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One and multi-compartments toxico-kinetic modeling to understand metals' organotropism and fate in *Gammarus fossarum*

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ABSTRACT

The use of freshwater invertebrates for biomonitoring has been increasing for several decades, but little is known about relations between external exposure concentration of metals and their biodistribution among different tissues. One and multi-compartments toxicokinetic (TK) models are powerful tools to formalize and predict how a contaminant is bioaccumulated. The aim of this study is to develop modeling approaches to improve knowledge on dynamic of accumulation and fate of Cd and Hg in gammarid's organs. Gammarids were exposed to dissolved metals ($11.1 \pm 1.2 \mu\text{g.L}^{-1}$ of Cd or $0.27 \pm 0.13 \mu\text{g.L}^{-1}$ of Hg) before a depuration phase. At each sampling days, their organs (caeca, cephalon, intestine and remaining tissues) were separated by dissection before analyses. Results allowed us to determine that i) *G. fossarum* takes up Cd as efficiently as the mussel *M. galloprovincialis*, but eliminates it more rapidly, ii) organs which accumulate and depurate the most, in terms of concentrations, are caeca and intestine for both metals; iii) the one-compartment TK models is the most relevant for Hg, while the multi-compartments TK model allows a better fit to Cd data, demonstrating dynamic transfer of Cd among organs.

1. Introduction

The environmental risk assessment aims at “contributing to the protection and management of the environment through scientifically credible evaluation of the ecological effects of human activities” (Suter, 2016). In this way, the European Water Framework Directive (WFD) 2000/60/EC qualified water as “a heritage that must be protected, defended and treated as such” and established a list of priority substances to be monitored, including cadmium (Cd) and mercury (Hg).

Although metallic contamination of water can occur through natural phenomena such as leaching or volcanism, the most important cause is anthropogenic through industrial and agricultural discharges (Zhou et al., 2008). While contamination of environment can be studied by direct analysis of chemicals in water or sediments these approaches are expensive and poorly integrative (Bertrand et al., 2019). Indeed, punctual samplings of water do not provide information on the

contamination history and none of the two matrices informs on bioavailability and the potential toxicity of the contaminant on the biota (e.g. Saleem et al., 2018). The use of sentinel species as bioindicators for biomonitoring of aquatic systems has been increasing for several decades (Kraak et al., 1991) and is currently recommended by WFD. Since the gammarid *Gammarus fossarum* is highly sensitive to various chemicals and can accumulate and transfer toxicants to upper trophic levels (Vellinger et al., 2012; Zhou et al., 2008), it represents an essential species for the biomonitoring. Active biomonitoring (caged organisms) of sentinel species to assess water quality has been largely deployed the last decade (Besse et al., 2013; Chaumot et al., 2015; Kunz et al., 2010).

The bioaccumulation in organisms results from the difference between uptake (from water or food for example) and elimination (as excretion or dilution by growth). In 1996, Amyot et al. (1996), underlined the necessity to understand the main pathways of uptake and distribution of contaminant among the different organs of invertebrates

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like amphipods. However, in the last 20 years, very few studies have been conducted in this area. The lack of data regarding bioaccumulation at the organ level of invertebrates results from the challenges of quantifying concentrations in small organisms (Grech et al., 2019). Indeed, mainly constrained by analytical detection thresholds, the studies on organism's tissues and organotropism's literature mainly focused on fishes for vertebrates, and we found only two studies concerning bivalves for invertebrates (Ju et al., 2011; Rocha et al., 2015). To date, almost all literature about metals' bioaccumulation concerning amphipods, and more generally invertebrates, are focused on the entire organism (Hare et al., 2003). Consequently, definitive conclusions regarding the bioaccumulation mechanisms such as tissue distribution in freshwater invertebrates, like crustacean amphipods, could not be established (O'Callaghan et al., 2019). The few studies measuring metals concentrations in the organs of gammarids, or phylogenetically related genus, have been carried out based on punctual field sampled organisms (Amyot et al., 1996; van Hattum et al., 1996), and not with toxicokinetic experimental designs such as those routinely used in fishes (Gao et al., 2019; Huang et al., 2012).

Toxicokinetic (TK) is a dynamic process measuring the concentration accumulated into organisms over time according to the contamination pressure, by integrating the different accumulation, distribution, metabolism and elimination processes (ADME) (Huang et al., 2012; Ratier et al., 2019). TK models, coupled to Bayesian Inference to estimate their kinetic parameters, are powerful tools to predict and to confirm how a contaminant is bioaccumulated by an organism over time, in relation to the contamination sources (Ratier et al., 2019). Therefore, kinetic parameters of such models reflect the rates of accumulation and depuration and their estimation can be compared between species revealing their respective bioaccumulation and/or depuration capacities.

Two types of TK models exist: i) the one compartment model considers the entire organism, a tissue or an organ, as a homogeneous "box" for which uptake and elimination are only occurring between this "box" and the environment; ii) in contrast, the multi-compartments model takes in consideration the distribution of the contaminant among the different tissues and/or organs of an organism.

The one-compartment model is easy to implement and widely used in ecotoxicology for both vertebrates and invertebrates. Indeed, this model allows to link, with a simple formalism, the concentration of contaminant effectively bioaccumulated by an organism in relation to its exposure, even when considering different contamination sources and depuration processes. In this way, such one-compartment model is very useful to i) validate the potential of species for contaminant accumulation (Pery, 2008) and to compare the accumulation and depuration rates among species (Ju et al., 2011). This model is also useful to select the most relevant species for active biomonitoring for example; ii) study and identify the bioavailable fraction of contaminants (Pellet et al., 2009); and iii) make simple links between exposure and effects on organisms in toxicokinetic/toxicodynamic (TK-TD) models. Currently, the TK part of TK-TD models developed on invertebrates, whether GUTS or DEBtox (Baudrot et al., 2018; Jager, 2020; Jager and Ashauer, 2018) models, use the one-compartment TK models in order to predict contaminant bioaccumulation thereby ignoring the contribution of each organ (Gao et al., 2019).

On the contrary, by assessing contaminant exchanges among organs and by predicting the concentrations reached in the different tissues (van Hattum et al., 1989), multi-compartments TK models are more accurate from a physiological point of view. Such multi-compartments TK models are thus very useful to i) understand and describe the distribution and the fate of contaminant into the organism (McCarty and Mackay, 1993); and ii) to identify which organs play a key role in the accumulation and/or the regulation of contaminant (Chen, 2016; Gao et al., 2019), as a first step to develop relevant exposure and effective biomarkers for example. However, the lack of bioaccumulation data at the organ level for invertebrates has restricted the use of multi-

compartments TK models to vertebrates, such as fishes (Grech et al., 2017; Stadnicka et al., 2012).

In this context, the aim of our study was to establish a proof-of-concept by i) quantifying the bioaccumulation of two metals in the organs of a sentinel crustacean species, the gammarid *Gammarus fossarum*; and ii) proposing one and multi-compartments models, with their inference process associated, to improve our knowledge on the dynamic of these two metals accumulation and fate in gammarid's organs. To this end, males *G. fossarum* were exposed to dissolved, non-lethal high concentrations of cadmium and mercury (to ensure an accurate metal detection in tiny gammarids tissues) for 4 and 7 days before a depuration phase of 14 and 21 days. At different time points during these two phases, gammarids were dissected to collect their organs for metal measurements. Using these results we derived, for each metal, a one-compartment model fitted to each organ separately. In addition, a multi-compartments model, in association with the iterative Bayesian inference method, was developed in order to analyze data when fitted to all organs simultaneously.

2. Material and methods

2.1. Experimental section

2.1.1. Reagents and chemicals

Cadmium ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$, > 98%) and mercury (Hg Certipur®, analytical standard at 1000 mg.L^{-1} in HNO_3 2 mol.L $^{-1}$) were purchased from Merck ®. Stock solutions of Cd (80 mg.L^{-1}) and Hg (1 mg.L^{-1}) were prepared in acidified (HNO_3 65% Suprapur®, 0.5%) Milli-Q® ultrapure quality water. Exposure concentrations, of $11.1 \pm 1.2 \text{ } \mu\text{g.L}^{-1}$ for cadmium and $0.27 \pm 0.13 \text{ } \mu\text{g.L}^{-1}$ for mercury, were obtained by diluting 70 μL of Cd stock solution or 200 μL of Hg stock solution into 500 mL of well water for which physico-chemical parameters are presented in Table S1. These low volumes of stock solution prevent any change in the pH of the water.

2.1.2. Collect, maintenance and selection of organisms

Adults *Gammarus fossarum* were collected, through 2- and 2.5-mm sieves, on a bygone watercress farm located in Saint-Maurice-de-Rémens (eastern central France). They were stored in plastic bottles containing ambient freshwater, and immediately transferred to the laboratories: Ecotoxicology Laboratory in INRAE Lyon for Hg experimentation and LIENSs in La Rochelle University for Cd experimentation. The organisms were acclimated in a 10 L aquarium filled with well water available in INRAE Lyon and with Evian® water (being the water with the closest characteristics to well-water) in La Rochelle, at 12 ± 0.5 °C, with a dark:light cycle of 8:16 h for 10 to 15 days and under constant aeration. Organisms were fed *ad libitum* with alder leaves (*Alnus glutinosa*) collected at a site with negligible anthropic pressure and previously conditioned by immersion in groundwater for 6 days (Ratier et al., 2019). Freeze-dried Tubifex® worms, were added twice a week as a dietary supplement.

Only males between 20 and 30 mg wet weight were selected for the following experiments.

2.1.3. Exposure of gammarids

All the experiments were carried out in 12 ± 0.5 °C water baths, temperature, pH and mortality were monitored. The design is presented in Figure S1.

Gammarids ($n = 450$ for Cd; $n = 630$ for Hg) were randomly placed in 500 mL-beakers (30 beakers for Cd, each containing 15 gammarids; and 63 beakers for Hg, each containing 10 gammarids). During the exposure phase, each medium was totally renewed every two days for Cd and daily for Hg to ensure a constant exposure. Gammarids were not fed to avoid a metal accumulation through trophic pathway. At sampling time-points (Table S2), renewed water was sampled (just after and before renewal) for checking concentration levels. Water samples were

acidified (HNO₃ 65% Suprapur®, 0.5%) and stored at 4 °C until analysis. During the depuration phase, each beaker was renewed only with uncontaminated water, well water for Hg experiment and Evian® water for Cd experiment, and organisms fed *ad libitum* with *A. glutinosa* leaves. At the same time, dead gammarids were counted and removed. The characteristics of waters used for these experiments are presented in Table S1.

2.1.4. Gammarids dissection

Organs of interest (cephalons, caeca, intestines and remaining tissues) were sampled at days 0, 2, 5, 7, 9, 15, 21 for Cd and 0, 1, 2, 4, 7, 10, 14, 24 for Hg (Fig. S1), then stored at −80 °C before freeze-drying. At each sampling day, amphipods (3 replicates of $10 \leq n \leq 18$ of each organ for Cd or $n = 15$ for Hg) were randomly sampled from all the beakers, to have a homogeneous decrease of organism numbers in each beaker, gently dried with paper towel and weighted ± 0.1 mg. Then, gammarids were dissected according to the procedure described in Fig. S2. The cephalon was first separated from the body. Then the urosome was separated from the abdomen, revealing the caeca and the intestine which were then isolated from each other. Finally, cephalons, caeca, intestines and remaining tissues (i.e. the thoracic, the abdomen and the urosome) from all individuals sampled per replicate were pooled and stored at −80 °C before freeze-drying waiting for their analyses.

To express the metal concentrations measured in each organ per gram of dry weight, we previously performed dissections on pool of 15 organisms to estimate the respective percentage of each dry organ regarding the total wet weight of the whole gammarid (Tables S3 and S4), with *G. fossarum* dry weight representing 25% of the wet weight (Eq. (1)):

$$W_i = 0.25 \times \frac{\sum_{z=1}^{15} x_{i,z}}{y_z} \times 100 \quad (1)$$

where W_i is the percentage of the dry organ i ($i = 1..4$) regarding the total dry weight of the whole organism (in mg dw), $x_{i,z}$ is the dry weight of the organ i ($i = 1..4$) for the organism z ($z = 1..15$) (mg dw), y_z is the total wet weight of the z organisms constituting the pool (mg ww). $i = 1$ corresponds to intestines, $i = 2$ to caecum, $i = 3$ to cephalon and $i = 4$ to remaining tissues.

All along the experiment, for Cd and Hg experiments respectively, a single gammarid weighted in average 24.2 ± 1.2 and 26.3 ± 1.1 mg ww, with a respective percentage of 2.2% for intestines, 5% for caeca, 14% for cephalons and 78.8% for the remaining tissues.

The weights and concentrations presented next will always be expressed in dry weight (dw).

2.1.5. Cd and Hg analyses

All chemical analyses were conducted at the Aquatic Chemistry Laboratory (LAMA) of INRAE, Lyon.

Waters and gammarids' organs samples which were contaminated by Hg were analyzed by a Direct Mercury Analyzer 80 (ThermoFisher Scientific). The limit of quantification (LOQ) for Hg was 0.025 ng or 0.010 $\mu\text{g.g}^{-1}$ of Hg for a 30 mg dry weight sample. The certified reference material (CRM) NIST® SRM® 2976 (mussel tissue) was used to check for method accuracy and precision. Samples which were

contaminated by Cd were prepared by microwave oven with nitric acid digestion (HNO₃ 65% Suprapur®) before being analyzed according to NF EN ISO 17294-2 (AFNOR, 2016) by inductively coupled plasma mass spectrometry with triple quadrupole technology (ThermoFisher Scientific iCAP™ TQ ICP-MS). The LOQ was 0.0010 $\mu\text{g.L}^{-1}$ for waters and between 0.005 and 0.125 $\mu\text{g.g}^{-1}$ for organs samples (depending on the sample dry weight between 3 and 80 mg). The CRM used were TM 27-4 (Ontario lake water) for waters and BCR-278R (mussel tissue) for gammarids' organs samples.

Blank tests were carried out systematically to detect any possible contamination along the analytical chains. The CRM results were generally well within certified values. Three replicates of each pooled sample were subjected to analysis and relative standard deviations of their analyses (including sampling and analytical variability) were generally below 20%.

2.2. Toxicokinetic modeling and inference

2.2.1. One-compartment models

Here, a compartment represents an organ. Since gammarids weren't fed during the accumulation phase, we considered that contaminants' bioaccumulation is only carried out from water. Furthermore, since organisms exposed are adults and the maximum duration of experiments is 24 days, we neglected the growth of gammarids. As expressed in Ratier et al. (Ratier et al., 2019), the variation of internal concentration in an organ during time can thus be described by:

$$\frac{dC_i(t)}{dt} = \begin{cases} -k_{e,i} \times C_i(t) & \text{for } 0 \leq t \leq t_c \\ -k_{e,i} \times C_i(t) & \text{for } t > t_c \end{cases} \quad (2)$$

$$\frac{dC_i(t)}{dt} = \begin{cases} -k_{e,i} \times C_i(t) & \text{for } 0 \leq t \leq t_c \\ -k_{e,i} \times C_i(t) & \text{for } t > t_c \end{cases} \quad (3)$$

where $C_i(t)$ is the internal concentration ($\mu\text{g.g}^{-1}$ dry weight) in the organ i ($i = 1..4$) at time t (days), $k_{u,i}$ the accumulation rate from water (day^{-1}) for the organ i , $C_w(t)$ the external concentration in water ($\mu\text{g.mL}^{-1}$) at time t , $k_{e,i}$ the elimination rate (day^{-1}) for the organ i and t_c the duration of the accumulation phase (days). As before, $i = 1$ corresponds to intestines, $i = 2$ to caecum, $i = 3$ to cephalon and $i = 4$ to remaining tissues.

As confirmed by the concentrations measured in water, we considered that C_w is constant during the experiment. As a consequence, Eqs. (2) and (3) can be analytically solved (Eqs. (S1) and (S2), SI).

2.2.2. Multi-compartments model

A multi-compartments model was developed to describe accumulation and fate of these metals through gammarid's organs, as shown in Fig. 1. The complete model therefore consists of 8 coupled ordinary differential equations (ODE): 4 for the accumulation phase (one for each organ) and 4 for the depuration phase (details in SI, Eqs. (S3) to (S10)), that can be generalized as:

$$\begin{cases} \frac{dC_i(t)}{dt} = k_{u,i} \times C_w + \sum_{j \neq i} k_{ji} \times C_j(t) - \sum_{j \neq i} k_{ij} \times C_i(t) - k_{e,i} \times C_i(t) & \text{for } 0 \leq t \leq t_c \end{cases} \quad (4)$$

$$\begin{cases} \frac{dC_i(t)}{dt} = \sum_{j \neq i} k_{ji} \times C_j(t) - \sum_{j \neq i} k_{ij} \times C_i(t) - k_{e,i} \times C_i(t) & \text{for } t > t_c \end{cases} \quad (5)$$

where k_{ji} is the passage rate of contaminant from organ j ($j = 1..4$, with $j \neq i$) to organ i .

2.2.3. Bayesian inference

We used the Bayesian inference method proposed by Ratier et al. (Ratier et al., 2019) to estimate parameters of toxicokinetic models. We briefly summarize here the main information (see (Ratier et al., 2019)

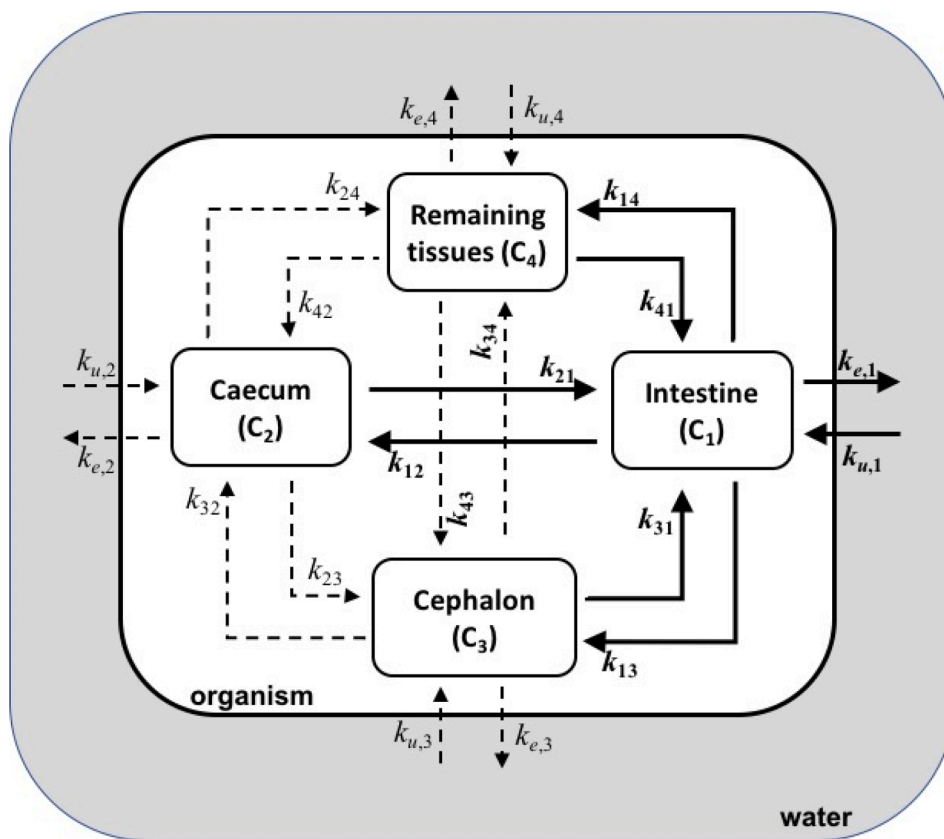


Fig. 1. General scheme of the multi-compartment model. The large grey outer border represents the medium surrounding the organism, here water, the white box represents the organism and inside it the organs of interest (cecum, cephalon, intestine, and remaining tissues), associated with their respective concentrations C_i ($i = 1$ for intestine, $i = 2$ for caecum, $i = 3$ for cephalon and $i = 4$ for remaining tissues). The different arrows represent the rates of uptake ($k_{u,i}$) and elimination ($k_{e,i}$) of contaminant between water and the organ i , and k_{ij} represents the flux from the organ i to the organ j . All the solid and dotted arrows form the complete model firstly tested (Eqs. (S3) to (S10)). Dotted arrows were successively fixed to zero to obtain, at the end, the “best” multi-compartment model consisting of arrows in solid line only.

for more technical details).

Stochasticity. We assumed a gaussian distribution of the contaminant concentration in an organ:

$$C_{obs,i}(t) \sim \mathcal{N}(C_i(t), \sigma_i) \quad (6)$$

where $C_{obs,i}(t)$ is the observed concentrations in the organ i ($i = 1..4$) at time t , \mathcal{N} stands for the Normal law, with a mean $C_i(t)$, the internal concentrations ($\mu\text{g.g}^{-1}$ dry weight) in the organ i predicted by the model at time t (Eqs. (2) and (3) or (4) and (5) according to the case), and the standard deviation σ_i for the organ i ($i = 1..4$). Definition of priors. According to the few information in the literature concerning

accumulation and depuration of gammarids' organs, we chose a priori non informative laws: a Uniform law on the decimal logarithm scale for parameters concerning accumulation and elimination rates (due to the lack of information about the order of magnitude of these parameters) and a Gamma law for uncertainty parameters (Tables 1 and S6).

Implementation of the model – MCMC simulations. Model computation was performed with JAGS and R software (Plummer, 2003; R Core Team, 2017). Models were fitted to data (from analytical solutions for the one-compartment models and by using a Euler integration scheme for the multi-compartment model) by using Bayesian Inference via Markov chain Monte-Carlo (MCMC) sampling. In each case, we started by running a short sampling (5000 iterations after a burn-in phase of

Table 1

Parameters estimates of the TK one-compartment model (Eqs. (2) and (3)) fitted separately to each organ of *Gammarus fossarum* exposed to dissolved Cd and Hg for 7 and 4 days, respectively, before being placed for 14 and 20 days, respectively, in depuration conditions.

Organs	Parameters	Priors	[Cd] = $11.1 \pm 1.2 \mu\text{g.L}^{-1}$			[Hg] = $0.27 \pm 0.13 \mu\text{g.L}^{-1}$		
			Median	Percentiles		Median	Percentiles	
				2.5%	97.5%		2.5%	97.5%
Intestines	$k_{u,1}$	log10.Unif (-5,5)	1917	849.4	5059	2853	1848	4062
Caeca	$k_{u,2}$		1571	1274	1891	1807	1404	2261
Cephalons	$k_{u,3}$		91.1	71.9	111	424	389	477
Remaining tissues	$k_{u,4}$		135	120	150	673	608	737
Intestines	$k_{e,1}$	log10.Unif (-5,5)	0.506	0.174	1.580	0.122	0.053	0.235
Caeca	$k_{e,2}$		0.060	0.033	0.092	0.097	0.057	0.151
Cephalons	$k_{e,3}$		0.053	0.023	0.086	1.79e-03	1.33e-05	0.014
Remaining tissues	$k_{e,4}$		0.026	0.013	0.039	0.022	0.014	0.034
Intestines	σ_1	Gamma (0.001, 0.001)	11	8.0	17	0.694	0.518	1.013
Caeca	σ_2		16	12	24	0.317	0.237	0.455
Cephalons	σ_3		1.0	0.74	1.5	0.064	0.047	0.090
Remaining tissues	σ_4		0.95	0.69	1.4	0.071	0.053	0.103

$k_{u,i}$ and $k_{e,i}$ are, respectively, the uptake and elimination rates (d^{-1}) of the organ i ($i = 1..4$); σ_i is the standard deviation of the Gaussian stochastic part associated to the organ i ($i = 1..4$); Priors: scale, law and interval of values tested during the inference process; Median and Percentiles: median and percentiles of the posterior distribution for each parameter, the percentiles corresponding to the lower and upper limit of the 95% credibility interval of each parameter.

10,000 iterations) using the Raftery and Lewis method (Raftery and Lewis, 1992) to set the necessary thinning and number of iterations to reach an accurate estimation of each parameter. MCMC sampling was based on 50,000 iterations for three chains after discarding the first 5000 iterations. Samples of 5000 iterations were stored to reduce autocorrelation. To monitor the convergence of the chains, we used a visual inspection as well as the Gelman criterion (Gelman et al., 1995).

Posteriors distribution and relevance of model predictions. From the joint posterior distribution, we deduce the marginal posterior distribution for each parameter, which is summarized by the median and the 95% credible intervals (Table 1). The accuracy of model parameter estimation is visualized by comparing prior and posterior distributions (Figure S3): a thin posterior distribution reflects that the data bring enough information to precisely estimate parameters.

To check the relevance of model predictions, we represent observed data superimposed on the model simulated with the median of the posterior distribution for each parameter and the 95% credible band of the predicted data considering parameter uncertainties and stochasticity. To obtain the 95% credible band, the predicted data were simulated with the model (Eqs. (2) and (3) or (4) and (5)) for each MCMC iteration and the stochastic model considered for observed data (Eq. (6)).

Selection of the multi-compartments model. The modeling can be viewed as one hypothesis to be tested on the experimental data, and the structure of the model is then altered until the fitting on the data is satisfactory. The inference starts with the complete model consisting in considering all links between organs and water (Fig. 1, Eqs. (S3) to (S10) summarized by Eqs. (4) and (5)). Then, parameter(s) estimated at values close to zero are successively fixed to zero. The successive fits were compared according to the precision of each parameter estimation and the relevance of model predictions through the deviance information criterion (DIC), a Bayesian measurement that weighs the quality of model fit with its complexity. Lower DIC values are expected to effectively balance between predictive capacity and complexity of the model

(Spiegelhalter et al., 2002). At the end, the selected multi-compartments model was constructed based on two guiding principles: it contains the fewest compartments to adequately describe all the data simultaneously and reflects realistic physiology processes.

3. Results and discussion

3.1. Experimental conditions

The low levels of environmental dissolved metals accumulation in small organs (i.e. caeca, cephalons, intestines and remaining tissues), represent an important challenge for accurate detection and quantification (O'Callaghan et al., 2019). In order to obtain accurate measurements for bioaccumulation modeling, we exposed the gammarids to $11.1 \pm 1.2 \mu\text{g.L}^{-1}$ of Cd or $0.27 \pm 0.13 \mu\text{g.L}^{-1}$ of Hg. Under these conditions, Cd concentration in water decreased from 12.09 ± 0.12 to $10.06 \pm 0.24 \mu\text{g.L}^{-1}$, a 17% loss in 48 h (Table S2), with bioaccumulation in gammarids accounting for 50% of the observed Cd loss. Mercury concentrations in water decreased from 0.379 ± 0.008 to $0.158 \pm 0.028 \mu\text{g.L}^{-1}$, a 58% loss in 24 h (Table S2). However, only 10% of the loss could be explained by metal bioaccumulation in gammarids. We think that adsorption of Hg onto polypropylene beakers walls or volatilization in the atmosphere due to Hg^{2+} transformation into volatile $\text{Hg}(0)$ (European Chemicals Agency (ECHA), 2008) may account for the majority of Hg observed loss from the water. The survival rates of gammarids over the total experiments duration were 76% for Cd and 78% for Hg.

3.2. Organotropism of Cd and Hg in *G. fossarum*

All Cd and Hg concentrations in *G. fossarum* samples were above the LOQ, regardless the tissue and the sampling time, allowing reliable measurements of metals concentrations. Fig. 2 and S4 and Tables S3 and S4 show the concentration observed in organs for both metals during the

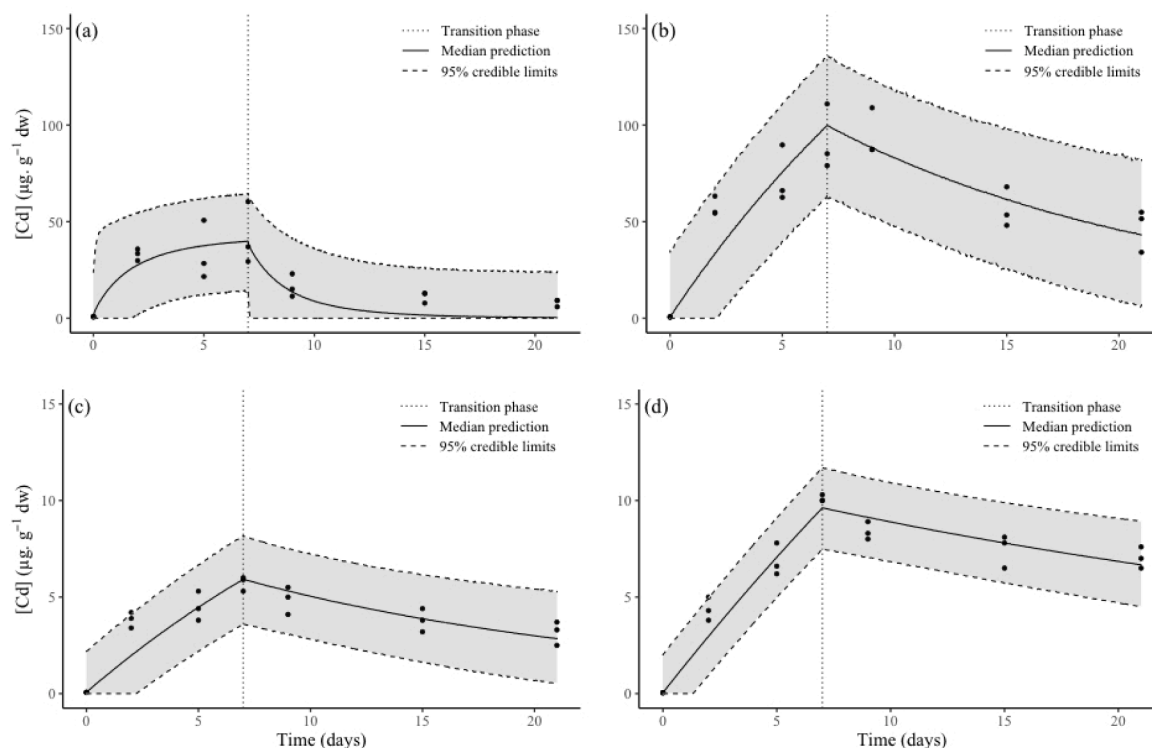


Fig. 2. Measured (dots) and predicted (solid and dashed) Cd concentrations with the one-compartment model (Eqs. (2) and (3)) for a) intestines; b) caeca; c) cephalons and d) remaining tissues of gammarids exposed to $11.1 \pm 1.2 \mu\text{g.L}^{-1}$, during the uptake (days 0–7) and depuration (days 7–21) phases (separated with the black dotted vertical line). Please note that the y-scale differs between the plots a-b and the plots c-d.

experiments.

Cadmium. Before exposure (i.e. day 0), the highest Cd concentrations were found in intestines ($0.806 \pm 0.160 \mu\text{g.g}^{-1}$) and caeca ($0.648 \pm 0.093 \mu\text{g.g}^{-1}$), then in cephalons ($0.067 \pm 0.006 \mu\text{g.g}^{-1}$) and the lowest concentrations were found in remaining tissues ($0.048 \pm 0.006 \mu\text{g.g}^{-1}$). During the exposure phase, the concentrations increased in all organs to reach maximal values at day 7 for intestines ($42.3 \pm 16.2 \mu\text{g.g}^{-1}$), caeca ($91.9 \pm 17.2 \mu\text{g.g}^{-1}$), cephalons ($5.7 \pm 0.4 \mu\text{g.g}^{-1}$) and remaining tissues ($10.1 \pm 0.2 \mu\text{g.g}^{-1}$) (Fig. 2 and Table S3). Then, during the 15 days of the depuration phase, the concentrations decreased by 83% in intestines, 51% in caeca, 44% in cephalons and 31% in remaining tissues (Fig. 2 and Table S3). Interestingly, the accumulation in the intestines reached a plateau on the second day of exposure and the elimination was extremely rapid, with 75% of the loss occurring during the first two days of the depuration phase.

Our results are consistent with those previously reported by Amyot et al. (1996) showing that caeca and intestines have the highest Cd concentrations in *Gammarus fasciatus* from Lake St Louis, Canada, suggesting that concentration were metal and tissue dependent. In addition, Pellet et al. (2009) have shown a similar accumulation and depuration of Cd in whole *Gammarus pulex* when compared with *Asellus aquaticus*. Regarding *A. aquaticus* organs, Cd concentrations range from hepatopancreas > intestines >> cephalons ≈ remaining tissues when they were sampled in highly polluted rivers (van Hattum et al., 1996). Notably, these values are of the same order of proportion as those recorded in our experiments (Fig. 2).

Bioaccumulation quantification in freshwater invertebrates' tissues is analytically more restrictive due to the very low weight of the samples when compared to fishes' tissues (Ju et al., 2011; Rocha et al., 2015). Consequently, a very limited number of studies have investigated Cd accumulation and depuration in the organs of freshwater amphipods, and in particular in gammarids which is considered a model organism for biomonitoring. For these reasons, modern studies on metal bioaccumulation quantification in freshwater invertebrates' tissues are using more advanced techniques such as radioisotopic metals. High sensitivity and traceability allow to measure very low levels present in small organisms or at environmental concentrations. Following exposure to dissolved ^{109}Cd autoradiography in the prawn *Macrobrachium australiense* revealed a high accumulation of the metal in hepatopancreas (Cresswell et al., 2017), an organ analog of gammarids' caeca.

Mercury. At day 0, the highest Hg concentrations were found in the intestines ($0.095 \pm 0.006 \mu\text{g.g}^{-1}$), then in the caeca ($0.032 \pm 0.004 \mu\text{g.g}^{-1}$), the remaining tissues ($0.016 \pm 0.001 \mu\text{g.g}^{-1}$) and the cephalons ($0.014 \pm 0.001 \mu\text{g.g}^{-1}$). At the end of the exposure phase, Hg concentrations increased in all organs, values in descending order were: intestines ($2.14 \pm 0.45 \mu\text{g.g}^{-1}$) ≈ caeca ($2.05 \pm 0.60 \mu\text{g.g}^{-1}$) >> remaining tissues ($0.63 \pm 0.09 \mu\text{g.g}^{-1}$) ≈ cephalons ($0.58 \pm 0.09 \mu\text{g.g}^{-1}$) (Fig. S4 and Table S4). After the 20 days depuration phase, the concentrations decreased by 82% in the intestines, 79% in the caeca, 24% in the cephalons and 42% in the remaining tissues (Fig. S4). Interestingly, depuration mostly occurred during the first 3 days and Hg concentrations in the cephalon remained constant thereafter suggesting a very slow process of depuration in this organ (Fig. S4 and Table S4).

It is well known that marine fishes can accumulate dissolved metals in their intestines by drinking to regulate their osmotic balance and avoid dehydration (Wood et al., 1999; Zhang and Wang, 2007). However, information on bioaccumulation of Hg in freshwater gammarid organs is lacking. Most of the studies to this date have focused on bivalves, shrimps, crabs and fishes (Boening, 2000) and revealed high Hg accumulation in the intestines of mollusk and fish species (Pelletier and Maheu, 1996; Peng et al., 2016; Wang and Wang, 2015). In view of our data, intestines can represent an initial site for metal accumulation in vivo and then serve as a secondary source allowing distribution of metals to other organs. In fact, this has been shown to be the case for Hg accumulation in the marine fish *Siganus canaliculatus* (Peng et al., 2016).

Aside from the intestines, caeca is well known for its capacity to

accumulate metals (Amyot et al., 1996) and represent the main site for storage, regulation and metallic detoxification in crustaceans (Correia et al., 2002).

The low Hg depuration efficiency in cephalons can be explained by previous studies showing that Hg in its methylmercury (MeHg) form depurates slower (~2.8 times) than in inorganic species (Kidd et al., 2012) and is highly concentrated in the ventral nerve cord of grass shrimp (Boening, 2000). Dutton and Fischer (Dutton and Fisher, 2011) found that MeHg easily cross the intestinal barrier in killifish, and showed that a decrease of Hg(II) and MeHg in the intestines coincides with an increase in the heads of killifishes. In the present study, i) the total Hg concentrations in gammarid cephalons confirmed the Hg tropism for nervous tissues while ii) the low elimination rate raises the possibility that Hg accumulates as MeHg form, suggesting an in vivo methylation of inorganic Hg in gammarid (Jereb et al., 2003). Further experiments are needed to confirm this hypothesis.

3.3. Modeling of Cd and Hg accumulation and fate predictions in *G. Fossarum*'s organs

For both metals, two types of toxicokinetic models were fitted to the experimental data of both accumulation and elimination: a one-compartment model (Eqs. (2) and (3)) on each organ dataset separately (Fig. 2 and Tables S3 and S4); and a multi-compartment model (Eqs. (4) and (5)) on all organ datasets simultaneously (Fig. 3 and Tables S3 and S4).

3.3.1. One-compartment model

For all fits, the inference process quickly converged, and thin posterior distributions were obtained for each kinetic parameter suggesting that data offer sufficient information to accurately estimate model parameters (Figure S3). Median values and 95% credible intervals for each parameter are summarized in Table 1 and the median model predictions associated to their 95% credible intervals, superimposed to observed data, are represented in Fig. 2 for each organ. For all organs, 95% credible intervals around model predictions encompassing between 96% and 100% of observed data. The estimates of accumulation rates ($k_{u,i}$ parameters, Table 1) were generally higher for Hg than for Cd. In accordance with differences of accumulation efficiency among organs discussed above, k_u of intestines and caeca were respectively i) 21 and 17 times higher compared to cephalons and 14 and 12 compared to remaining tissues, for Cd; and ii) 6.7 and 4.2 times higher compared to cephalons and 4.2 and 2.7 compared to remaining tissues, for Hg.

Regarding the elimination rates ($k_{e,i}$ parameters), values (Table 1) were in the same order of magnitude between both metals for all organs, except for cephalons that showed a very low value of k_e with Hg. As for k_u , we can observe differences between organs, with intestines and then caeca showing the highest k_e estimates: i) with Cd, the k_e value of intestines was 8.4, 9.5 and 19 times higher when compared to the ones of caeca, cephalons and the remaining tissues; and ii) for Hg, intestines and caeca showed similar k_e values but respectively 68 and 54 times higher when compared to cephalons and 5.5 and 4.4 times higher when compared to the remaining tissues.

We must notice here that k_u and k_e parameters determined for each organ (considered as a single homogeneous compartment) showed a high correlation (Table S5) but it is intrinsic to the writing of the model (Eqs. (2) and (3)). Such structural correlation justifies the use of Bayesian inference to simultaneously estimate them from accumulation and depuration data, taking into account their correlation in their estimation (Ratier et al., 2019).

The last parameters σ_i (Table 1) correspond to the uncertainty around concentrations measured for each organ (Eq. (6)) and thus reflect the variability of the measured data. Indeed, the less variable the data, the more precise are the predictions and therefore the lower is the uncertainty around these predictions. Intestines and caeca showed the highest σ values, suggesting that uncertainty around model predictions

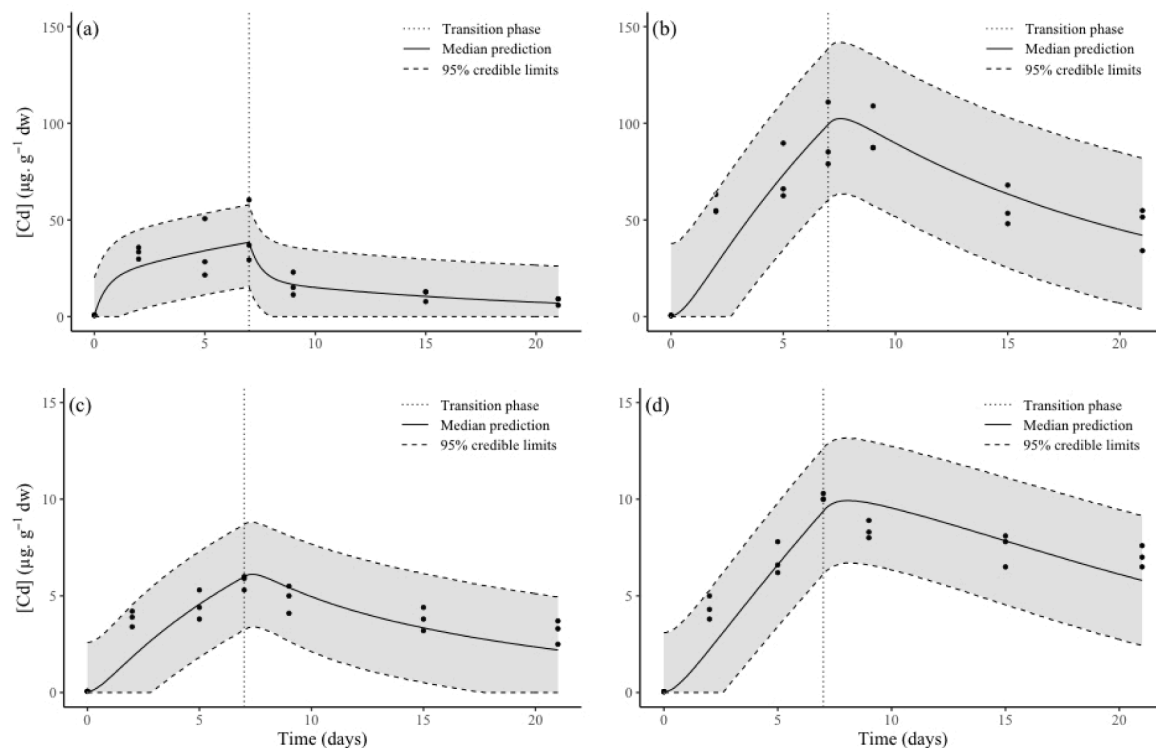


Fig. 3. Measured (dots) and predicted (solid and dashed curves) Cd concentrations with the multi-compartments model (Eqs. (4) and (5)) in a) intestines b) caeca c) cephalons and d) remaining tissues of gammarids exposed to $11.1 \pm 1.2 \mu\text{g} \cdot \text{L}^{-1}$, during the uptake (days 0–7) and depuration (days 7–21) phases (separated with the black dotted vertical line). Please note that the y-scale differs between the plots a-b and the plots c-d.

for both of these organs are higher than the ones for cephalons and the remaining tissues. This is confirmed with the 95% credible band around model predictions showed in Fig. 2, the width of this band is directly linked to the corresponding σ value. The high variability observed with the intestines and the caeca data can reflect measurements on very low average weights (Eq. (1)), since the intestines and the caeca represent only 2.2% and 5% of an entire gammarid weight.

To our knowledge, there are few studies estimating the toxicokinetic parameters at the organ level in freshwater or marine invertebrates, and most studies have been performed on bivalves (Ju et al., 2011; Rocha et al., 2015). Ju et al. (2011) studied the bioaccumulation of Cd in the intestines of the trout *Oncorhynchus mykiss* exposed to 0.6, 2.2 and $30.3 \mu\text{g} \cdot \text{Cd} \cdot \text{L}^{-1}$. They estimated k_u to 0.018, 0.028 and 0.013 d^{-1} and k_e to 0.124, 0.185 and 0.0747 d^{-1} , respectively. In the present study, values of k_u for *G. fossarum* are 70,000 to 150,000 times higher than *O. mykiss*, whereas k_e value were only 2.7 to 6.7 times higher (Table 1). In the same study (Ju et al., 2011), the bioaccumulation of Cd in i) the liver of the trout *O. mykiss* exposed to 5, 25 and $50 \mu\text{g} \cdot \text{Cd} \cdot \text{L}^{-1}$, demonstrated a k_u of 1.18, 0.72 and 1.01 d^{-1} and k_e of $1\text{e-}05$, $6\text{e-}06$ and $1\text{e-}06 \text{ d}^{-1}$, respectively; and ii) the digestive gland of the clam *Ruditapes decussatus* exposed to 4 and $40 \mu\text{g} \cdot \text{Cd} \cdot \text{L}^{-1}$, had an estimated k_u of 175 and 122 d^{-1} and k_e of $7\text{e-}07$ and $9\text{e-}07 \text{ d}^{-1}$, respectively while k_u was 20.9 and 16.2 d^{-1} respectively for the remaining tissues and k_e of 0.003 d^{-1} for both exposure.

Rocha et al. (Rocha et al., 2015), studied the bioaccumulation of Cd in the digestive gland and remaining tissues of the mussel *Mytilus galloprovincialis* exposed to $10 \mu\text{g} \cdot \text{Cd} \cdot \text{L}^{-1}$ for 21 days. They estimated k_u to 1390 d^{-1} and 120 d^{-1} , respectively but no k_e could be determined for both organs.

For the caeca of *G. fossarum*, the value of k_u was similar to the digestive gland of *M. galloprovincialis*, but 10 times higher than *R. decussatus* and 1000 times higher than the liver of *O. mykiss*, while the values of k_e was 67,000 to 86,000 times higher than clams and 1000 times higher than trouts. For the remaining tissues, k_u value was similar

to *M. galloprovincialis* but was 6–8 times higher than *R. decussatus* and the values of k_e was 8.6 times higher than clams.

Based on comparisons between bivalves and trout, data confirm that *G. fossarum* is as high of a Cd accumulator as the mussel *M. galloprovincialis*, but also an efficient depurator species.

These results are based on the fit of a one-compartment model to each organ separately, as if each organ bioaccumulates metal independently from one another. Going back to the model predictions superimposed to observed data (Fig. 2), and particularly the fit concerning the intestines (Fig. 2a), it is noteworthy that the model under-estimates observed Cd data between days 15 and 21. Indeed, the median prediction is at 0 from day 15 whereas data observed, while they are in the upper half of the 95% credible intervals of model predictions, seem to be at a plateau of $11 \mu\text{g} \cdot \text{g}^{-1}$ from day 15 to day 21. This suggests that the intestines eliminate Cd very rapidly, but their concentrations remained at a minimal threshold presumably because of a constant flow from the other tissues. This observation highlights the fact that the one-compartment model undermines more complex metal transfer among different organs.

The use of one-compartment models in adults' organs has limits in the quantification and comprehension of ADME processes (Gao et al., 2019). As a result, it will be better to use a multi-compartment model (Hare et al., 2003) because more parameters can be considered in analyses and interpretations (Adams et al., 2010). In view of the role of the intestines, underlined with the data and the model fit, we can assume that i) the intestines play a very important role in accumulation and depuration, even for dissolved pathway; and ii) the existence of a strong link of exchanges between the different organs, mostly between the intestines and the other organs making the use of multi-compartments models relevant.

3.3.2. Multi-compartments model

Because metals' distribution is not homogeneous among the different organs, multi-compartments models are used to make assumptions

about the likely related organs and their exchanges rates (Grech et al., 2017).

The best multi-compartments model selected, based notably on fits, thin posterior distributions for all parameters, simultaneously estimated and the smallest DIC value is the one represented by solid arrows in Fig. 1. Notably, the same model was retained at the end for Cd and Hg. The estimates of kinetic parameters are presented in Table S6, with respective median and their 95% credible intervals. The multi-compartment model for Cd in the intestines between days 15 and 21 allows a better fit of the model to the data (Fig. 3), than with one compartment model (Fig. 2), without reducing the quality of fit for the other organs. The multi-compartments model that best fits the data for Cd shows that i) accumulation of dissolved metals from water and depuration would occur through the intestines, with a $k_{u,1}$ two times higher for the intestines in the multi-compartments model than those of one compartment model but with the same $k_{e,1}$ for both models; and ii) other organs of the gammarids would all accumulate from and all depurate into the intestines (Table S6), with a strong accumulation rates from the intestines to caeca. All these conclusions are consistent with the role of each organs and their physiology in gammarids (Figure S2).

Even if the selected multi-compartments model fit well the data for both metals (Fig. 3 for Cd and S5 for Hg) with 98.8% and 91.7% of observed data for Cd and for Hg, respectively, included in the 95% credible intervals of the model predictions, we found a better fit to observed data when using the one compartment model for Hg. These results confirm that for Hg, the four organs have a better fit when they are modeled individually using a one compartment model, than when using a multi-compartments model connecting all the organs together. This suggests that gammarids manage Cd and Hg differently. Hg contamination, accumulation and depuration rates of each organ seem to be independent of each other. Altogether, our results underline the fact that the intestines play a key role in the accumulation process of Hg, while the cephalon may be more important as an organ storage for Hg, as previously proposed in the literature (Dutton and Fisher, 2011; Jereb et al., 2003).

4. Conclusions

By using high concentrations of dissolved metals (regarding *in situ* contamination levels), we were able to quantify their concentrations in the different organs of *G. fossarum* and determine which organs accumulate and depurate the most. Our results demonstrate that the caeca and the intestines are the most important for both metals. Consequently, the TK model that is most relevant for Hg is the mono-compartment one, while the multi-compartments one allows a better fit to the Cd data. The fact that the one-compartment TK models are the ones which fitted the best Hg data suggests that the organs act independently from one another with regard to bioaccumulation of this metal. The best multi-compartment TK model for Cd demonstrates a central role of intestines. This work was set as a proof-of-concept aiming to demonstrate that we can quantify and model the fate of different trace elements (i.e. Cd and Hg) among organs of a small sentinel invertebrate. This study provided a general framework to fit TK multi-compartments models and to select the optimal one that describe the best all organs' data simultaneously, and thus help in understanding the role of each organ. Our study further shows that the models' outputs are metal-specific, confirming contrasting organotropism, fate and regulation of metals in a freshwater invertebrate species. Such results are particularly relevant to identify key organ on which biomarkers of contamination effects would be developed to link exposure to effect (TK-TD models) but would request next steps of improvement by i) integrating gills apart from remaining tissues in the TK models, considering their direct contact with contaminated environment and their role in the ADME processes and fate of metals (McDonald et al., 2020); ii) setting up experiments at environmentally relevant concentrations of the order of ng.L^{-1} , by using more sensitive techniques such as radioisotopic metals (Cresswell et al.,

2017; Lanctôt et al., 2017); and/or iii) extending this method to other metals such as zinc, allowing a comparison between known contrasting physiologically-based management of trace elements.

CRedit authorship contribution statement

Ophélie Gestin: Conceptualization, Methodology, Formal analysis, Writing - original draft. **Thomas Lacoue-Labarthe:** Supervision, Conceptualization, Methodology, Writing - review & editing. **Marina Coquery:** Methodology. **Nicolas Delorme:** Methodology. **Laura Garnero:** Methodology. **Lysiane Dherret:** Methodology. **Théo Ciccica:** Methodology. **Olivier Geffard:** Supervision, Conceptualization, Methodology, Writing - review & editing. **Christelle Lopes:** Supervision, Conceptualization, Formal analysis, Methodology, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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References

- Adams, W.J., Blust, R., Borgmann, U., Brix, K.V., DeForest, D.K., Green, A.S., Meyer, J.S., McGeer, J.C., Paquin, P.R., Rainbow, P.S., Wood, C.M., 2010. Utility of tissue residues for predicting effects of metals on aquatic organisms. *Integr. Environ. Assess. Manag.* 7, 75–98. <https://doi.org/10.1002/ieam.108>.
- AFNOR, 2016. Qualité de l'eau - Application de la spectrométrie de masse avec plasma à couplage inductif (ICP-MS) - Partie 2: dosage des éléments sélectionnés y compris les isotopes d'uranium. NF EN ISO. AFNOR, Paris, pp. 17294–17302.
- Amyot, M., Pinel-Aloul, B., Campbell, P.G.C., Déry, J.C., 1996. Total metal burdens in the freshwater amphipod *Gammarus fasciatus*: contribution of various body parts and influence of gut contents. *Freshw. Biol.* 35, 363–373. <https://doi.org/10.1046/j.1365-2427.1996.00493.x>.
- Baudrot, V., Veber, P., Gence, G., Charles, S., 2018. Fit reduced GUTS models online: from theory to practice. *Integr. Environ. Assess. Manag.* 14, 625–630. <https://doi.org/10.1002/ieam.4061>.
- Bertrand, L., Monferrán, M.V., Valdés, M.E., Amé, M.V., 2019. Usefulness of a freshwater macrophyte (*Potamogeton pectinatus*) for an environmental risk assessment in a multi-source contaminated basin. *Chemosphere* 222, 1003–1016. <https://doi.org/10.1016/j.chemosphere.2019.02.018>.
- 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>.
- Boening, D.W., 2000. Ecological effects, transport, and fate of mercury: a general review. *Chemosphere* 40, 1335–1351. [https://doi.org/10.1016/S0045-6535\(99\)00283-0](https://doi.org/10.1016/S0045-6535(99)00283-0).
- Chaumot, A., Geffard, O., Armengaud, J., Maltby, L., 2015. Gammarids as Reference Species for Freshwater Monitoring. *Aquatic Ecotoxicology: Advancing Tools for Dealing with Emerging Risks*. Elsevier Inc. <https://doi.org/10.1016/B978-0-12-800949-9.00011-5>.
- Chen, W.Y., 2016. Toxicokinetic modeling challenges for aquatic nanotoxicology. *Front. Mar. Sci.* 2, 1–5. <https://doi.org/10.3389/fmars.2015.00114>.

- Correia, A.D., Costa, M.H., Ryan, K.P., Nott, J.A., 2002. Studies on biomarkers of copper exposure and toxicity in the marine amphipod *Gammarus locusta* (Crustacea): I. Copper-containing granules within the midgut gland. *J. Mar. Biol. Assoc. United Kingdom* 82, 827–834. <https://doi.org/10.1017/S0025315402006197>.
- Cresswell, T., Mazumder, D., Callaghan, P.D., Nguyen, A., Corry, M., Simpson, S.L., 2017. Metal transfer among organs following short- and long-term exposures using autoradiography: cadmium bioaccumulation by the freshwater prawn *Macrobrachium australiense*. *Environ. Sci. Technol.* 51, 4054–4060. <https://doi.org/10.1021/acs.est.6b06471>.
- Dutton, J., Fisher, N.S., 2011. Bioaccumulation of As, Cd, Cr, Hg(II), and MeHg in killifish (*Fundulus heteroclitus*) from amphipod and worm prey. *Sci. Total Environ.* 409, 3438–3447. <https://doi.org/10.1016/j.scitotenv.2011.05.022>.
- European Chemicals Agency (ECHA), 2008. Guidance on Information Requirements and Chemical Safety Assessment - Appendix R.7.13-2: Environmental risk assessment for metals and metal compounds, European Chemicals Agency.
- Gao, Y., Zhang, Y., Feng, J., Zhu, L., 2019. Toxicokinetic–toxicodynamic modeling of cadmium and lead toxicity to larvae and adult zebrafish. *Environ. Pollut.* 251, 221–229. <https://doi.org/10.1016/j.envpol.2019.05.003>.
- Gelman, A., Carlin, J.B., Stern, H.S., Rubin, D.B., 1995. Bayesian Data Analysis, first ed. Chapman and Hall/CRC. <https://doi.org/10.1201/9780429258411>.
- Grech, A., Brochot, C., Dorne, J., Lou, Quignot, N., Bois, F.Y., Beaudouin, R., 2017. Toxicokinetic models and related tools in environmental risk assessment of chemicals. *Sci. Total Environ.* 578, 1–15. <https://doi.org/10.1016/j.scitotenv.2016.10.146>.
- Grech, A., Tebby, C., Brochot, C., Bois, F.Y., Bado-Nilles, A., Dorne, J.L., Quignot, N., Beaudouin, R., 2019. Generic physiologically-based toxicokinetic modelling for fish: Integration of environmental factors and species variability. *Sci. Total Environ.* 651, 516–531. <https://doi.org/10.1016/j.scitotenv.2018.09.163>.
- Hare, L., Tessier, A., Borgmann, U., 2003. Metal sources for freshwater invertebrates: pertinence for risk assessment. *Hum. Ecol. Risk Assess.* 9, 779–793. <https://doi.org/10.1080/713610009>.
- Huang, S.S.Y., Strathe, A.B., Hung, S.S.O., Boston, R.C., Fadel, J.G., 2012. Selenocompounds in juvenile white sturgeon: Estimating absorption, disposition, and elimination of selenium using Bayesian hierarchical modeling. *Aquat. Toxicol.* 109, 150–157. <https://doi.org/10.1016/j.aquatox.2011.11.005>.
- Jager, T., 2020. Revisiting simplified DEBtox models for analysing ecotoxicity data. *Ecol. Modell.* 416, 108904. <https://doi.org/10.1016/j.ecolmodel.2019.108904>.
- Jager, T., Ashauer, R., 2018. Modelling survival under chemical stress to the GUTS framework.
- Jereb, V., Horvat, M., Drobne, D., Pihlar, B., 2003. Transformations of mercury in the terrestrial isopod *Porcellio scaber* (Crustacea). *Sci. Total Environ.* 304, 269–284. [https://doi.org/10.1016/S0048-9697\(02\)00574-0](https://doi.org/10.1016/S0048-9697(02)00574-0).
- Ju, Y.-R., Chen, W.-Y., Singh, S., Liao, C.-M., 2011. Trade-offs between elimination and detoxification in rainbow trout and common bivalve molluscs exposed to metal stressors. *Chemosphere* 85, 1048–1056. <https://doi.org/10.1016/j.chemosphere.2011.07.033>.
- Kidd, K., Clayden, M., Jardine, T., 2012. Bioaccumulation and biomagnification of mercury through food webs. In: Liu, G., Cai, Y., O'Driscoll, N. (Eds.), *Environmental Chemistry and Toxicology of Mercury*. John Wiley & Sons, pp. 453–499. <https://doi.org/10.1002/9781118146644.ch14>.
- Kraak, M.H.S., Scholten, M.C.T., Peeters, W.H.M., Kock, W.C. De, 1991. Biomonitoring of heavy metals in the western European rivers rhine and meuse using the freshwater mussel *Dreissena polymorpha*. *Environ. Pollut.* 74, 101–114. [https://doi.org/10.1016/0269-7491\(91\)90107-8](https://doi.org/10.1016/0269-7491(91)90107-8).
- Kunz, P.Y., Kienle, C., Gerhardt, A., 2010. *Gammarus* spp. in aquatic ecotoxicology and water quality assessment: toward integrated multilevel tests. In: *Reviews of environmental contamination and toxicology*. Springer, New York. https://doi.org/10.1007/978-1-4419-5623-1_1.
- Lancôt, C.M., Cresswell, T., Melvin, S.D., 2017. Uptake and tissue distributions of cadmium, selenium and zinc in striped marsh frog tadpoles exposed during early post-embryonic development. *Ecotoxicol. Environ. Saf.* 144, 291–299. <https://doi.org/10.1016/j.ecoenv.2017.06.047>.
- McCarty, L.S., Mackay, D., 1993. Enhancing ecotoxicological modeling and assessment. *Environ. Sci. Technol.* 27, 1718–1728. <https://doi.org/10.1021/es00046a001>.
- McDonald, S., Cresswell, T., Hassell, K., 2020. Bioaccumulation kinetics of cadmium and zinc in the freshwater decapod crustacean *Paratya australiensis* following multiple pulse exposures. *Sci. Total Environ.* 720, 137609. <https://doi.org/10.1016/j.scitotenv.2020.137609>.
- O'Callaghan, L., Harrison, S., Fitzpatrick, D., Sullivan, T., 2019. The freshwater isopod *Asellus aquaticus* as a model biomonitor of environmental pollution: a review. *Chemosphere* 235, 498–509. <https://doi.org/10.1016/j.chemosphere.2019.06.217>.
- Pellet, B., Geffard, O., Lacour, C., Kerma, 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. <https://doi.org/10.1897/09-042.1>.
- Pelletier, E., Maheu, S., 1996. Cinétique d'accumulation et rétention d'espèces du mercure chez l'étoile de mer *Leptasterias polaris* : une expérience de transfert trophique à long terme. *Rev. des Sci. l'eau / J. Water Sci.* 9, 351–364. <https://doi.org/10.7202/705257ar>.
- Peng, X., Liu, F., Wang, W.X., 2016. Organ-specific accumulation, transportation, and elimination of methylmercury and inorganic mercury in a low Hg accumulating fish. *Environ. Toxicol. Chem.* 35, 2074–2083. <https://doi.org/10.1002/etc.3363>.
- Pery, A., 2008. Évaluation du risque écotoxicologique dans les milieux aquatiques à partir d'expérimentations de laboratoire. Perspectives offertes par la modélisation. *Tech. Sci. Méthodes* 37–49. <https://doi.org/10.1051/tsm/200809037>.
- Plummer, M., 2003. JAGS: a program for analysis of Bayesian graphical models using Gibbs sampling JAGS: just another Gibbs sampler. In: *3rd International Workshop on Distributed Statistical Computing*. Vienne, Austria, p. 8.
- R Core Team, 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienne, Austria.
- Raftery, A.E., Lewis, S.M., 1992. Comment: One long run with diagnostics: implementation strategies for Markov Chain Monte Carlo. *Stat. Sci.* 7, 493–497. <https://doi.org/10.1214/ss/1177011143>.
- Ratier, A., Lopes, C., Labadie, P., Budzinski, H., Delorme, N., Quéau, H., Peluhet, L., Geffard, O., Babut, M., 2019. A Bayesian framework for estimating parameters of a generic toxicokinetic model for the bioaccumulation of organic chemicals by benthic invertebrates: proof of concept with PCB153 and two freshwater species. *Ecotoxicol. Environ. Saf.* 180, 33–42. <https://doi.org/10.1016/j.ecoenv.2019.04.080>.
- Rocha, T.L., Gomes, T., Pinheiro, J.P., Sousa, V.S., Nunes, L.M., Teixeira, M.R., Bebianno, M.J., 2015. Toxicokinetics and tissue distribution of cadmium-based Quantum Dots in the marine mussel *Mytilus galloprovincialis*. *Environ. Pollut.* 204, 207–214. <https://doi.org/10.1016/j.envpol.2015.05.008>.
- Saleem, M., Iqbal, J., Akhter, G., Shah, M.H., 2018. Fractionation, bioavailability, contamination and environmental risk of heavy metals in the sediments from a freshwater reservoir, Pakistan. *J. Geochemical Explor.* 184, 199–208. <https://doi.org/10.1016/j.gexplo.2017.11.002>.
- Spiegelhalter, D.J., Best, N.G., Carlin, B.P., Van Der Linde, A., 2002. Bayesian measures of model complexity and fit. *J. R. Stat. Soc. Ser. B Stat. Methodol.* 64, 583–639. <https://doi.org/10.1111/1467-9868.00353>.
- Stadnicka, J., Schirmer, K., Ashauer, R., 2012. Predicting concentrations of organic chemicals in fish by using toxicokinetic models. *Environ. Sci. Technol.* 46, 3273–3280. <https://doi.org/10.1021/es2043728>.
- Suter II, G.W., 2016. *Ecological Risk Assessment*, second ed. CRC Press.
- van Hattum, B., de Voogt, P., van den Bosch, L., van Straalen, N.M., Joosse, E.N.G., Govers, H., 1989. Bioaccumulation of cadmium by the freshwater isopod *Asellus aquaticus* (L.) from aqueous and dietary sources. *Environ. Pollut.* 62, 129–151. [https://doi.org/10.1016/0269-7491\(89\)90183-8](https://doi.org/10.1016/0269-7491(89)90183-8).
- van Hattum, B., van Straalen, N.M., Govers, H.A.J., 1996. Trace metals in populations of freshwater isopods: Influence of biotic and abiotic variables. *Arch. Environ. Contam. Toxicol.* 31, 303–318. <https://doi.org/10.1007/BF00212669>.
- Vellinger, C., Parant, M., Rousselle, P., Usseglio-Polatera, P., 2012. Antagonistic toxicity of arsenate and cadmium in a freshwater amphipod (*Gammarus pulex*). *Ecotoxicology* 21, 1817–1827. <https://doi.org/10.1007/s10646-012-0916-1>.
- Wang, X., Wang, W.X., 2015. Physiologically based pharmacokinetic model for inorganic and methylmercury in a marine fish. *Environ. Sci. Technol.* 49, 10173–10181. <https://doi.org/10.1021/acs.est.5b02301>.
- Wood, C.M., Playle, R.C., Hogstrand, C., 1999. Physiology and modeling of mechanisms of silver uptake and toxicity in fish. *Environ. Toxicol. Chem.* 18, 71–83. <https://doi.org/10.1002/etc.5620180110>.
- Zhang, L., Wang, W.X., 2007. Waterborne cadmium and zinc uptake in a euryhaline teleost *Acanthopagrus schlegelii* acclimated to different salinities. *Aquat. Toxicol.* 84, 173–181. <https://doi.org/10.1016/j.aquatox.2007.03.027>.
- Zhou, Q., Zhang, J., Fu, J., Shi, J., Jiang, G., 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. *Anal. Chim. Acta* 606, 135–150. <https://doi.org/10.1016/j.aca.2007.11.018>.