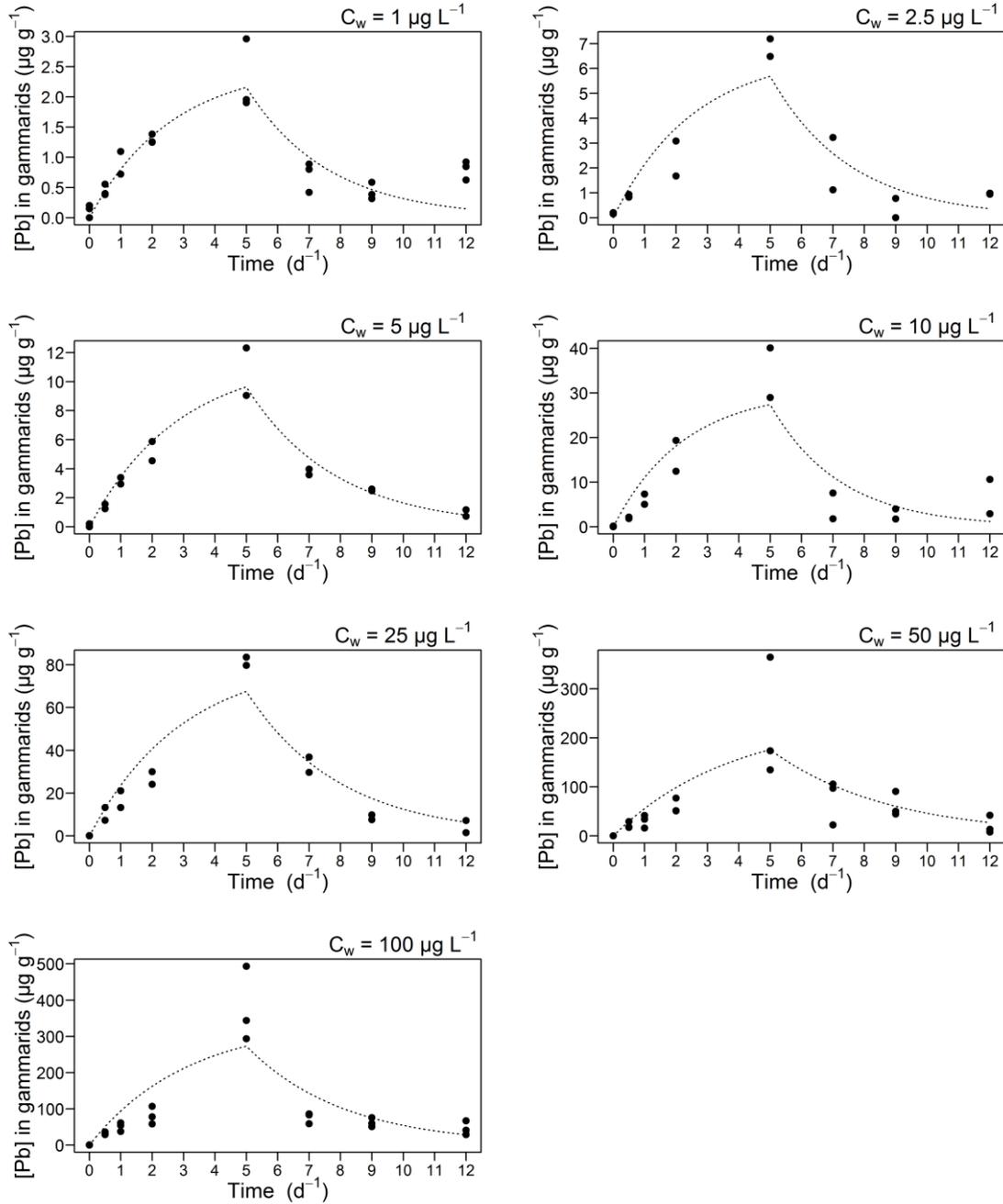


Fig. 2. Accumulation and depuration kinetics of Pb in *G. pulex* for each exposure condition (C_w). Black points are replicates each composed by a pool of 5 gammarids and dotted lines represent the best model fit of Eq. (2) (exposure phase) and Eq. (5) (depuration phase) on the Pb contents in gammarids.

3.2. Influence of water cationic composition on Pb uptake

Neither Mg^{2+} nor Na^+ had a significant effect on Pb uptake ($p = 0.92$ and 0.74 , respectively, Kruskal-Wallis, data not shown). Inversely, the k_m value for Pb decreased from 1.25 to 0.34 $L\ g^{-1}\ d^{-1}$ with increasing Ca^{2+} concentrations from 11.5 (initial content in the mineral water) to 154 $mg\ L^{-1}$ (Fig. 3). In *D. magna*, Komjarova and Blust (2009) showed that the Pb uptake doubled with high sodium content (184 $mg\ L^{-1}$) and sharply decreased with a high Ca^{2+} concentration (100 $mg\ L^{-1}$) (Komjarova and Blust, 2009). It is well known that some trace metals are able to enter cells via routes for the uptake of major metal ions (Rainbow, 1997). Previous studies in fish have demonstrated that Pb is preferentially taken up via Ca^{2+} channels and consequently

competes with Ca^{2+} for penetration into organisms (Macdonald et al., 2002; Niyogi and Wood, 2004; Rogers et al., 2003; Rogers and Wood, 2004).

Derived from Eq. (6), the effect of Ca^{2+} concentration observed on k_{in} value is given by:

$$k_{in} = k_{in\ max} / \left(1 + \frac{C_{Ca}}{Ki_{Ca}} \right) \quad (9)$$

where the model parameters were estimated at the following values: $k_{in\ max} = 1.38\ \text{L g}^{-1}\ \text{d}^{-1}$ [1.00; 1.76] and $Ki_{Ca} = 60\ \text{mg L}^{-1}$ [18; 101]. The model is shown in Fig. 3. Thus, accounting for the effect of only Ca^{2+} , bioaccumulation of metal from the solution in the steady-state ($t \rightarrow \infty$) (Eq. (7)) is expressed as follows:

$$C_{ss} = \frac{k_{in\ max} / \left(1 + \frac{C_{Ca}}{Ki_{Ca}} \right)}{k_{out}} \cdot C_w \quad (10)$$

In *G. pulex*, the effect of Ca^{2+} has also been observed for Cd with a $Ki_{Ca} = 66.4\ \text{mg L}^{-1}$ and for Ni with an affinity constant equal to $181.5\ \text{mg L}^{-1}$ (Lebrun et al., 2011; Pellet et al., 2009). The similarity between the affinity constants of Pb and Cd underlined the comparable behaviour of these two metals to enter biological surfaces and especially the use of the same entry pathway via Ca^{2+} channels as reported for Cd in bivalves or in freshwater snails (Croteau and Luoma, 2007; Qiu et al., 2005). Moreover, Pb uptake in *G. pulex* probably does not involve Na^+ or Mg^{2+} channels, as indicated by the Na^+ and Mg^{2+} concentrations having no effect on the k_{in} value.

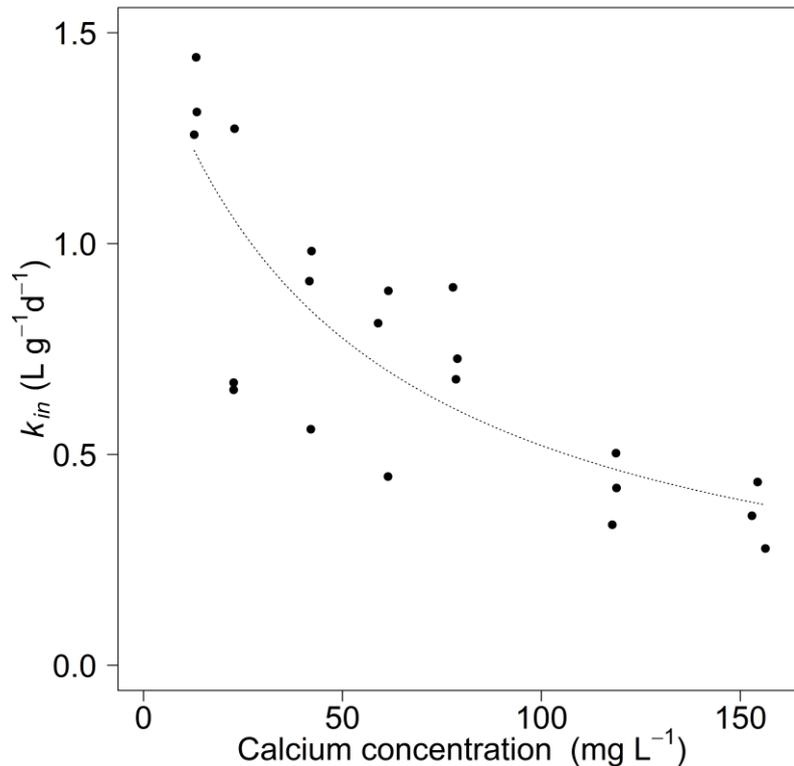


Fig. 3. Inhibition of the Pb uptake by increasing Ca^{2+} concentrations in *G. pulex* exposed to $10\ \text{mg L}^{-1}$ Pb. The dashed line represents the best fit of a competitive model (Eq. (6)) to data. Points are replicates each composed by a pool of 5 gammarids.

3.3. *In situ* model validation

Our field study aimed to assess whether experimentally-derived kinetic parameters could be suitable to estimate Pb contents in native gammarids. Samples of native gammarids in 21 sites were collected from the Seine watershed on which Pb contents were measured. Assuming that *in situ* organisms are in equilibrium with their medium, waterborne Pb bioaccumulation at steady state was calculated with a kinetic model calibrated in the laboratory taking into account or not the effect of calcium. As presented in Table 1, concentrations of dissolved Pb in the 21 sites ranged between 0.03 and 1.60 mg L⁻¹, only two sites exhibited Pb concentrations higher than the related recent EQS (1.2 mg L⁻¹ (Directive, 2013/39/EC)). Pb contents in native gammarids ranged between 0.84 and 3.43 µg g⁻¹, i.e., above the background concentration observed in *G. pulex* used to calibrate the model in the laboratory (0.64 µg g⁻¹) (Table 1). Clason et al. (2004) reported comparable Pb concentrations measured in *Gammarus oceanicus* of up to 2 µg g⁻¹ in northern Norway. Calcium profiles were contrasted with a minimum concentration of 47 mg L⁻¹ and a maximum of 159 mg L⁻¹ (Table 1), which is consistent with the limestone geology of the Seine watershed.

Table 1

Summary of the dissolved contamination levels of Pb in waters (mg L⁻¹), Pb concentrations in native gammarids (µg g⁻¹) and concentrations of the major cations Ca²⁺, Mg²⁺ and Na⁺ (mg L⁻¹) at the 21 sampling sites for which bioaccumulation was predicted by the kinetic model.

Sampling sites		Measured concentrations				
Year	River	Pb in gammarids (µg g ⁻¹)	dissolved Pb (µg L ⁻¹)	Ca ²⁺ (mg L ⁻¹)	Mg ²⁺ (mg L ⁻¹)	Na ⁺ (mg L ⁻¹)
2008	Le Lieutel	1.21	0.64	119	23	34
2008	L'Essonne	3.12	0.51	117	5	47
2008	La Sausseron	1.49	0.29	116	20	12
2008	La Juine	1.96	0.11	104	4	9
2008	La Gondoire	1.75	0.12	114	10	21
2008	La Rimarde	0.84	0.06	113	4	13
2008	L'Ecole	1.05	0.08	114	4	17
2008	Ru de Senneville	0.90	0.47	120	17	15
2008	La Mauldre	2.93	0.83	116	18	63
2008	Ru d'Ancueil	1.03	0.08	153	8	75
2008	Ru de Gally	3.43	1.60	118	12	61
2008	L'Oeuf	0.98	0.03	120	5	8
2008	L'Epte	1.60	0.15	120	5	20
2008	La Levrière	2.06	1.10	122	8	10
2009	L'Essonne	2.31	1.60	124	7	47
2009	Ru de Fourville	1.19	0.37	120	20	14
2009	La Sausseron	1.14	0.53	120	20	14
2012	Le Rognon	0.92	0.06	47	3	4
2012	L'Auve	0.84	0.05	130	2	8
2013	Ru de Gally	1.64	0.51	159	19	77
2013	Le Rognon	1.10	0.26	98	8	16

Fig. 4A displays the observed bioaccumulation levels compared with the estimated bioaccumulation levels calculated with Eq. (3) using k_{in} and k_{out} of 1.01 L g⁻¹ d⁻¹ and 0.33 d⁻¹. Results show a good agreement between observations and estimations, with 85% of the estimations between the dashed lines representing a twofold deviation above or below the theoretical 1:1 relationship. The calculated SRMSE was equal to 71%. Overall, 15% of the points were above the dashed line, which means that bioaccumulated concentrations were overestimated at those sites. These results suggest that other factors could influence the metal uptake, typically calcium concentrations. Besides, the kinetic model assumes that the concentrations to which organisms are exposed are constant. However, although the metal exposure can be easily verified in the laboratory, it is sometimes not certain *in situ* because of the temporal fluctuations of the contamination that may occur during particular incidents such as accidental discharge, floods or flushing. Thus, a single measure of the dissolved metal concentration *in situ* could introduce a bias in the estimated bioaccumulation and may explain some discrepancies between predictions and observations. Fig. 4B depicts the observed bioaccumulation levels compared with the estimated bioaccumulation levels taking into account the effect of Ca²⁺. Bioaccumulated concentration was calculated with Eq. (10) with $k_{in\ max} = 1.38$ L g⁻¹ d⁻¹, $k_{out} = 0.33$ d⁻¹, $Ki_{Ca} =$

60 mg L⁻¹, experimentally derived in the laboratory for this study and the lead concentration C_w and the calcium concentration C_{Ca} measured at the sampling site. The consideration of Ca²⁺ resulted in a tightening of the plot around the theoretical 1:1 relationship. The calculated SRMSE was equal to 40%, which means that the consideration of Ca²⁺ improves the estimation accuracy by about 44%. Thus, calcium concentrations in the ambient water seem to be an important factor influencing bioaccumulation of Pb since the consideration of the effect of calcium has permitted to improve bioaccumulation modelling.

As presented in Fig. 4B, the model taking into account Ca²⁺ concentrations under-estimates the bioaccumulation in 14% of the cases. The sites for which the bioaccumulation was underestimated were not geographically close and no other factors than those given in Table 1 were available to support any explanation. These observations may be related to a lower impact of Ca²⁺ concentrations under field conditions as compared with observed effects under laboratory conditions. Another explanation could be the contribution of the trophic route at these sites. In the literature, an increasing number of studies have found that, for particular metal/ species combinations, diet is an important contributory factor in metal bioaccumulation, and the contribution of the trophic route needs to be integrated to improve the accuracy of any biodynamic model. This is, for example, the case of Cd, Zn or Ag in the marine lugworm, *Arenicola marina* (Casado-Martinez et al., 2009) or in marine and freshwater bivalves (Luoma and Rainbow, 2005). To date, bioaccumulation data of Pb are limited in freshwater species and the question of the contribution of the trophic route is yet not univocal. Thus, studies assessing the dietary route of Pb in gammarids will bring complementary information to the limited body of literature concerning dietary route of Pb in aquatic organisms.

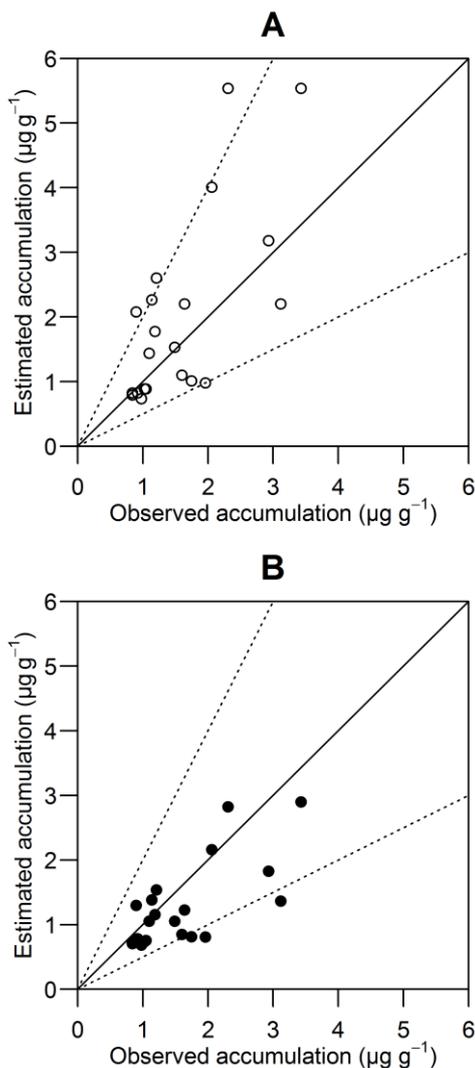


Fig. 4. Observed bioaccumulation levels of Pb in native gammarids against estimated bioaccumulation levels by the kinetic model using Eq. (3) ($k_{in} = 1.01 \text{ L g}^{-1} \text{ d}^{-1}$ and $k_{out} = 0.33 \text{ d}^{-1}$, established in the Volvic® mineral water containing a basal Ca²⁺ concentration of 11.5 mg L⁻¹) for the plot A and using Eq. (10) ($k_{in \text{ max}} = 1.38 \text{ L g}^{-1} \text{ d}^{-1}$ and $k_{out} = 0.33 \text{ d}^{-1}$ and $Ki_{Ca} = 60 \text{ mg L}^{-1}$) for the plot B (white and black symbols are mean values, $n = 3$ pools of 5 gammarids). The solid line represents the theoretical model fit 1:1 and dashed lines display a deviation of 2-fold above and below the relationship 1:1.

In this study, the experimentally derived kinetic parameters were highly applicable to environmental conditions encountered in the Seine watershed, in spite of ignoring the dietary route of uptake. In the perspective to tend towards the use of a generalised model to predict Pb bioaccumulation, it could be opportune to validate the kinetic parameters over a larger geographical scale and in contrasted hydrosystems (variability of size, metal contamination, geochemical parameters...). However, the validation of the model can be limited because they cannot be tested if native gammarids are absent. To overcome this issue, the use of transplanted organisms can be relevant. Indeed, this approach allows for studies at sites devoid of native gammarids. Moreover, the use of transplanted organisms limits biological variability, which can hinder the interpretation of bioaccumulation, as organisms are collected from the same population (Besse *et al.*, 2012, 2013; Bourgeault *et al.*, 2010; Mersch *et al.*, 1996). This approach also enables control of the exposure during the transplantation period, and facilitates robust inter-site comparisons (Besse *et al.*, 2012). Therefore, in further studies, the use of transplanted gammarids could be an interesting approach to assess the performances of the model at large spatial scales.

4. Conclusion

An experimentally-derived kinetic model was used to predict waterborne bioaccumulation of Pb in native populations of *G. pulex* from the Seine watershed. Results showed a relatively robust agreement between the predicted and measured bioaccumulation levels, highlighting the applicability of the kinetic parameters for *G. pulex* to environmental conditions. The results of the present study also demonstrate that the use of dissolved metal concentrations allows for accurate predictions of Pb accumulation in *G. pulex*. Moreover, the consideration of the effect of calcium concentrations into the model has improved Pb bioaccumulation modelling. This supports that water chemistry needs to be taken into account when interpreting field data of bioaccumulation in this species. Finally, the relevance of the model was successful in the environmental conditions presented in this study. Through the use of transplanted gammarids in large geographical scale, the application of a generalised predictive model will be assessed in further investigations.

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