# Organ-specific accumulation of cadmium and zinc in *Gammarus fossarum* exposed to environmentally relevant metal concentrations

Ophélia Gestin[a,b,c] (ophelia.gestin@etu.univ-lyon1.fr), Christelle Lopes[a] (christelle.lopes@univ-lyon1.fr), Nicolas Delorme[c] (nicolas.delorme@inrae.fr), Laura Garnero[c] (laura.garnero@inrae.fr), Olivier Geffard[c] (olivier.geffard@inrae.fr) and Thomas Lacoue-Labarthe[b,*] (tlacouel@univ-lr.fr)

[a] Univ Lyon, *Université Lyon 1, CNRS, Laboratoire de Biométrie et Biologie Evolutive UMR 5558, 69622 Villeurbanne, France*

[b] *Littoral Environnement et Sociétés (LIENSs), UMR 7266 CNRS - Université de la Rochelle, 2 rue Olympe de Gouges, 17000 La Rochelle, France*

[c] *INRAE, RiverLy, Ecotoxicology Laboratory, 5 Avenue de la Doua, CS20244, 69625 Villeurbanne Cedex, France*

[*] Corresponding author: Thomas Lacoue-Labarthe – Littoral Environnement et Sociétés (LIENSs) UMR7266, CNRS, 17 000 La Rochelle, France; Phone: +33 (0)5 46 45 83 88; Email: tlacouel@univ-lr.fr

**Abstract**

One of the best approaches for improving the assessment of metal toxicity in aquatic organisms is to study their organotropism (i.e., the distribution of metals among organs) through a dynamical approach (i.e., *via* kinetic experiments of metal bioaccumulation), to identify the tissues/organs that play a key role in metal regulation (e.g., storage or excretion). This study aims at comparing the organ-specific metal accumulation of a non-essential (Cd) and an essential metal (Zn), at their environmentally relevant exposure concentrations, in the gammarid *Gammarus fossarum*. Gammarids were exposed for 7 days to $^{109}$Cd- or $^{65}$Zn-radiolabeled water at a concentration of 52.1 and 416 ng.L$^{-1}$ (stable equivalent), respectively, and then placed in clean water for 21 days. At different time intervals, the target organs (i.e., caeca, cephalons, intestines, gills, and remaining tissues) were collected and $^{109}$Cd or $^{65}$Zn contents were quantified by gamma-spectrometry. A one-compartment toxicokinetic (TK) model was fitted by Bayesian inference to each organ/metal dataset in order to establish TK parameters. Our results indicate: i) a contrasting distribution pattern of concentrations at the end of the accumulation phase (7$^{th}$ day): gills > caeca ≈ intestines > cephalons > remaining tissues for Cd and intestines > caeca > gills > cephalons > remaining tissues for Zn; ii) a slower elimination of Cd than of Zn by all organs, especially in the gills in which the Cd concentration remained constant during the 21-day depuration phase, whereas Zn concentrations decreased sharply in all organs after 24 h in the depuration phase; iii) a major role of intestines in the uptake of waterborne Cd and Zn at environmentally relevant concentrations.

**Keywords:** Amphipods, Metal, Uptake rate, Elimination rate, Toxicokinetic model, Bayesian Inference

## 1. Introduction

Naturally present in the earth's crust and potentially released through erosion and leaching, metals are persistent elements due to their non-degradability (Cresswell et al., 2017; Lebrun et al., 2017). They can also be introduced into aquatic ecosystems by anthropogenic activities, such as emissions and runoff from the industrial, urban, and agricultural sectors (Filipović Marijić et al., 2016; Lebrun et al., 2014; Soegianto et al., 2013; Zhang and Reynolds, 2019). Since both essential and non-essential elements can become toxic even at low concentrations following anthropogenic contamination, there is a need for comprehensive research into their bioaccumulation processes and their effects (Lebrun et al., 2017; Ramiro Pastorinho et al., 2009). The deleterious effects resulting from an acute or a chronic exposure to metals are well described in crustaceans. At the molecular and cellular levels, Zn can trigger structural, histological, and immunocytochemical damage with, for example, a deterioration of the cytoskeleton or a large augmentation of their vacuoles (Issartel et al., 2010a; Soegianto et al., 2013). Deleterious effects of Cd on DNA integrity have been reported in several decapods (Frías-Espericueta et al., 2022). Histological analyses have shown an increased cell proliferation in the gills of gammarids, shrimps, and crabs following Cd exposure (Dayras et al., 2017). Moreover, Zn and Cd may, among other things, cause osmoregulation disorders, the induction of reactive oxygen species (ROS), and a decrease in ionoregulation (Frías-Espericueta et al., 2022; Ren et al., 2019). It has been reported that Cd and Zn can also have an impact on oxygen consumption and ammonium excretion (Frías-Espericueta et al., 2022; Jakob et al., 2017). At the organism level, the presence of metals will lead to a decrease in genetic diversity, organism size, and reproduction with, for example, a shorter life span, lower fecundity, and behavioral changes. All these changes can affect the population levels, which in the long-term may decrease overall species survival and richness (Júdová, 2006; Kadiene et al., 2019).

Cadmium and Zn, whose environmental concentrations are generally estimated in the literature to be less than 1 $\mu g.L^{-1}$ and 50 $\mu g.L^{-1}$, respectively (McDonald et al., 2020), are both qualified as being "directly ecotoxicologically important" and are on the list of priority elements of the European Water Framework Directive (EC, 2000). To overcome the analytical difficulties of detecting metal levels in water, organisms (named "sentinel species" or "bioindicators") are proposed as a targeted matrix for contamination surveys (Besse et al., 2012). Such species are known to be net accumulators of metals present in their environment (including food), and are used to evaluate the fraction of bioavailable metals (Besse et al., 2012). Biomonitoring of metal contamination using freshwater invertebrates is frequently done by measuring the amounts or concentrations of metals in the whole-body sentinel organism (Besse et al., 2013) because they are more temporally and spatially integrative than water or sediment samples. Finally, whether they are essential metals (and thus potentially actively taken up and/or regulated to meet metabolic needs; Rainbow, 2002) or not may influence their rate of uptake and elimination in organisms, as well as their distribution among different organs (Cresswell et al., 2015). Therefore, to gain a better understanding of the processes governing the mechanism of bioaccumulation, it is essential to work at the organ level. Indeed, studying the behavior and the role of organs in the bioaccumulation mechanisms of metals can be used to determine which organs to focus on in order to develop biomarkers of the exposure and effects of metals on organisms.

In recent years, studies have been performed at the organ level for fish, and toxicokinetic (TK) models adapted from these studies have been developed (Grech et al., 2019, 2017). TK models describe how accumulated internal concentrations vary in time according to the external exposure concentration. These models have helped to identify target organs: i) to fill the existing gaps in knowledge of the mechanisms influencing bioconcentration in organs (Grech

et al., 2019); and ii) to better understand and describe the bioaccumulation processes, and for the future to better predict toxicity.

Despite their ecological importance, to date freshwater invertebrates have not received such attention in this area of research. This can be explained by the fact that although they have strong bioaccumulation capacities, the low organ weights of small invertebrates imply a low amount of metals, which presents a significant detection challenge for accurate quantification (O'Callaghan et al., 2019). The use of gamma-emitting isotopes such as $^{109}$Cd or $^{65}$Zn allows us not only to work at relevant environmental concentrations, but also to measure concentrations in the organs of small aquatic invertebrates such as crustaceans, as was done for the decapod *Paratya australiensis* (McDonald et al., 2020). There are very few data on other orders of crustaceans such as amphipods, despite their well-known ecological importance. The species *Gammarus fossarum* is of particular interest to freshwater ecosystems due to their function as a detritivore, giving them a central role in freshwater ecosystems, and in particular in aquatic food webs as an important link between detritus and fish (Filipović Marijić et al., 2016; Kunz et al., 2010). They also have a wide distribution, are present in abundance, and, because of their size and ease of identification, they are easy to sample and handle in the laboratory (Dayras et al., 2017; Issartel et al., 2010b; Lebrun et al., 2017). In addition, they are known to be net accumulators of metals, which explains why gammarids are regularly used to monitor aquatic contaminations (Besse et al., 2013; Conti et al., 2016; Lebrun et al., 2015).

It has been shown in crustaceans that essential (Zn) and non-essential (Cd) metals are distributed, managed, and detoxified through different pathways (Nunez-Nogueira et al., 2006). However, there are still many gaps in understanding of the mechanisms that govern the exchange and fate of metals among various organs. To our knowledge, our previous study (Gestin et al., 2021) was the only work to show a dynamic view of metal bioaccumulation along uptake and elimination time course in a freshwater invertebrate (gammarids), focusing on the

126 distribution, toxicokinetic, and fate of Cd among organs over time. This previous study
127 considered four organs: cephalons, caeca, intestines, and remaining tissues. However, it was
128 conducted at a high Cd concentration (i.e., 11 µg.$L^{-1}$) and had not isolated the gills, which are
129 known to be involved in respiration, osmoregulation, excretion, and pH regulation as well as
130 being considered the primary pathway in the accumulation of dissolved metals (Henry et al.,
131 2017; Nunez-Nogueira et al., 2006).
132 In this context, the aim of the present work was to investigate the organotropism (i.e., the
133 distribution of metals among organs) and accumulation and elimination rates, at organ level, of
134 a non-essential (Cd) and an essential metal (Zn) in the crustacean *G. fossarum* exposed to
135 environmentally relevant concentrations of these metals. We compared the organotropism,
136 toxicokinetic, and fate of a non-essential and an essential metal in the gills, caeca, intestines,
137 and cephalons of gammarids. Males of *G. fossarum* were exposed for 7 days (uptake phase) to
138 $^{109}$Cd- and $^{65}$Zn-radiolabeled water at a concentration of 52 ng.$L^{-1}$ and 416 ng.$L^{-1}$, respectively,
139 and were then placed in clean water for 21 days (depuration phase). At several sampling times,
140 the target organs (i.e., caeca, cephalons, intestines, gills, and remaining tissues) were recovered
141 and their Cd or Zn content quantified by gamma-spectrometry. A one-compartment TK model
142 was fitted by Bayesian inference to each organ/metal dataset to estimate the TK parameters.

## 2. Material and methods

### *2.1 Collection, maintenance and selection of organisms*

Adult male gammarids (*Gammarus fossarum*) between 20 and 30 mg wet weight were selected from a bygone watercress farm located in Saint-Maurice-de-Rémens (France). They were stored in plastic bottles containing ambient freshwater and transferred to the LIENSs in La Rochelle University. The organisms were acclimated for 7 days in Evian® water (see characteristics in Table S1), under constant aeration, at 12 ± 0.5°C and with a dark:light cycle of 8:16h. Alder leaves (*Alnus glutinosa*) were used to fed *ad libitum* the organisms.

### *2.2 Reagents and chemicals*

All the material used was decontaminated all along the experiments with HCl solution (Hydrochloric acid S.G. 32 %, certified AR for analysis; Fischer Scientific®) and a Decon® 90 solution, both diluted to 1/10 with MilliQ water (18.2 MΩ.cm$^{-1}$). The radiotracers $^{109}$Cd and $^{65}$Zn were both obtained in their chloride form (i.e., $CdCl_2$ and $ZnCl_2$), respectively 0.1 M and 0.5M HCl, from Eckert & Ziegler Isotope Products Inc., Valencia, USA. Both solutions are carrier-free, allowing to work with the smallest equivalent stable concentration as possible (coefficient ng/Bq = 0.182 for $^{109}$Cd and 27.96 for $^{65}$Zn). Both solutions were diluted to obtain intermediate solution named "D1 solutions" allowing spikes of 20 µl to reach 15 Bq.mL$^{-1}$ for $^{109}$Cd or $^{65}$Zn in the experimental polypropylene beakers during the exposure phase (corresponding to 3 and 420 ng.L$^{-1}$ equivalent stable, respectively). The final Cd exposure concentration was increased from 3 to 52.1 ng.L$^{-1}$ by adding stable cadmium ($CdCl_2$ 2.5$H_2O$, > 98 %; Merck®; stock solution at 85 mg.L$^{-1}$, 0.5 M HCl) to the $^{109}$Cd D1 solution. These final concentrations were chosen for their environmental relevance and based on concentrations found in low impacted freshwater media (i.e., <100 and <500 ng.L$^{-1}$ for Cd and Zn, respectively) (Cresswell et al., 2014b; Urien et al., 2016).

168 The 0.0065 % change of pH following D1 addition was considered to have negligible impact
169 on organisms.
170
171 *2.3 Uptake and depuration phases*
172 All along the experiment, the water was maintained at 12 ± 0.5°C, aerated and renewed every
173 two days. Initially, 20 beakers were set up for Cd experiment and 40 for Zn, with each beaker
174 containing 8 gammarids (for a total of gammarids of n = 160 for $^{109}$Cd and n=320 for $^{65}$Zn). In
175 each beaker, the 8 gammarids were individually separated by handmade baskets (i.e., plastic
176 mesh with a height of 11 cm and a diameter of 8.6 cm, with a mesh size of 0.5 cm, see Fig. S1b.)
177 to avoid cannibalism.
178 The experimental procedure was composed of two phases (Fig. S1a.): i) a 7-day accumulation
179 phase during which gammarids were exposed to $^{109}$Cd or $^{65}$Zn dissolved in water and ii) a 21-
180 day depuration phase during which gammarids were maintained in clean water (i.e., without
181 radiotracer). During the 7-days exposure phase, beakers were filled with 0.200 L of Evian®
182 water contaminated with 20 Bq.mL$^{-1}$ (i.e., 50 ng.L$^{-1}$ in stable equivalent) of $^{109}$Cd or 15 Bq.mL$^{-}$
183 $^{1}$ (i.e., 416 ng.L$^{-1}$ in stable equivalent) of $^{65}$Zn. The dissolved radiotracers concentrations were
184 monitored twice a day by sampling randomly 10 mL of water in 5 beakers (Tables S2 and S3).
185 If necessary, radiotracers were added to compensate the loss due to ad- and absorption, and thus
186 maintain an exposure pressure as constant as possible (Fig. S1b. and S1c.). Only during this
187 first phase, gammarids were not fed to avoid accumulation through dietary pathway, by
188 adsorption of radiotracers on the food. At the end of the accumulation phase, gammarids were
189 transferred into clean baskets and clean polypropylene beakers filled with uncontaminated
190 Evian® water and fed with alder leaves (Fig. S1a.). Water sample was collected randomly from
191 5 beakers and radiocounted daily to check possible radiotracer desorption from gammarids to
192 the water.

193 The mortality was monitored every day. A gammarid is considered as dead when its pleopods
194 do not beat anymore (i.e., related to the ventilatory activity of the organisms to uptake oxygen,
195 which is around 150 beat.min$^{-1}$; Vellinger et al., 2012), even after a stimulation (i.e., gently
196 push with clean tweezers).
197
198 *2.4 Gammarids dissection and collection of sampled organs*
199 Gammarids were collected at days 2, 5, 7, 9, 14, 17, 21, 28 for $^{109}$Cd and days 1, 2, 3, 4, 7, 8,
200 9, 11, 15, 17, 21, 28 for $^{65}$Zn (Fig. S1a.). There is more sampling time-points for the experiment
201 with $^{65}$Zn, as Zn is an essential metal well regulated by gammarids. The fact that the data were
202 collected at different times of accumulation and depuration phases between Cd and Zn
203 experiments, does not impact the TK modelling outputs (i.e., uptake and elimination rates, see
204 below). Indeed, the dynamic approach allows to disregard the data points in themselves, as long
205 as there are enough data points to obtain accurate kinetic parameters (i.e., see their precision in
206 Table 1).
207 At each sampling time, twenty gammarids (4 replicates of 5 pooled organisms) were randomly
208 sampled from all the beakers, placed in clean water (free of $^{109}$Cd or $^{65}$Zn) for maximum one
209 minute, gently dried with paper towel and weighed (± 0.1 mg). Then, gammarids were dissected
210 to separate and collect the organs of interest (caeca, cephalons, gills, intestines and remaining
211 tissues) according to the procedure described in Gestin *et al.* (2021), modified to separate the
212 gills from the remaining tissues in the last step (Fig. S2). These organs were chosen for their
213 presumed functional relevance: the intestines and gills involved in the metal uptake and loss,
214 the caeca in detoxification/storage functions. At the end, all gammarids tissues were analyzed
215 since the exoskeleton and the muscle are included in the remaining tissues. All the same five
216 organs sampled per replicate were pooled and stored in 500 µL of HCl (3,4 %) at ambient

temperature before gamma-counting (i.e., counting of the gamma-ray emissions to determine the amount of metal in the sample).

In average, gammarids weights were 23.1 ± 1.8 and 23.9 ± 2.5 mg wet weight for $^{109}$Cd and $^{65}$Zn experiments respectively (Tables S4 and S5). Considering that *G. fossarum* dry weight represents 25 % of the wet weight, the weights of the organs were calculated from estimation of the respective percentage of each dry organ regarding the whole body gammarid total wet weight, i.e., 1.3 % for gills, 2.2 % for intestines, 5 % for caeca, 14 % for cephalons and 77.5 % for the remaining tissues (Tables S4 and S5).

### *2.5 Gamma-spectrometry: $^{109}$Cd and $^{65}$Zn detection*

The radioactivity of each isotope was determined using calibrated inhouse standards with the appropriate sample geometry, i.e.: i) a "water-counting" Caubères® geometry, a large cylindrical container filled with 10 mL of acidified water (HCl; 3,4 %); and ii) an "organ-counting" Caubères® geometry, a narrow cylindrical container filled with 0.5 mL of acidified water (HCl; 3,4 %). Samples were analyzed on NaI detector coupled to InterWinner 7.0 software (ITECH Instruments®). Counting time was adjusted to obtain counting uncertainties below 5 % with runs ranged from 10 minutes to 48 hours of counting. All organ samples were counted with less than 5 % of errors for both radioisotopes, except for two $^{109}$Cd intestines samples at the end of the depuration phase. The radiotracer activity (expressed in Bq) measured in each organ was then converted to obtain the concentrations of Cd and Zn in stable equivalent (µg of metal.g of organ$^{-1}$; Tables S4 and S5).

### *2.6 One-compartment toxicokinetic modelling*

A one-compartment TK model was fitted to each metal/organ data set independently, according to the methodology already described in Gestin *et al.* (2021), in order to estimate the

accumulation and depuration capacities of each organ independently to each other through a dynamical view (i.e., integration of metal concentration over time). Since gammarids were not fed during the accumulation phase, we considered that bioaccumulation of contaminants occurs only from water. Furthermore, since exposed organisms were adults and there is no weight gain or loss over the total duration of the experiments (Tables S4b and S5b), gammarid growth was considered negligible.

Briefly, the variation of internal concentration in an organ during time is described by:

$$\frac{dC_i(t)}{dt} = \begin{cases} k_{u,i} \times C_w(t) - k_{e,i} \times C_i(t) & \text{for } 0 \leq t \leq t_c \quad (1) \\ -k_{e,i} \times C_i(t) & \text{for } t > t_c \quad (2) \end{cases}$$

where $C_i(t)$ is the internal concentration (µg.g$^{-1}$ dry weight) in the organ $i$ ($i$=1..5) 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 (ng.L$^{-1}$) at time $t$, $k_{e,i}$ the elimination rate (day$^{-1}$) for the organ $i$ and $t_c$ the duration of the accumulation phase (7 days). $i$ = 1 corresponds to intestines, $i$ = 2 to caeca, $i$ = 3 to cephalon, $i$ = 4 to remaining tissues and $i$ = 5 to gills.

As confirmed by the concentrations measured in water (Tables S2 and S3), we considered that $C_w$ is constant during the accumulation phase. Therefore, Eqs. (1) and (2) can be analytically solved (Eqs. (S2) and (S3)).

For the stochasticity part, a gaussian distribution of the metal concentration in each organ was used:

$$C_{obs,i}(t) \sim \mathcal{N}(C_i(t), \sigma_i) \qquad (3)$$

where $C_{obs,i}(t)$ is the measured concentrations in the organ $i$ ($i$ = 1..5) at time $t$, $\mathcal{N}$ stands for the Normal law, with a mean $C_i(t)$, the internal concentrations (µg.g$^{-1}$ dry weight) in the organ $i$ predicted by the model at time $t$ (Eqs. (1) and (2)), and the standard deviation $\sigma_i$ for the organ $i$ ($i$ = 1..5).

This model was fitted to each organ data set using Bayesian inference with R software and JAGS (Plummer, 2003; R Core Team, 2017), thus leading to an estimate of kinetic parameters ($k_{u,i}$ and $k_{e,i}$) for each organ. According to available information in the literature concerning uptake and elimination at the level of gammarids' organs, mostly at environmentally relevant pressure of contamination, we chose non informative priors: i) for uncertainty parameters a Gamma law (Tables 1 and S6); and ii) for parameters concerning uptake and depuration rates a Uniform law on the decimal logarithm scale (due to the limited information available on the kinetic parameters). For more details, see Gestin *et al.* (2021).

## 3. Results

### *3.1 Experimental conditions*

The dissolved concentrations of $^{109}Cd$ and $^{65}Zn$ in water were variable during the accumulation phase (Tables S2 and S3). A loss of 60 ± 33% of Cd concentration and 67 ± 22% of Zn concentration in water was measured between two re-adjustments, each one occurring at approximately 12 ± 5.7 h (Tables S2 and S3). The bioaccumulation of metals by gammarids could explain only 8% of the Cd and 22% of the Zn losses, suggesting a strong adsorption of elements on the polypropylene beaker walls and plastic baskets. Gammarids were exposed to 52.1 ± 27.3 ng.L$^{-1}$ of Cd and 416 ± 264 ng.L$^{-1}$ of Zn in stable equivalent. To simplify the model implementation during the accumulation phase, we considered $C_w$ as a constant exposure of 52.1 ng.L$^{-1}$ for Cd and 416 ng.L$^{-1}$ for Zn. During the depuration phase, the concentration of metals measured in water were 1.1 ± 2.3 ng.L$^{-1}$ of Cd and 0.6 ± 1 ng.L$^{-1}$ of Zn, considered as negligible.

Over the total experiment duration, the survival rates of gammarids were 97 % for Cd and 91 % for Zn.

### *3.2 Uptake and elimination kinetics of Cd and Zn in gammarid organs*

**Cadmium (Fig. 1, left panel)**. During the accumulation phase, the concentration of Cd in each organ reached maximal values on day 7 (Fig. 1 and Table S4). The rank of organs from the highest to the lowest concentrations of Cd was similar throughout the period, reaching the following values on day 7: gills (1.5 ± 0.97 µg.g$^{-1}$) >> intestines (0.39 ± 0.24 µg.g$^{-1}$) ≈ caeca (0.33 ± 0.071 µg.g$^{-1}$) >> cephalons (0.035 ± 0.018 µg.g$^{-1}$) > remaining tissues (0.013 ± 0.0044 µg.g$^{-1}$). This rank did not change at the end of the depuration phase (28$^{th}$ day). However, although the intestines and caeca still had higher concentrations than the cephalons and remaining tissues at the end of the depuration phase, the Cd concentrations in these organs

298 decreased the most, with a drop of 88 and 66%, respectively, during this phase. The Cd
299 concentrations in cephalons and the remaining tissues decreased by 55 and 34% of their
300 maximal values, respectively. Surprisingly, the Cd concentrations in gills did not decrease
301 during the whole depuration phase.
302
303 **Zinc (Fig. 1, right panel)**. The maximal concentrations of Zn were observed between day 4
304 and day 7 of the uptake phase depending on the organ, meaning that the accumulation quickly
305 reached a steady state (Fig. 1 and Table S5). From day 4, the order of the organs from the highest
306 concentration of Zn to the lowest concentration was: intestines ($5.0 \pm 7.75$ µg.g$^{-1}$) ≈ caeca
307 ($3.6 \pm 3.3$ µg.g$^{-1}$) > gills ($0.85 \pm 0.49$ µg.g$^{-1}$) > cephalons ($0.34 \pm 0.14$ µg.g$^{-1}$) ≈ remaining
308 tissues ($0.21 \pm 0.11$ µg.g$^{-1}$). Nevertheless, it is worth noting that the Zn concentrations in
309 intestines reached a plateau from day 2 onward of the accumulation phase. After 21 days of
310 depuration (i.e., on day 28), the concentrations drastically decreased for all organs, reaching
311 similarly low values: intestines at $0.13 \pm 0.054$ µg.g$^{-1}$, caeca at $0.10 \pm 0.0051$ µg.g$^{-1}$, gills at
312 $0.098 \pm 0.025$ µg.g$^{-1}$, cephalons at $0.068 \pm 0.013$ µg.g$^{-1}$, and remaining tissues at
313 $0.062 \pm 0.0091$ µg.g$^{-1}$.
314
315 *3.3 Modeling the toxicokinetics of Cd and Zn for each G. fossarum organ*
316 To study the question of bioaccumulation from a dynamic point of view and to estimate kinetic
317 parameters (uptake and elimination rates, termed $k_u$ and $k_e$, respectively), a one-compartment
318 TK model was fitted to each metal/organ dataset separately. The median predictions of the
319 concentration in each organ over time (and their 95% credible intervals) are presented in
320 Figure 1 and superimposed onto the observed data. For all organs and for both metals, between
321 94% and 98% of the observed data are in the 95% credible intervals of the model predictions.
322 Except for the intestines of the Zn dataset, the inference process quickly converged and thin

323 posterior distributions were obtained for all kinetic parameters (Fig. S3 and S4). A summary of
324 each marginal posterior distribution is given in Table 1, with the median of each parameter and
325 their respective 95% credible interval. First, it is noteworthy that, except for the Cd uptake rate
326 of gills ($k_{u,5}$), which is 2.6-fold higher than that of Zn, the $k_u$ and $k_e$ values for Zn were higher
327 than those for Cd in all organs: i) from 2.9-fold higher in the remaining tissues to 695-fold in
328 intestines for $k_u$; and ii) from 3.6-fold higher in remaining tissues and cephalons to 341-fold in
329 intestines for $k_e$ (Table 1).
330 Overall, the highest estimated $k_u$ values were different between the two metals tested. In view
331 of the credibility intervals, for Cd, the intestines and the gills were the two tissues with the most
332 accumulation, while for Zn, the $k_u$ of the intestines only was prominent. For both metals, the
333 highest $k_e$ median values among organs were those of the intestines. Concerning the particular
334 case of the kinetic parameters of Zn in the intestines, both $k_u$ and $k_e$ had a large credible interval
335 (between an order of $10^4$ and $10^{10}$ for $k_{u,5}$ and between an order of $10^0$ and $10^4$ for $k_{e,5}$) (Tables
336 1 and Fig. S4), meaning that the depuration rate might be overestimated and consequently also
337 the accumulation rate (Tables S6). This high uncertainty resulted from the very fast
338 accumulation and depuration of Zn in the intestines (Fig. 1b), as shown by the highest $k_u$ and
339 $k_e$ values and the uptake kinetics reaching a plateau within a few hours (Fig. 1b).
340
341 *3.4 Cd and Zn distribution among organs during the uptake and loss phases*
342 The proportions of metal amount found in each organ at all sampling times are presented in
343 Figure 2. These distribution patterns highlighted the contrasting organotropism between the two
344 elements. It is noteworthy that, during the accumulation phase, more than 30% of the total
345 amount of accumulated Cd was found in the gills, despite their very small size (and low weight).
346 This proportion increased to more than 50% during the depuration phase, while the proportion
347 remained stable or decreased in the other compartments. These results suggest that gills provide

348 a key contribution to the Cd bioaccumulation efficiency in gammarids. Aside from the gills, Cd
349 was found in the caeca and the remaining tissues (i.e., up to 31% and 20% for Cd on day 9,
350 respectively). In the depuration phase, Zn was mainly found in the remaining tissues and the
351 cephalons (i.e., up to 55% and 22% for Cd on day 9, respectively).

352 **4. Discussion**

353 First, using radioisotopes as tracers allowed us to accurately quantify the low
354 concentrations of bioaccumulated metals in the tiny organs of small invertebrates (e.g., ~ 1.54
355 mg for each pool of gills) exposed to environmentally relevant concentrations. Indeed, the
356 gammarid exposure concentrations of 52.1 and 416 $ng.L^{-1}$ for Cd and Zn, respectively, are
357 assessed as environmentally similar to the concentrations measurable in rivers (i.e., <100 and
358 <500 $ng.L^{-1}$ for Cd and Zn, respectively) (Cresswell et al., 2014b; Urien et al., 2016). Thus, the
359 low mortality rate observed throughout the experiment attests to the good rearing conditions
360 and the absence of acute metal toxicity for gammarids.

361

362 One of the main goals of this study was to compare bioaccumulation behavior, at the organ
363 level, of a non-essential (Cd) and an essential (Zn) metal. Greatly contrasting contamination
364 patterns were found between the two elements. First, our data strongly suggest a fast regulation
365 of Zn, as has been reported at the whole-body level in *Gammarus fasciatus*,
366 *Echinogammarus marinus,* and *Gammarus pulex* (Amyot et al., 1994; Ramiro Pastorinho et al.,
367 2009; Xu and Pascoe, 1993). This is a common feature for Zn in crustacean species (Rainbow,
368 2002) but also shared with other essential metals (e.g., copper and iron), in order to maintain a
369 constant internal level to meet the metabolic needs (Lebrun et al., 2017). Indeed, Zn was taken
370 up very quickly during the accumulation phase and was lost during the depuration phase
371 (Fig. 1): 72% of the total Zn eliminated during the depuration phase was lost during the first
372 24 h (Fig. 2 and Table S5). This efficient depuration has already been demonstrated in
373 *Hyalella azteca* at the scale of the whole organism, which depurated Zn mainly in the first 24 h,
374 and reached its baseline after 5 days of depuration (Shuhaimi-Othman and Pascoe, 2007). This
375 efficient excretion, confirmed by high $k_e$ values, indicated a fast regulation capacity of Zn by
376 gammarids at the organ level. Moreover, following the rapid 24-h loss, the measured data

(Fig. 1 and Tables S4 and S5) showed that Zn concentrations in all organs reached a plateau on day 9 until the end of that phase, implying that elimination drastically slowed down or stopped. This peculiar pattern suggests two pools of accumulated Zn in gammarid organs: the first one is very labile and rapidly eliminated, while the second one seems to be retained much longer and eliminated more slowly (White and Rainbow, 1984). This latter pattern would correspond to metabolically available Zn, which is required for essential metabolic purposes (i.e., co-factor of enzymes, DNA; Dixit and Witcomb, 1983), or to some elements reversily detoxified by metalloproteins such as metallothioneins; Rainbow and Luoma, 2011).

The remaining tissues showed the lowest Cd concentration throughout the experiment, which contained around 20% of the total body burden (Fig. 2). This is in contrast to our previous results that showed approximately twofold higher Cd concentrations in the remaining tissues than in the cephalons of gammarids exposed to 11 $\mu g.L^{-1}$ of Cd (i.e., where remaining tissues included gills) (Gestin et al., 2021). Moreover, the $k_u$ values of Cd were 2.87-fold higher and the $k_e$ values 1.77-fold lower in the remaining tissues of gammarids exposed to 11 $\mu g.L^{-1}$ compared to the values calculated for 52 $ng.L^{-1}$. When considered alone, the gills displayed a high value and null values of $k_u$ and $k_e$, respectively, implying an efficient bioaccumulation and a strong retention of metal. The differences in kinetic parameter values for the remaining tissues in the two studies could thus be attributed to the presence of gills in the remaining tissues in the first study, which, despite their tiny size, accumulated significant amounts of Cd as the first organ susceptible to waterborne uptake (see Discussion below).

Concerning Zn, the amount of metal in the remaining tissues accounted for one third of the total Zn body burden (Fig. 2) during the exposure phase. In the literature, Nunez-Nogueira and Rainbow (2005) reported that 40% of Zn is associated with the exoskeleton of decapods, *Penaeus indicus* (i.e., the exoskeleton that mainly comprises, with muscles, the "remaining

402 tissues" compartment in our study). Following depuration, the proportion increased to ~ 70%
403 of the total amount of Zn (Fig. 2). The same value has also been shown in the gammarids
404 *G. fasciatus*, from Lake St. Louis (Canada), in which 68% of the total Zn body burden was
405 found in the remaining tissues after 24 h of depuration (Amyot et al., 1996). This is consistent
406 with a controlled distribution of Zn in the organisms. Indeed, at the end of the depuration phase,
407 the increase in the relative contribution of the remaining tissues and cephalons is explained by
408 a more rapid depuration of Zn by the other tissues.
409
410 Regarding the other organs, the results obtained here suggest that the caeca and intestines often
411 play a key role in metal regulation, with the highest concentrations reached at the end of the
412 accumulation phase for both metals (Fig. 1).
413 In terms of metal amounts, the caeca accounted for around one third of the total metal body
414 burden at the end of the accumulation phase (Fig. 2), which is similar to the proportion already
415 reported for the caeca of amphipods: i) *Orchestia gammarellus* stored 30% of the Zn body
416 burden at higher concentrations (i.e., 20 $\mu g.L^{-1}$) (Nassiri et al., 2000; Weeks and Rainbow,
417 1991); and ii) *G. fasciatus* stored 38% of the Cd body burden (Amyot et al., 1996). The
418 elimination rate ($k_e$) of Zn by the caeca is six-fold greater than that of Cd. The caeca are known
419 to be an organ of metal detoxification, through various sequestration mechanisms. Subcellular
420 mechanisms have already been described in amphipods, including binding to metallothioneins,
421 insoluble granules, or lysosomes (Nunez-Nogueira et al., 2006). Among these processes, some
422 lead to a long retention of non-essential metals, such as Cd, and a short retention of essential
423 metals, such as Zn. Indeed, to decrease their metabolic bioavailability, and thereby any possible
424 toxicity, Cd and Zn bind to two different groups of metallothionein, type C and B, respectively,
425 in the caeca of crustaceans, suggesting that they are controlled and detoxified differently
426 (Nunez-Nogueira et al., 2006). For both metals, the elimination rates of the caeca were the

second highest (i.e., 0.077 and 0.877 $d^{-1}$ for Cd and Zn, respectively), after those of the intestines (i.e., 0.352 and 120 $d^{-1}$ for Cd and Zn, respectively). It is already known that metals can be temporarily stored in the lysosomes of the caeca before being eliminated in the lumen of the intestines, making the latter tissue a major organ in the elimination of metals (Schaller et al., 2011).

It is noteworthy that the literature tends to summarize the role of the intestines in the uptake of metal only in cases of trophic exposure (Ahearn et al., 2004), presenting the gills as the primary pathway for accumulation of metals from waterborne contamination (Henry et al., 2017; Nunez-Nogueira et al., 2006). For Cd, the 95% credible interval around the median prediction of the intestine $k_u$ value encompasses that of gills, making them the two dominant pathways of Cd accumulation. Surprisingly, the $k_u$ value of Zn for intestines is 1,220 times higher than that of gills. These results support the idea that intestines are a predominant uptake pathway of waterborne metals, when gammarids drink water contaminated to environmentally relevant concentrations.

Concerning the gills, one of the major results of this study was the highest Cd concentration found in the gills when compared to the other organs, with more than 30% of the total metal amount in the accumulation phase, despite their very small size and low weight. Indeed, the gills displayed a very high bioconcentration capacity of Cd, with maximal concentrations measured on day 7 up to 3.5-fold higher than those recorded in the caeca and the intestines. This high bioaccumulation of Cd in the gills was already reported in other genera of crustaceans, such as the prawns *P. australiensis* and *Macrobrachium australiense*, with gills accumulating four times more Cd than caeca (Cresswell et al., 2017; McDonald et al., 2020). This can be explained by the fact that most of the Cd taken up by the gills would remain in this

tissue, even during depuration. Regarding the kinetics parameters, the $k_u$ values of Cd in the gills for *Mytilus galloprovincialis*, *Ruditapes decussatus,* and *Oncorhynchus mykiss* (Ju et al., 2011; Rocha et al., 2015) are, respectively, 14, 22, and 326 times lower than the values for *G. fossarum*. This suggests that the gills of gammarids accumulate Cd more rapidly than those of bivalves and fish. However, regarding the elimination rate ($k_e$) of Cd by the gills, the values were null or very low ($k_e = 6.93.10^{-7}$ $d^{-1}$) in the bivalves *M. galloprovincialis* and *R. decussatus,* respectively*,* suggesting that Cd is not eliminated from the gills as observed in gammarids. On the contrary, the trout *O. mykiss* eliminates Cd more rapidly, with a significantly higher $k_e$ value of 0.32 $d^{-1}$. Cresswell et al. (2017) showed that after 6 h of exposure to 0.56 ± 0.14 $\mu g.L^{-1}$ of Cd, the concentration in the gills of *M. australiense* decreases rapidly during the depuration phase, whereas it decreases much more slowly when the shrimp were exposed for 7 days. Nevertheless, for gammarids, bioaccumulation data at the organ scale are still lacking for determining whether the absence or very low depuration of Cd by the gills: i) is characteristic of the invertebrate group as opposed to fish; and/or ii) is instead related to the duration of exposure, as already discussed for the crustacean *M. australiense* (Cresswell et al., 2017). Some authors make the assumption that the gills of crustaceans store Cd for a later elimination by the exuviae (Amyot et al., 1994; Nunez-Nogueira et al., 2006). However, there is no consensus on this topic, as other authors have determined that the molting phenomenon has no influence on Cd efflux (Cresswell et al., 2014a). The amount of Cd contained in the exuviae was not measured in this study. Nevertheless, this absence of apparent Cd depuration from the highly concentrated gills leads to two hypotheses: i) the accumulated metal is tightly bound to the cellular components of gills resulting in a long-term storage of Cd (Table 1); and ii) alternatively, stable Cd concentrations during the depuration phase could result from a dynamic balance between the influx rate from the other organs into the gills and the efflux rate from the gills toward the medium. Considering that the other organs showed very low levels of

Cd concentrations at the end of the depuration phase, while concentrations in the gills remained constant, we assume that the gills of gammarids are characterized by a high Cd accumulation and retention capacity. This would make it an independent organ from the rest of the gammarid, in terms of Cd uptake and elimination.

The development of a multicompartment modeling approach could help to further investigate the hypothesis of linkages and exchanges between the gills and other organs.

This study provides the base for understanding the organotropism and toxicokinetic of essential and non-essential metals in a sentinel species. Firstly, the measurements obtained for Zn confirmed its good regulation by all organs of gammarids and consistent with the essential character of this element whose accumulation has to meet the metabolomic needs (Amyot et al., 1996; Rainbow and Luoma, 2011). Moreover, this regulation is very rapid, with 65% of the Zn lost in the first 24 h of the depuration phase for all organs. This implies that in the context of biomonitoring, the duration of exposure will not have an impact on the Zn concentrations measured in gammarids. Instead, these concentrations will tend to reflect a constant or a very recent contamination in the environment. On the contrary, the absence of Cd elimination in the gills during the 21 days of the depuration phase shows that this tissue integrates the contamination changes to which the organism is exposed. Indeed, at the end of the depuration phase, it appears that the concentration in the gills is still the same as that measured at the end of the accumulation phase and represents 55 $\pm$ 8.0% of the total dissolved Cd of the whole body. The fact that gills are an organ of Cd accumulation in gammarids is consistent with previous work conducted on *G. pulex* and *G. fossarum* (Felten et al., 2008; Issartel et al., 2010c). The gills can therefore be considered a very good indicator of aqueous Cd contamination, assuming no loss of Cd during the molting event. This storage function makes them an organ of great interest in biomonitoring, but their low mass and difficult extraction

from other tissues make this tissue of choice somewhat difficult to use in routine compared to the whole organism, as is currently done. Secondly, the gills are an organ essential for maintaining homeostasis and respiration, which makes them particularly vulnerable to metal-induced toxic effects. Indeed, environmental Cd contamination leads in particular to a decrease in iono- and osmoregulation, linked to the induction of critical cellular damage after exposure (Felten et al., 2008; Issartel et al., 2010c).

Finally, one of the major objectives in the field of ecotoxicology is the development of biomarkers to help understand and predict the impact of metal contamination on organisms. The study of organotropism and toxicokinetic can be useful for identifying key organs in the accumulation, storage, or regulation of metals. Thus, in gammarids, the ability of the gills to integrate non-essential metals, such as Cd, may make them a tissue of interest for the development of biomarkers of the effect of dissolved metal contamination. The caeca, whose detoxification role enables the establishment of molecular responses to regulate metals, both essential and non-essential, would instead be an organ in which biomarkers of metal exposure could be developed. However, in this work we raised the issue that some studies may show that the trophic pathway is the main route of metal accumulation in invertebrate freshwater species or fish (Cresswell et al., 2014a; Mijošek et al., 2020). It will therefore be necessary in the future to improve the mechanistic understanding of the processes governing organotropism, toxicokinetic, and the fate of metals (Wang and Rainbow, 2008) so as to consider the trophic pathway that can have a major impact on the bioaccumulation mechanisms (Vijver et al., 2004).

## 5. Conclusions

This study provides a proof of concept that organotropism of metals in a tiny invertebrate species, *G. fossarum*, can be studied at environmentally relevant concentrations. Our results demonstrate that gammarid organs handle Zn and Cd very differently. Whereas Zn is quickly accumulated and depurated, Cd is more persistently retained, especially in the gills and caeca, which may be explained by the essential (Zn) or non-essential (Cd) character of the two metals studied here. These results of metal-specific bioaccumulation are consistent with other studies on freshwater crustaceans performed on whole organisms.

In addition, we showed that the bioaccumulation of these two metals is strongly organ-specific in *G. fossarum*, with undoubtedly contrasting distribution and management. The main findings were that gills represent the major site of persistent Cd accumulation, while the intestines and caeca are central organs for both Cd and Zn accumulation and depuration.

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**Author statement**

Ophélia Gestin: Methodology, Formal Analysis, Writing - original draft, Visualization.

Christelle Lopes: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition

Nicolas Delorme: Resources

Laura Garnero: Resources

Olivier Geffard: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition

Thomas Lacoue-Labarthe: Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition

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Table 1. Parameter estimates of the TK one-compartment model (Eqs. (1) and (2)) fitted separately to each organ of *Gammarus fossarum* exposed to dissolved Cd and Zn for 7 days before being placed in depuration conditions for 21 days.

| | | | [Cd] = 52.1 ± 27.3 ng.L$^{-1}$ | | | [Zn] = 416 ± 264 ng.L$^{-1}$ | | |
|---|---|---|---|---|---|---|---|---|
| Organs | Parameters | Priors | **Median** | Percentiles | | **Median** | Percentiles | |
| | | | | 2.5% | 97.5% | | 2.5% | 97.5% |
| Intestines | $k_{u,1}$ | log10.Unif (-5, 10) | **2648** | 1439 | 8168 | **1,841,000** | 17911 | 1,058,000,000 |
| Caeca | $k_{u,2}$ | log10.Unif (-5, 5) | **1249** | 833 | 1733 | **6901** | 3733 | 52770 |
| Cephalons | $k_{u,3}$ | | **101** | 71 | 136 | **326** | 219 | 483 |
| Remaining tissues | $k_{u,4}$ | | **47** | 37 | 57 | **138** | 88 | 223 |
| Gills | $k_{u,5}$ | | **3868** | 3412 | 4321 | **1509** | 993 | 2422 |
| Intestines | $k_{e,1}$ | log10.Unif (-5, 5) | **0.352** | 0.016 | 1.38 | **120** | 1.11 | 70730 |
| Caeca | $k_{e,2}$ | | **0.077** | 0.033 | 0.142 | **0.877** | 0.460 | 7.92 |
| Cephalons | $k_{e,3}$ | | **0.068** | 0.030 | 0.12 | **0.242** | 0.136 | 0.406 |
| Remaining tissues | $k_{e,4}$ | | **0.046** | 0.023 | 0.072 | **0.165** | 0.077 | 0.341 |
| Gills | $k_{e,5}$ | | **0** | 0 | 0 | **0.591** | 0.367 | 1.02 |
| Intestines | $\sigma_1$ | Gamma (0.001, 0.001) | **0.12** | 0.10 | 0.16 | **3.5** | 2.9 | 4.4 |
| Caeca | $\sigma_2$ | | **0.13** | 0.09 | 0.16 | **1.4** | 1.2 | 1.7 |
| Cephalons | $\sigma_3$ | | **0.009** | 0.007 | 0.011 | **0.12** | 0.10 | 0.15 |
| Remaining tissues | $\sigma_4$ | | **0.003** | 0.003 | 0.004 | **0.08** | 0.06 | 0.10 |
| Gills | $\sigma_5$ | | **0.42** | 0.33 | 0.55 | **0.28** | 0.24 | 0.35 |

$k_{u,i}$ and $k_{e,i}$ are, respectively, the uptake and elimination rates (d$^{-1}$) of the organ *i* (*i*=1..5); $\sigma_i$ is the standard deviation of the Gaussian stochastic part associated to the organ *i* (*i* =1..5); 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.

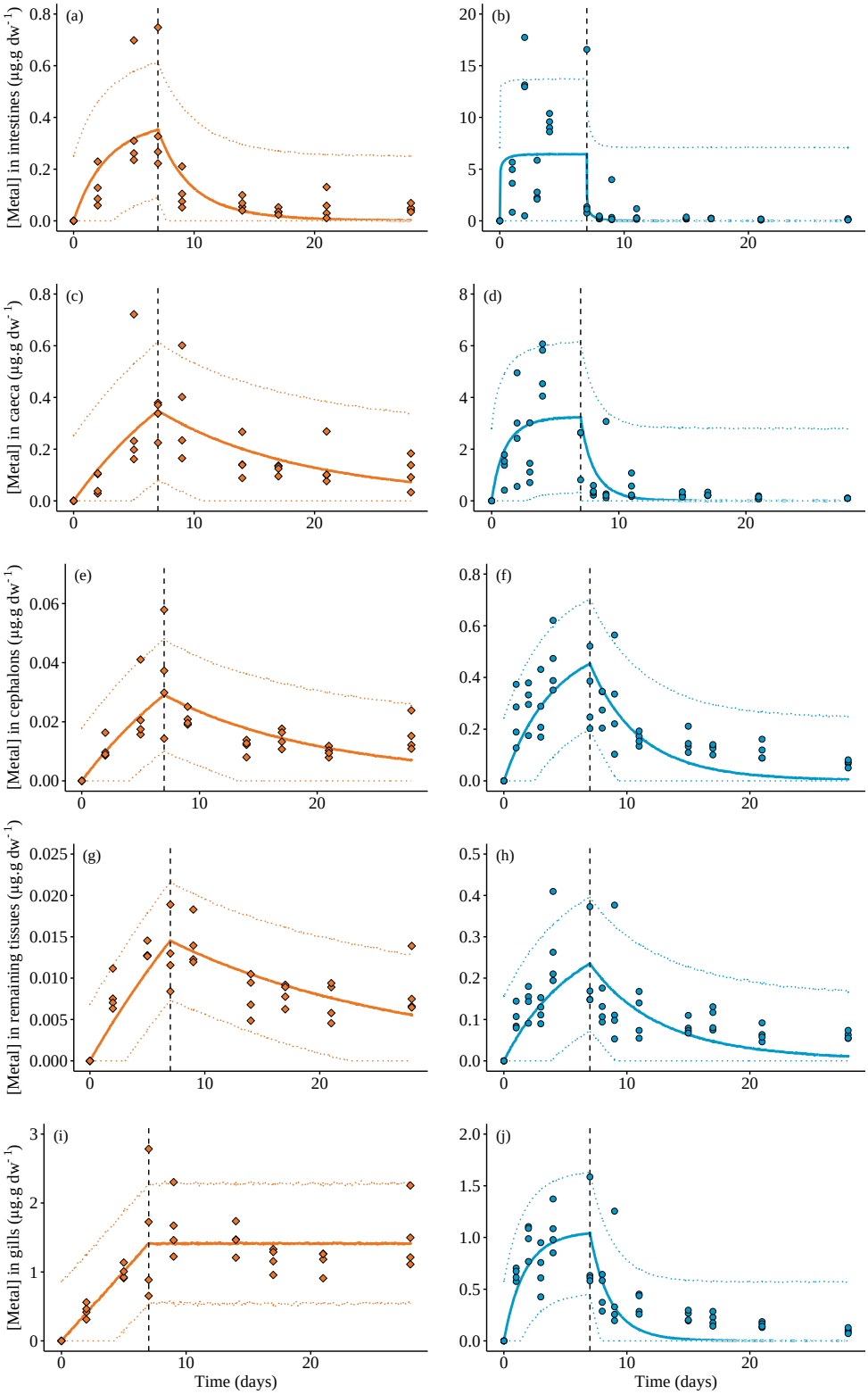



Figure 1. Measured (diamonds for Cd; dots for Zn) and predicted (solid line for the median and dashed lines for the 95% credible interval) concentrations of metals with the one-compartment model (Eqs. (1) and (2)) for a) and b) intestines; c) and d) caeca; e) and f) cephalons; g) and h) remaining tissues; and i) and j) gills of gammarids exposed to 52.1 ± 27.3 ng.L$^{-1}$ of Cd (left column in orange) and 416 ± 264 ng.L$^{-1}$ of Zn (right column in blue) during the uptake phase (days 0–7) followed by a depuration phase (days 7–28). These two phases are separated by the black dotted vertical line. Please note that the y-scale differs between the plots.

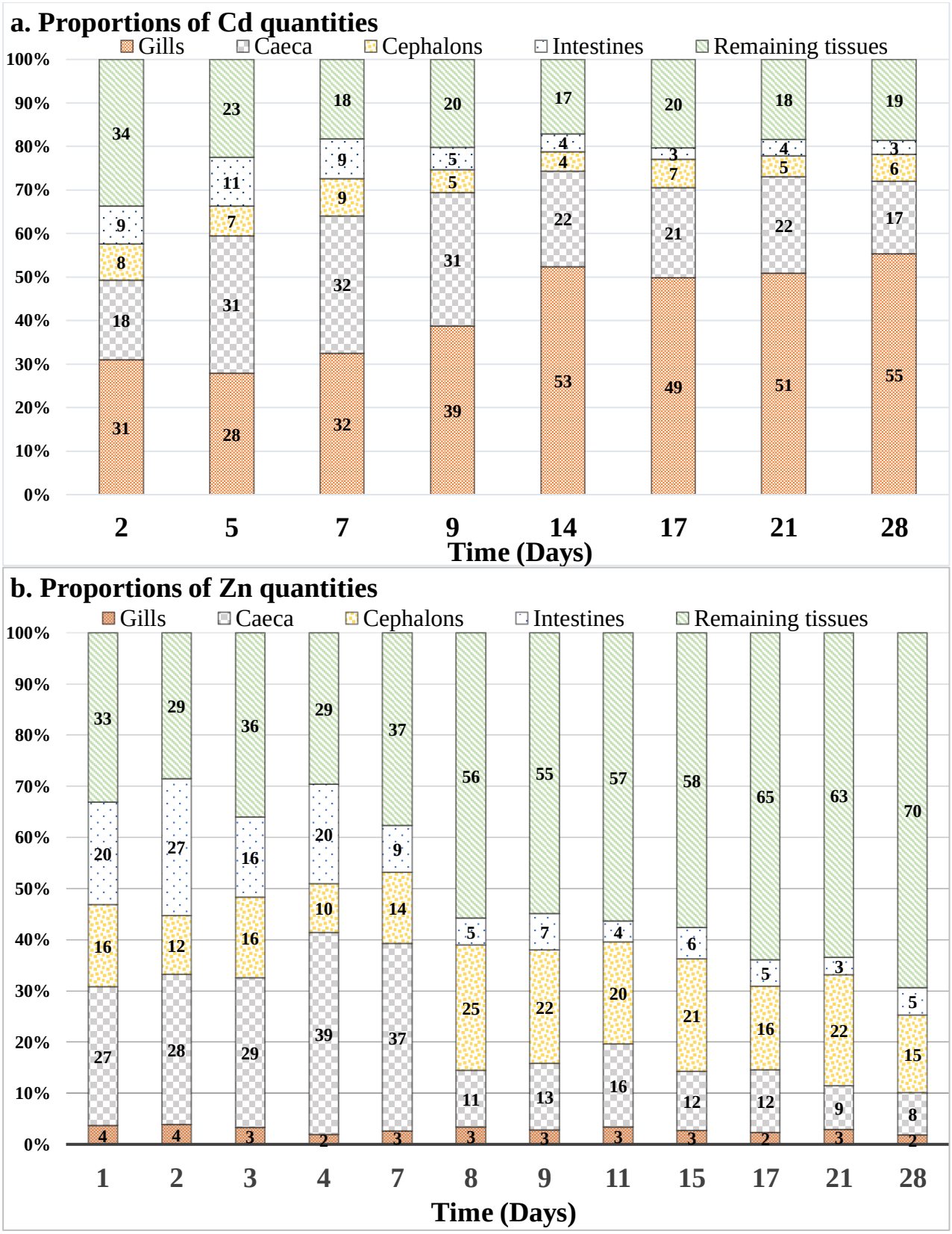


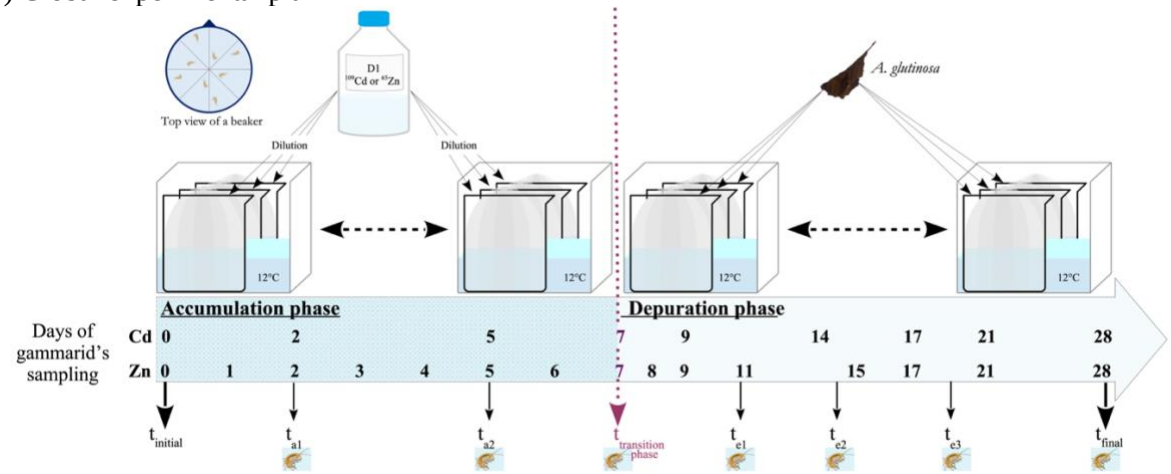

Figure 2. Mean of the relative proportions (%) of metal burdens (figure a. for Cd and b. for Zn) per organ (gills, caeca, cephalons, intestines, and remaining tissues) with respect to the whole-body burden at each sampling time (days) for accumulation (days 0–7) and depuration phases (days 8–28).

# Supplementary data

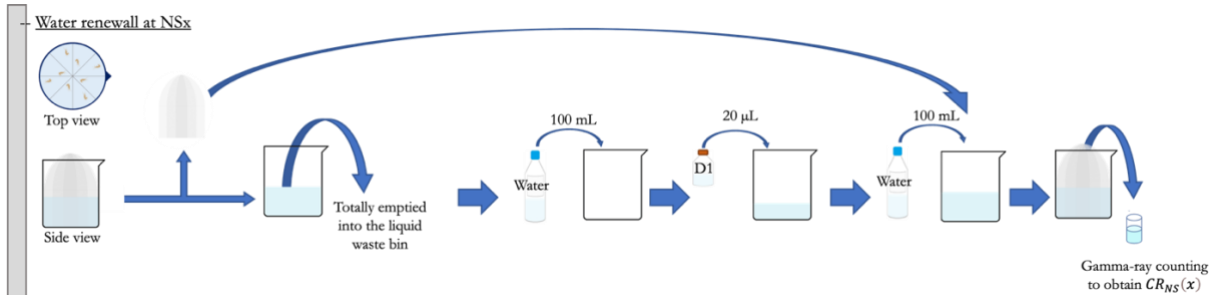
Table S1. Characteristics of the Évian® water used for the experiments.

| | Concentrations ($mg.L^{-1}$) |
|---|---|
| Bicarbonates $HCO_3^-$ | 360 |
| Calcium $Ca^{2+}$ | 80 |
| Chlorides $Cl^-$ | 10 |
| Magnesium $Mg^{2+}$ | 26 |
| Nitrates $NO_3^-$ | 3.8 |
| Potassium $K^+$ | 1 |
| Silica $SiO_2$ | 15 |
| Sodium $Na^+$ | 6.5 |
| Sulfates $SO_4^{2-}$ | 14 |
| pH = 7.2 | |

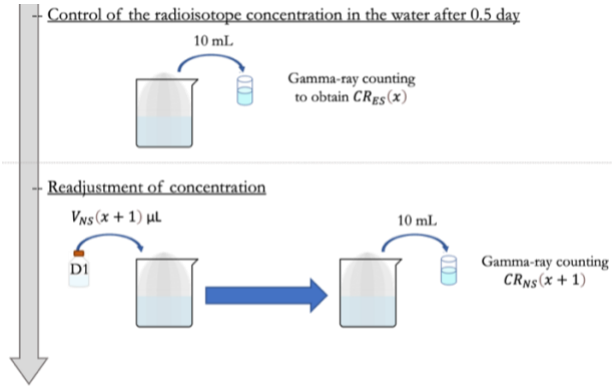
a) Global experimental plan




b) Water renewals




c) Monitoring of $^{109}$Cd and $^{65}$Zn concentrations




$$V_{NS}(x+1) = \frac{(CR_I - CR_{ES}(x)) \times V_{max}}{CR_I} \quad (S1)$$

Figure S1. Main parts of the experimental plan. a) Global experimental plan, where $t_{initial}$=0 is the beginning of the experiment, $t_{a1}$ and $t_{a2}$ are sampling timesteps during the accumulation phase, $t_c$ is the duration of the accumulation phases ($t_c$ = 7 days), $t_{e1}$, $t_{e2}$ and $t_{e3}$ are sampling timesteps during the depuration phase and $t_{final}$ is the total duration of the experiment ($t_{final}$ = 28 days). During the accumulation phase, there will be no food placed. Moreover, the individualized exposure avoids inter-individual cannibalism; b) For water renewal, the baskets were placed in another "transition" beaker and the contaminated water was thrown. To begin, 100 mL of uncontaminated Évian® water was poured into the beaker, then 20 µL of $^{109}$Cd or $^{65}$Zn D1 solution followed by another 100 mL of uncontaminated Evian water. The basket containing the gammares was then repositioned in the beaker that had just been prepared. The operation was repeated for each beaker, every 2 days; and c) Monitoring of $^{109}$Cd and $^{65}$Zn concentrations in real time. To compensate the loss of $^{109}$Cd or $^{65}$Zn, due to ad- and uptake, and thus keep the exposure concentration as constant as possible, the activity concentration in water was monitored twice a day (Tables S4 and S5). Around every 0.5 days after the last "new spike" (abbreviated NS), 5 samples of 10 mL of water were taken in 5 different beakers for gamma-counting, called "end spike" (abbreviated ES). To control and readjust the contamination pressure, the difference between the theoretical concentration and the average of the measured concentrations at ES, was added in each beaker. The volume of D1 required was calculated according to Eq. (S1): where $V_{NS}(x+1)$ is the volume of D1 solution to be added to the beaker for the x+1$^{th}$ spike; $CR_I$ is the nominal concentration to be reached (i.e. 15 Bq.mL$^{-1}$); $CR_{ES}(x)$ is the concentration measured at the end of the x$^{th}$ spike (just before the x+1$^{th}$ spike); and $V_{max}$ is the maximum volume that can be spiked to reach the $CR_I$ (i.e. $V_{max}$ = 0.02 mL).

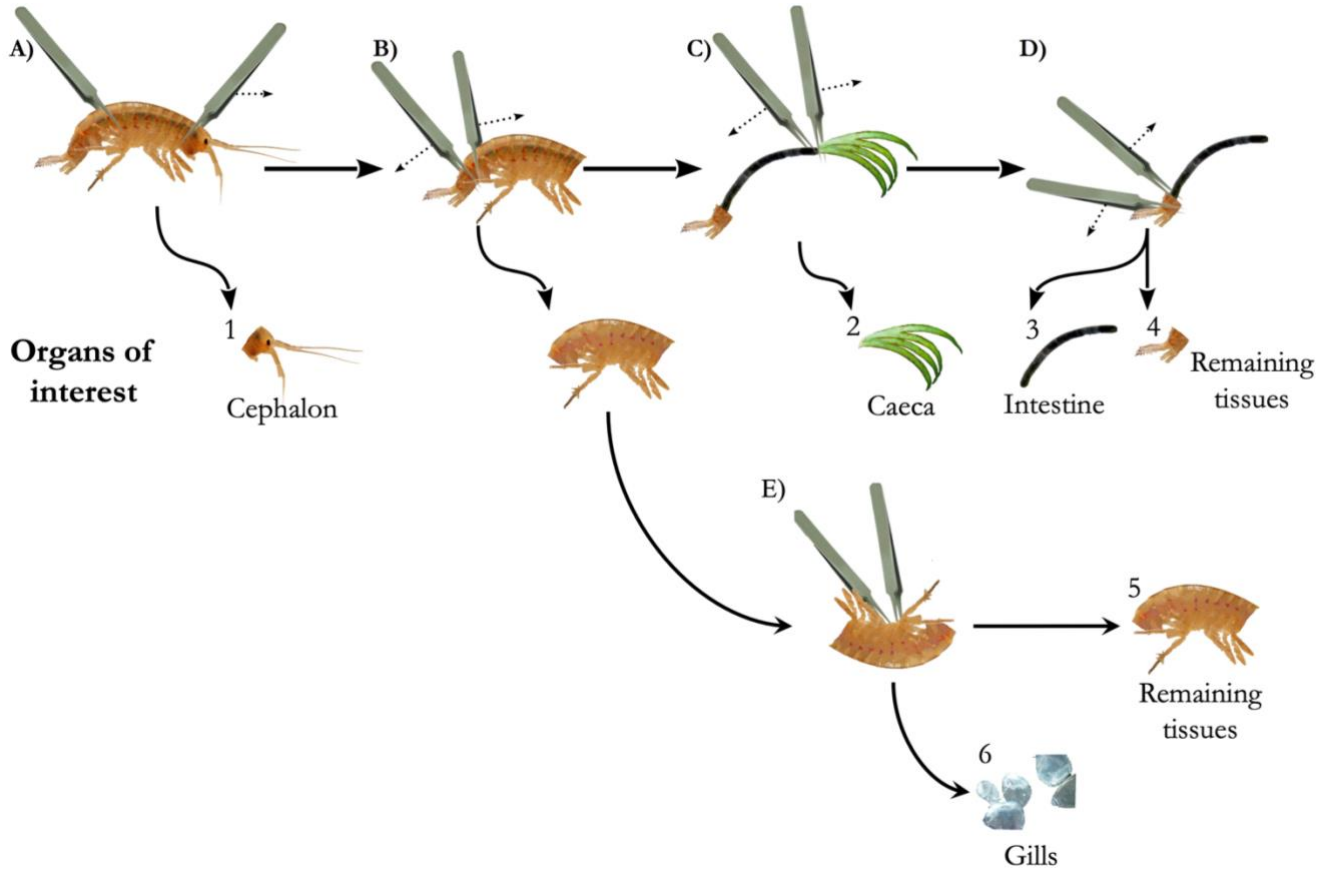



Figure S2. Dissection steps. A) separation of the cephalon from the rest of the organism; B) separation of the thorax and the abdomen from the urosome; C) separation of the caeca from the intestine; D) separation between the urosome and the intestine; and E) separation of the gills from the thorax and abdomen. To recover the organs of interest, with: 1 = cephalon, 2 = caeca, 3 = intestine, 4 = abdomen + thorax, 5 = urosome (4 + 5 = remaining tissues) and 6 = gills.

Table S2. Measured concentrations of $^{109}Cd$ (Mean ± SD; $Bq.mL^{-1}$) and calculated concentrations of Cd in stable equivalent (Mean ± SD; $ng.L^{-1}$) just after (New spike) and just before (End spike) the addition of D1 solution of $^{109}Cd$ in waters during the 7 days of exposure.

| Time of sampling (in hour from the gammarids are put in the beakers) | Moment of sampling | Concentrations | | | | | |
|---|---|---|---|---|---|---|---|
| | | $^{109}Cd$ ($Bq.mL^{-1}$) | | | Cd in stable equivalent ($ng.L^{-1}$) | | |
| | | Nominale | Measured | Mean measured | Nominale | Calculated | Mean calculated |
| -60.0 | New spike 1 | 15 | 22.1 ± 2.26 | 18.6 ± 11.0 | 50 | 53.3 ± 5.65 | 52.1 ± 27.3 |
| 0 | End spike 1 | | 2.27 ± 2.38 | | | 5.67 ± 5.96 | |
| 0 | New spike 2 | | 17.8 ± 2.49 | | | 44.5 ± 6.22 | |
| 7.3 | End spike 2 | | 11.8 ± 3.72 | | | 29.4 ± 9.29 | |
| 7.3 | New spike 3 | | 18.9 ± 1.13 | | | 47.3 ± 2.82 | |
| 23.2 | Middle spike 3 | | 17.1 ± 5.01 | | | 42.8 ± 12.5 | |
| 29.2 | End spike 3 | | 12.1 ± 2.46 | | | 30.3 ± 6.14 | |
| 29.2 | New spike 4 | | 30.5 ± 5.85 | | | 76.2 ± 14.6 | |
| 48.5 | Middle spike 4 | | 23.1 ± 1.94 | | | 57.7 ± 14.8 | |
| 53.0 | Middle spike 4 bis | | 29.6 ± 9.09 | | | 74.0 ± 22.7 | |
| 71.8 | Middle spike 4 ter | | 33.5 ± 16.6 | | | 83.8 ± 41.5 | |
| 80.3 | End spike 4 | | 16.2 ± 6.12 | | | 40.4 ± 15.3 | |
| 80.3 | New spike 5 | | 34.5 ± 5.17 | | | 86.2 ± 12.9 | |
| 101.5 | End spike 5 | | 23.6 ± 11.9 | | | 58.9 ± 29.6 | |
| 101.5 | New spike 6 | | 23.2 ±2.13 | | | 58.1 ± 5.34 | |
| 120.5 | End spike 6 | | 7.83 ± 6.32 | | | 19.6 ± 15.8 | |
| 120.5 | New spike 7 | | 28.9 ± 5.65 | | | 72.3 ± 14.1 | |
| 127.5 | End spike 7 | | 5.52 ± 8.82 | | | 13.8 ± 22.0 | |
| 127.5 | New spike 8 | | 25.5 ± 6.26 | | | 63.9 ± 15.6 | |
| 144.8 | End spike 8 | | 5.44 ± 5.77 | | | 13.6 ± 14.4 | |
| 144.8 | New spike 9 | | 19.1 ± 4.87 | | | 47.7 ± 12.2 | |
| 151.8 | End spike 9 | | 7.04 ± 5.26 | | | 17.6 ± 13.2 | |
| 151.8 | New spike 10 | | 24.7 ± 5.24 | | | 61.8 ± 13.1 | |
| 167.0 | End spike 10 | | 6.89 ± 5.45 | | | 17.2 ± 13.6 | |

Table S3. Measured concentrations of $^{65}Zn$ (Mean ± SD; $Bq.mL^{-1}$) and calculated concentrations of Zn in stable equivalent (Mean ± SD; $ng.L^{-1}$) just after (New spike) and just before (End spike) the addition of D1 solution of $^{65}Zn$ in waters during the 7 days of exposure.

| Time of sampling (in hour from the gammarids are put in the beakers) | Moment of sampling | Concentrations | | | | | |
|---|---|---|---|---|---|---|---|
| | | $^{65}Zn$ ($Bq.mL^{-1}$) | | | Zn in stable equivalent ($ng.L^{-1}$) | | |
| | | Nominale | Measured | Mean measured | Nominale | Calculated | Mean calculated |
| -68.0 | New spike 1 | | 14.62 ± 1.250 | | | 408.7 ± 34.94 | |
| -60.0 | End spike 1 | | 10.21 ± 1.283 | | | 285.6 ± 35.88 | |
| -60.0 | New spike 2 | | 23.47 ± 3.029 | | | 656.7 ± 84.68 | |
| 0.0 | End spike 2 | | 1.214 ± 0.4714 | | | 33.9 ± 13.18 | |
| 0.0 | New spike 3 | | 14.53 ± 0.9808 | | | 406.2 ± 27.42 | |
| 7.2 | End spike 3 | | 7.397 ± 0.8870 | | | 206.8 ± 24.80 | |
| 7.2 | New spike 4 | | 19.42 ± 2.362 | | | 543.0 ± 66.04 | |
| 23.4 | End spike 4 | | 8.665 ± 1.157 | | | 242.2 ± 32.35 | |
| 23.4 | New spike 5 | | 24.79 ± 5.110 | | | 693.1 ± 142.9 | |
| 29.8 | Middle spike 5 | | 14.90 ± 2.281 | | | 416.6 ± 63.78 | |
| 48.6 | End spike 5 | | 10.25 ± 1.757 | | | 286.6 ± 49.12 | |
| 48.6 | New spike 6 | | 16.24 ± 1.133 | | | 454.0 ± 31.69 | |
| 55.0 | End spike 6 | | 7.432 ± 3.969 | | | 207.8 ± 111.0 | |
| 55.0 | New spike 7 | | 22.44 ± 6.707 | | | 627.3 ± 187.5 | |
| 72.1 | End spike 7 | | 6.276 ± 6.937 | | | 175.5 ± 193.9 | |
| 72.1 | New spike 8 | 15 | 20.81 ± 9.351 | 14.89 ± 9.3 | 420 | 581.8 ± 261.4 | 416 ± 263.8 |
| 78.7 | End spike 8 | | 10.52 ± 9.721 | | | 294.1 ± 271.8 | |
| 78.7 | New spike 9 | | 23.47 ± 11.07 | | | 656.2 ± 309.5 | |
| 96.0 | End spike 9 | | 10.55 ± 10.57 | | | 295.0 ± 295.6 | |
| 96.0 | New spike 10 | | 25.68 ± 15.14 | | | 717.9 ± 423.2 | |
| 102.7 | End spike 10 | | 16.45 ± 13.26 | | | 459.9 ± 370.6 | |
| 102.7 | New spike 11 | | 22.47 ± 15.19 | | | 628.2 ± 424.8 | |
| 121.3 | End spike 11 | | 15.83 ± 11.51 | | | 442.6 ± 321.8 | |
| 121.3 | New spike 12 | | 18.15 ± 5.009 | | | 507.5 ± 140.0 | |
| 126.8 | End spike 12 | | 6.210 ± 6.576 | | | 173.6 ± 183.8 | |
| 126.8 | New spike 13 | | 19.42 ± 7.644 | | | 542.8 ± 213.7 | |
| 143.9 | End spike 13 | | 3.710 ± 3.171 | | | 103.7 ± 88.64 | |
| 143.9 | New spike 14 | | 18.24 ± 7.702 | | | 510.0 ± 215.3 | |
| 150.8 | End spike 14 | | 10.46 ± 6.144 | | | 292.5 ± 171.8 | |
| 150.8 | New spike 15 | | 18.89 ± 7.179 | | | 528.2 ± 200.7 | |
| 167.9 | End spike 15 | | 11.39 ± 7.945 | | | 318.5 ± 222.1 | |

Table S4. Data sets of Cd, with for each organ: n = 5, except for the last sampling time (day 28) where n = 4. a) Measured quantities of $^{109}$Cd (Mean ± SD; Bq) in organs of gammarids; b) weight of the gammarids organs sampled from dissections estimated for each organ (Mean ± SD; mg) from the total weights weighed; and c) concentrations of Cd in organs calculated in stable equivalent (Mean ± SD; µg Cd.g organ in dry weight$^{-1}$).

a)

| Day of sampling | Mean quantities of $^{109}$Cd measured (Bq) | | | | |
|---|---|---|---|---|---|
| | Caeca | Cephalons | Gills | Intestines | Remaining tissues |
| 2 | 42.13 ± 22.94 | 19.11 ± 5.745 | 69.65 ± 16.75 | 20.57 ± 11.19 | 76.58 ± 19.24 |
| 5 | 195.2 ± 159.6 | 39.42 ± 20.13 | 147.8 ± 16.33 | 57.59 ± 27.61 | 118.4 ± 12.68 |
| 7 | 191.2 ± 42.33 | 56.01 ± 27.69 | 219.9 ± 136.4 | 59.83 ± 35.11 | 113.4 ± 35.42 |
| 9 | 196.7 ± 94.06 | 31.92 ± 2.839 | 236.7 ± 48.26 | 33.53 ± 19.63 | 121.4 ± 22.26 |
| 14 | 93.19 ± 39.47 | 18.69 ± 4.558 | 217.7 ± 25.57 | 16.90 ± 5.093 | 70.90 ± 25.00 |
| 17 | 77.27 ± 12.39 | 24.34 ± 4.629 | 187.0 ± 36.95 | 9.903 ± 2.272 | 76.30 ± 17.01 |
| 21 | 76.91 ± 52.41 | 14.62 ± 2.055 | 160.3 ± 26.80 | 12.52 ± 9.305 | 59.90 ± 20.93 |
| 28 | 43.05 ± 25.26 | 15.92 ± 5.558 | 144.2 ± 44.15 | 7.687 ± 3.353 | 48.88 ± 18.00 |

b)

| Day of sampling | Mean weight of samples (mg) | | | | |
|---|---|---|---|---|---|
| | Caeca | Cephalons | Gills | Intestines | Remaining tissues |
| 2 | 6.347 ± 0.5749 | 17.69 ± 1.602 | 1.590 ± 0.1440 | 1.689 ± 0.1529 | 95.94 ± 8.689 |
| 5 | 5.947 ± 0.4428 | 16.57 ± 1.234 | 1.490 ± 0.1109 | 1.582 ± 0.1178 | 89.89 ± 6.692 |
| 7 | 5.823 ± 0.2368 | 16.23 ± 0.6599 | 1.459 ± 0.05932 | 1.549 ± 0.06300 | 88.02 ± 3.580 |
| 9 | 5.839 ± 0.6426 | 15.21 ± 1.743 | 1.447 ± 0.1602 | 3.105 ± 0.2839 | 86.56 ± 9.633 |
| 14 | 5.977 ± 0.2990 | 15.94 ± 0.9188 | 1.486 ± 0.07601 | 2.638 ± 0.2339 | 89.19 ± 4.642 |
| 17 | 6.334 ± 0.5525 | 16.89 ± 1.562 | 1.575 ± 0.1384 | 2.794 ± 0.4059 | 94.51 ± 8.363 |
| 21 | 5.574 ± 0.1479 | 14.87 ± 0.6868 | 1.386 ± 0.04102 | 2.457 ± 0.4029 | 83.17 ± 2.664 |
| 28 | 3.838 ± 0.1685 | 10.32 ± 0.3490 | 0.9556 ± 0.03990 | 1.569 ± 0.3609 | 57.40 ± 2.306 |

c)

| Day of sampling | Mean concentration of Cd in organs, calculated in stable equivalent (µg Cd.g organ dw$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Caeca | Cephalons | Gills | Intestines | Remaining tissues |
| 2 | 0.06899 ± 0.04162 | 0.01089 ± 0.003644 | 0.4377 ± 0.1035 | 0.1255 ± 0.07469 | 0.008012 ± 0.002159 |
| 5 | 0.3282 ± 0.2639 | 0.02369 ± 0.01176 | 0.9942 ± 0.1044 | 0.3761 ± 0.2169 | 0.01317 ± 0.0009170 |
| 7 | 0.3284 ± 0.07127 | 0.03484 ± 0.01813 | 1.512 ± 0.9656 | 0.3910 ± 0.2419 | 0.01296 ± 0.004395 |
| 9 | 0.3505 ± 0.1946 | 0.02115 ± 0.002753 | 1.665 ± 0.4629 | 0.1106 ± 0.06998 | 0.01412 ± 0.002916 |
| 14 | 0.1587 ± 0.07610 | 0.01169 ± 0.002566 | 1.470 ± 0.2160 | 0.06559 ± 0.02560 | 0.007901 ± 0.002561 |
| 17 | 0.1223 ± 0.01872 | 0.01451 ± 0.003128 | 1.183 ± 0.1686 | 0.03641 ± 0.01203 | 0.008032 ± 0.001342 |
| 21 | 0.1364 ± 0.08885 | 0.009873 ± 0.001634 | 1.154 ± 0.1677 | 0.05729 ± 0.05270 | 0.007162 ± 0.002362 |
| 28 | 0.1117 ± 0.06423 | 0.01551 ± 0.005840 | 1.519 ± 0.5172 | 0.04859 ± 0.01483 | 0.008611 ± 0.003551 |

Table S5. Data sets of Zn, with for each organ: n = 5, except for the last sampling time (day 28) where n = 6. a) Measured quantities of $^{65}$Zn (Mean ± SD; Bq) in organs of gammarids; b) weight of the gammarids organs sampled from dissections estimated for each organ (Mean ± SD; mg) from the total weights weighed; and c) concentrations of Zn in organs calculated in stable equivalent (Mean ± SD; µg Zn.g organ in dry weight$^{-1}$).

a)

| Day of sampling | Mean quantities of $^{65}$Zn measured (Bq) | | | | |
|---|---|---|---|---|---|
| | Caeca | Cephalons | Gills | Intestines | Remaining tissues |
| 1 | 71.23 ± 35.21 | 37.30 ± 15.09 | 8.939 ± 1.247 | 55.39 ± 30.03 | 86.16 ± 20.38 |
| 2 | 145.6 ± 90.96 | 44.38 ± 11.71 | 13.40 ± 1.977 | 157.8 ± 103.1 | 116.1 ± 26.93 |
| 3 | 85.02 ± 50.64 | 41.78 ± 16.21 | 9.488 ± 3.247 | 46.86 ± 23.09 | 100.5 ± 19.97 |
| 4 | 304.0 ± 53.86 | 75.89 ± 19.41 | 15.92 ± 2.899 | 149.0 ± 16.14 | 240.5 ± 80.19 |
| 7 | 208.8 ± 183.8 | 55.00 ± 21.91 | 12.36 ± 6.587 | 74.61 ± 114.8 | 183.4 ± 88.22 |
| 8 | 16.91 ± 6.650 | 34.71 ± 7.489 | 5.268 ± 1.690 | 7.870 ± 2.884 | 85.39 ± 21.94 |
| 9 | 40.14 ± 62.40 | 35.28 ± 21.61 | 5.482 ± 5.391 | 24.04 ± 40.33 | 104.1 ± 93.42 |
| 11 | 28.68 ± 21.20 | 26.13 ± 4.048 | 5.166 ± 1.354 | 6.752 ± 5.213 | 93.85 ± 42.25 |
| 15 | 14.12 ± 6.175 | 24.11 ± 7.598 | 3.678 ± 0.9541 | 7.694 ± 3.007 | 75.62 ± 22.17 |
| 17 | 13.93 ± 2.959 | 18.82 ± 3.041 | 2.920 ± 0.8441 | 6.320 ± 0.6768 | 84.79 ± 22.71 |
| 21 | 6.996 ± 2.546 | 17.67 ± 4.262 | 2.253 ± 0.4939 | 2.656 ± 0.5041 | 55.31 ± 12.09 |
| 28 | 8.755 ± 0.5529 | 15.41 ± 3.397 | 2.102 ± 0.5579 | 5.973 ± 2.470 | 78.73 ± 7.978 |

b)

| Day of sampling | Mean weight of samples (mg) | | | | |
|---|---|---|---|---|---|
| | Caeca | Cephalons | Gills | Intestines | Remaining tissues |
| 1 | 6.203 ± 0.3586 | 17.28 ± 0.9992 | 1.554 ± 0.08982 | 1.650 ± 0.09539 | 93.76 ± 5.420 |
| 2 | 6.076 ± 0.3052 | 16.93 ± 0.8504 | 1.522 ± 0.07645 | 1.616 ± 0.08119 | 91.83 ± 4.613 |
| 3 | 6.177 ± 0.3308 | 17.21 ± 0.9217 | 1.547 ± 0.08286 | 1.643 ± 0.08800 | 93.37 ± 5.000 |
| 4 | 6.656 ± 0.2082 | 18.55 ± 0.5800 | 1.667 ± 0.05214 | 1.771 ± 0.05537 | 100.6 ± 3.146 |
| 7 | 6.557 ± 0.2033 | 18.27 ± 0.5666 | 1.642 ± 0.05093 | 1.744 ± 0.05409 | 99.11 ± 3.073 |
| 8 | 5.103 ± 0.2964 | 13.33 ± 0.7356 | 1.265 ± 0.07263 | 2.662 ± 0.3452 | 75.70 ± 4.311 |
| 9 | 5.013 ± 0.2584 | 13.19 ± 0.7184 | 1.244 ± 0.06451 | 2.479 ± 0.2363 | 74.51 ± 3.886 |
| 11 | 6.503 ± 0.5247 | 17.95 ± 1.318 | 1.626 ± 0.1291 | 1.973 ± 0.4690 | 98.02 ± 7.681 |
| 15 | 6.858 ± 0.4331 | 17.89 ± 1.130 | 1.700 ± 0.1071 | 3.609 ± 0.3807 | 101.7 ± 6.402 |
| 17 | 6.343 ± 0.2886 | 16.76 ± 0.7007 | 1.575 ± 0.07005 | 3.033 ± 0.4997 | 94.40 ± 4.132 |
| 21 | 6.494 ± 0.6792 | 17.53 ± 1.765 | 1.618 ± 0.1682 | 2.562 ± 0.3697 | 97.23 ± 10.06 |
| 28 | 9.711 ± 0.7834 | 25.26 ± 2.041 | 2.405 ± 0.1941 | 5.227 ± 0.4536 | 143.9 ± 11.61 |

c)

| Day of sampling | Mean concentration of Zn in organs, calculated in stable equivalent (µg Zn.g organ $dw^{-1}$) | | | | |
|---|---|---|---|---|---|
| | Caeca | Cephalons | Gills | Intestines | Remaining tissues |
| 1 | 1.277 ± 0.6006 | 0.2440 ± 0.1081 | 0.6416 ± 0.05790 | 3.773 ± 2.136 | 0.1038 ± 0.02955 |
| 2 | 2.735 ± 1.810 | 0.2956 ± 0.08714 | 0.9871 ± 0.1551 | 11.09 ± 7.401 | 0.1423 ± 0.03749 |
| 3 | 1.573 ± 1.011 | 0.2741 ± 0.1159 | 0.6857 ± 0.2227 | 3.245 ± 1.761 | 0.1209 ± 0.02689 |
| 4 | 5.120 ± 0.9813 | 0.4584 ± 0.1198 | 1.072 ± 0.2219 | 9.396 ± 0.7698 | 0.2692 ± 0.09812 |
| 7 | 3.638 ± 3.332 | 0.3392 ± 0.1444 | 0.8526 ± 0.4893 | 4.961 ± 7.748 | 0.2095 ± 0.1092 |
| 8 | 0.3749 ± 0.1577 | 0.2922 ± 0.06764 | 0.4718 ± 0.1688 | 0.3357 ± 0.1261 | 0.1271 ± 0.03616 |
| 9 | 0.9188 ± 1.437 | 0.3059 ± 0.1966 | 0.5029 ± 0.5053 | 1.027 ± 1.684 | 0.1595 ± 0.1468 |
| 11 | 0.5129 ± 0.4173 | 0.1632 ± 0.02506 | 0.3577 ± 0.1011 | 0.4300 ± 0.4148 | 0.1091 ± 0.05364 |
| 15 | 0.2264 ± 0.08451 | 0.1498 ± 0.04349 | 0.2403 ± 0.04987 | 0.2348 ± 0.06989 | 0.08235 ± 0.01900 |
| 17 | 0.2471 ± 0.06142 | 0.1253 ± 0.01755 | 0.2077 ± 0.06268 | 0.2361 ± 0.03012 | 0.1006 ± 0.02757 |
| 21 | 0.1248 ± 0.05742 | 0.1143 ± 0.03446 | 0.1553 ± 0.02518 | 0.1199 ± 0.03754 | 0.06480 ± 0.01943 |
| 28 | 0.1010 ± 0.005138 | 0.06804 ± 0.01321 | 0.09776 ± 0.02527 | 0.1280 ± 0.05441 | 0.06160 ± 0.009058 |

***One-compartment models***

As confirmed by the concentrations measured in water (Tables S4 and S5), we consider that concentration in water ($C_w$) is constant during the experiment. As a consequence, Eqs. (1) and (2) can be analytically solved:

$$C_i(t) = \begin{cases} \frac{k_{u,i} \times C_w}{k_{e,i}} + \left( C_{0,i} - \frac{k_{u,i} \times C_w}{k_{e,i}} \right) \times e^{-k_{e,i} \times t} & \text{for } 0 \le t \le t_c \quad \text{(S2)} \\ \frac{k_{u,i} \times C_w}{k_{e,i}} \times e^{-k_{e,i} \times (t - t_c)} + \left( C_0 - \frac{k_{u,i} \times C_w}{k_{e,i}} \right) \times e^{-k_{e,i} \times t} & \text{for } t > t_c \quad \text{(S3)} \end{cases}$$

where $C_{0,i}$ is the gammarids' initial internal concentration in the organ i, at the beginning of the experiment.

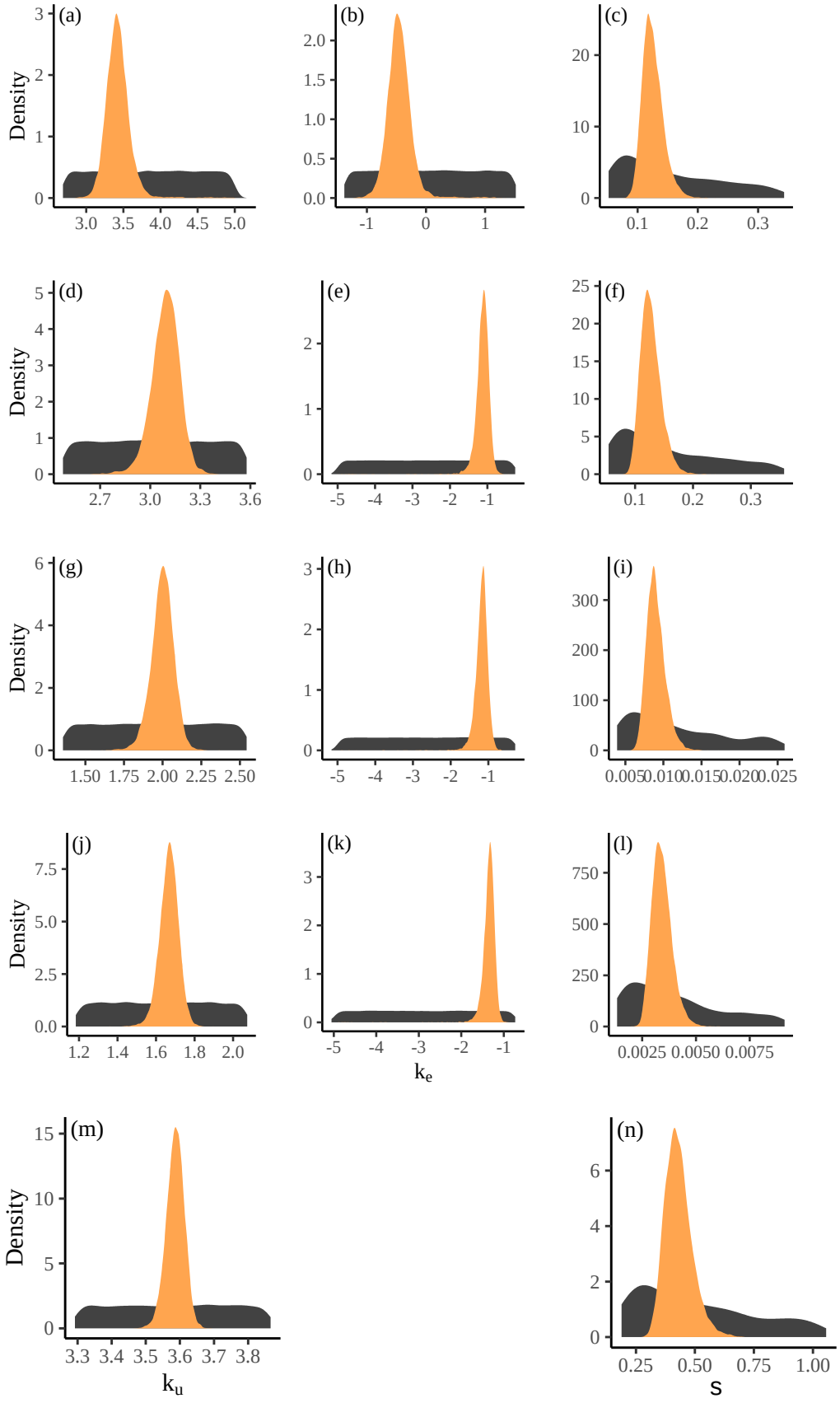



Figure S3. Representation of prior (dark grey) and posterior (orange) distributions of each parameter of the one-compartment model (Eqs. (1) and (2)) fitted to each organ for Cd data set: first line with a), b) and c) for intestines; second line with d) e) and f) for caeca; third line with g), h) and i) for cephalons; fourth line with j), k) and l) for remaining tissues; and last line with m) and n) for gills.

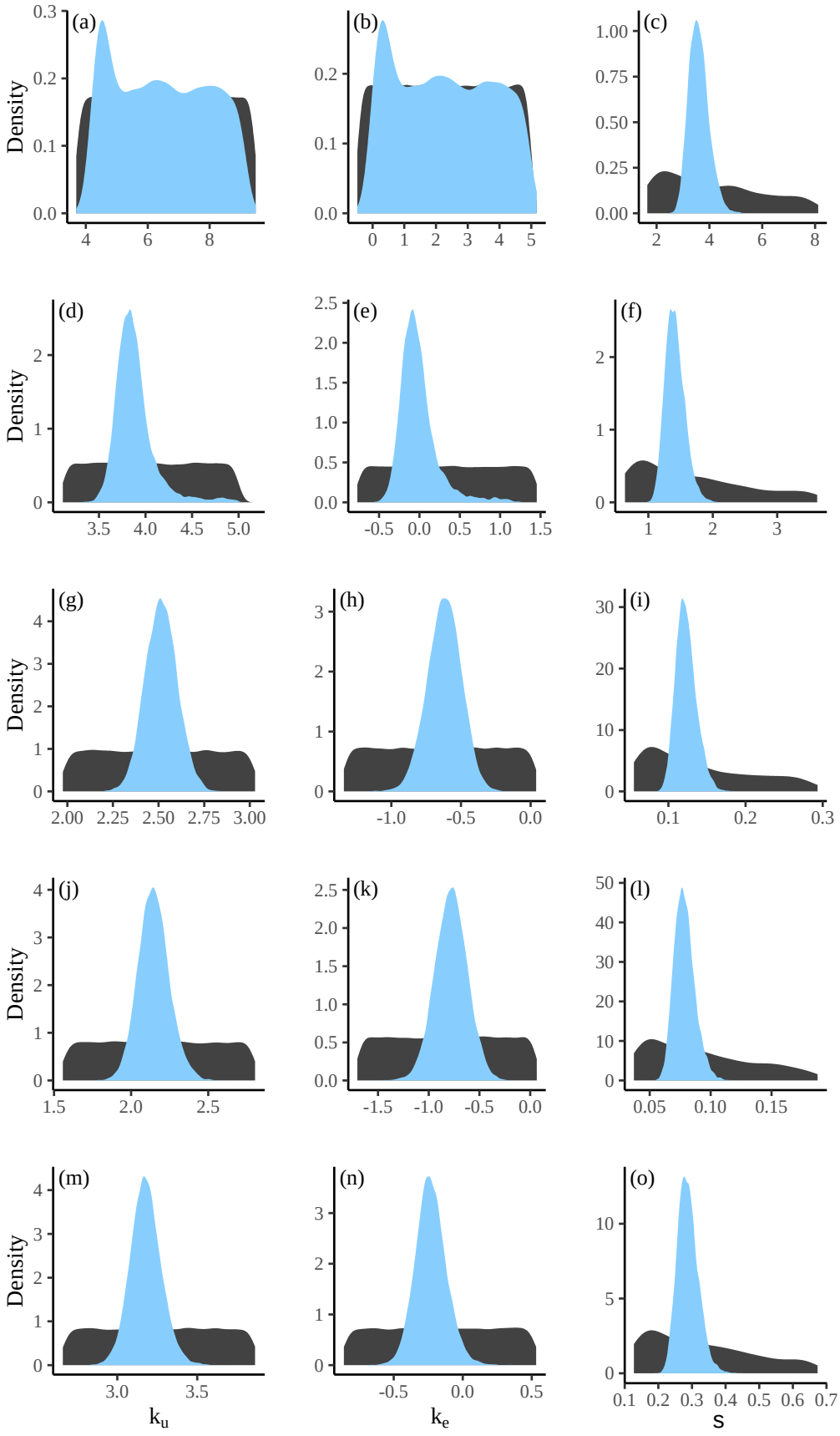



Figure S4. Representation of prior (dark grey) and posterior (blue) distributions of each parameter of the one-compartment model (Eqs. (X) and (Y)) fitted to each organ for Zn data set: first line with a), b) and c) for intestines; second line with d) e) and f) for caeca; third line with g), h) and i) for cephalons; fourth line with j), k) and l) for remaining tissues; and last line with m), n) and o) for gills.

Table S6. Posterior correlations between the parameters $k_{u,i}/k_{e,i}$, $k_{u,i}/\sigma_i$ and $k_{e,i}/\sigma_i$ (i=1 for intestines, i=2 for caeca, i=3 for cephalons, i=4 for remaining tissues and i=5 for gills) estimated by one compartment models for 52.1 ± 27.3 ng $Cd.L^{-1}$ or 416 ± 264 ng $Zn.L^{-1}$.

| | i = 1 - Intestines | | i = 2 - Caeca | | i = 3 - Cephalons | | i = 4 - Remaining tissues | | i = 5 - Gills | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cd | Zn | Cd | Zn | Cd | Zn | Cd | Zn | Cd | Zn |
| $k_{u,i}/k_{e,i}$ | 0.985 | 0.991 | 0.814 | 0.989 | 0.842 | 0.930 | 0.833 | 0.930 | / | 0.947 |
| $k_{u,i}/\sigma_i$ | 0.160 | 0.007 | - 0.053 | 0.145 | - 0.035 | 0.006 | - 0.071 | 0.017 | - 0.026 | 0.036 |
| $k_{e,i}/\sigma_i$ | 0.164 | 0.008 | - 0.017 | 0.147 | - 0.015 | 0.017 | - 0.059 | 0.029 | / | 0.029 |