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

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28 **Abstract**

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

48

49

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

52 **1. Introduction**

53 Naturally present in the earth's crust and potentially released through erosion and leaching,
54 metals are persistent elements due to their non-degradability (Cresswell et al., 2017; Lebrun et
55 al., 2017). They can also be introduced into aquatic ecosystems by anthropogenic activities,
56 such as emissions and runoff from the industrial, urban, and agricultural sectors (Filipović
57 Marijić et al., 2016; Lebrun et al., 2014; Soegianto et al., 2013; Zhang and Reynolds, 2019).
58 Since both essential and non-essential elements can become toxic even at low concentrations
59 following anthropogenic contamination, there is a need for comprehensive research into their
60 bioaccumulation processes and their effects (Lebrun et al., 2017; Ramiro Pastorinho et al.,
61 2009). The deleterious effects resulting from an acute or a chronic exposure to metals are well
62 described in crustaceans. At the molecular and cellular levels, Zn can trigger structural,
63 histological, and immunocytochemical damage with, for example, a deterioration of the
64 cytoskeleton or a large augmentation of their vacuoles (Issartel et al., 2010a; Soegianto et al.,
65 2013). Deleterious effects of Cd on DNA integrity have been reported in several decapods
66 (Frías-Espericueta et al., 2022). Histological analyses have shown an increased cell
67 proliferation in the gills of gammarids, shrimps, and crabs following Cd exposure (Dayras et
68 al., 2017). Moreover, Zn and Cd may, among other things, cause osmoregulation disorders, the
69 induction of reactive oxygen species (ROS), and a decrease in ionoregulation (Frías-
70 Espericueta et al., 2022; Ren et al., 2019). It has been reported that Cd and Zn can also have an
71 impact on oxygen consumption and ammonium excretion (Frías-Espericueta et al., 2022; Jakob
72 et al., 2017). At the organism level, the presence of metals will lead to a decrease in genetic
73 diversity, organism size, and reproduction with, for example, a shorter life span, lower
74 fecundity, and behavioral changes. All these changes can affect the population levels, which in
75 the long-term may decrease overall species survival and richness (Júdová, 2006; Kadiene et al.,
76 2019).

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

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

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

103 Despite their ecological importance, to date freshwater invertebrates have not received such
104 attention in this area of research. This can be explained by the fact that although they have
105 strong bioaccumulation capacities, the low organ weights of small invertebrates imply a low
106 amount of metals, which presents a significant detection challenge for accurate quantification
107 (O'Callaghan et al., 2019). The use of gamma-emitting isotopes such as ^{109}Cd or ^{65}Zn allows
108 us not only to work at relevant environmental concentrations, but also to measure
109 concentrations in the organs of small aquatic invertebrates such as crustaceans, as was done for
110 the decapod *Paratya australiensis* (McDonald et al., 2020). There are very few data on other
111 orders of crustaceans such as amphipods, despite their well-known ecological importance. The
112 species *Gammarus fossarum* is of particular interest to freshwater ecosystems due to their
113 function as a detritivore, giving them a central role in freshwater ecosystems, and in particular
114 in aquatic food webs as an important link between detritus and fish (Filipović Marijić et al.,
115 2016; Kunz et al., 2010). They also have a wide distribution, are present in abundance, and,
116 because of their size and ease of identification, they are easy to sample and handle in the
117 laboratory (Dayras et al., 2017; Issartel et al., 2010b; Lebrun et al., 2017). In addition, they are
118 known to be net accumulators of metals, which explains why gammarids are regularly used to
119 monitor aquatic contaminations (Besse et al., 2013; Conti et al., 2016; Lebrun et al., 2015).

120 It has been shown in crustaceans that essential (Zn) and non-essential (Cd) metals are
121 distributed, managed, and detoxified through different pathways (Nunez-Nogueira et al., 2006).

122 However, there are still many gaps in understanding of the mechanisms that govern the
123 exchange and fate of metals among various organs. To our knowledge, our previous study
124 (Gestin et al., 2021) was the only work to show a dynamic view of metal bioaccumulation along
125 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 $\mu\text{g}\cdot\text{L}^{-1}$) 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 $\text{ng}\cdot\text{L}^{-1}$ and 416 $\text{ng}\cdot\text{L}^{-1}$, 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.

143 **2. Material and methods**

144 *2.1 Collection, maintenance and selection of organisms*

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

151

152 *2.2 Reagents and chemicals*

153 All the material used was decontaminated all along the experiments with HCl solution
154 (Hydrochloric acid S.G. 32 %, certified AR for analysis; Fischer Scientific®) and a Decon® 90
155 solution, both diluted to 1/10 with MilliQ water ($18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$). The radiotracers ^{109}Cd and
156 ^{65}Zn were both obtained in their chloride form (i.e., CdCl_2 and ZnCl_2), respectively 0.1 M and
157 0.5M HCl, from Eckert & Ziegler Isotope Products Inc., Valencia, USA. Both solutions are
158 carrier-free, allowing to work with the smallest equivalent stable concentration as possible
159 (coefficient ng/Bq = 0.182 for ^{109}Cd and 27.96 for ^{65}Zn). Both solutions were diluted to obtain
160 intermediate solution named “D1 solutions” allowing spikes of 20 μl to reach $15 \text{ Bq}\cdot\text{mL}^{-1}$ for
161 ^{109}Cd or ^{65}Zn in the experimental polypropylene beakers during the exposure phase
162 (corresponding to 3 and 420 $\text{ng}\cdot\text{L}^{-1}$ equivalent stable, respectively). The final Cd exposure
163 concentration was increased from 3 to 52.1 $\text{ng}\cdot\text{L}^{-1}$ by adding stable cadmium ($\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$,
164 > 98 %; Merck®; stock solution at 85 $\text{mg}\cdot\text{L}^{-1}$, 0.5 M HCl) to the ^{109}Cd D1 solution. These final
165 concentrations were chosen for their environmental relevance and based on concentrations
166 found in low impacted freshwater media (i.e., <100 and <500 $\text{ng}\cdot\text{L}^{-1}$ for Cd and Zn,
167 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 $12 \pm 0.5^\circ\text{C}$, 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 $20 \text{ Bq}\cdot\text{mL}^{-1}$ (i.e., $50 \text{ ng}\cdot\text{L}^{-1}$ in stable equivalent) of ^{109}Cd or $15 \text{ Bq}\cdot\text{mL}^{-1}$
183 1 (i.e., $416 \text{ ng}\cdot\text{L}^{-1}$ 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 ¹⁰⁹Cd and days 1, 2, 3, 4, 7, 8,
200 9, 11, 15, 17, 21, 28 for ⁶⁵Zn (Fig. S1a.). There is more sampling time-points for the experiment
201 with ⁶⁵Zn, 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 ¹⁰⁹Cd or ⁶⁵Zn) 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 μ L of HCl (3,4 %) at ambient

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

219 In average, gammarids weights were 23.1 ± 1.8 and 23.9 ± 2.5 mg wet weight for ^{109}Cd and
220 ^{65}Zn experiments respectively (Tables S4 and S5). Considering that *G. fossarum* dry weight
221 represents 25 % of the wet weight, the weights of the organs were calculated from estimation
222 of the respective percentage of each dry organ regarding the whole body gammarid total wet
223 weight, i.e., 1.3 % for gills, 2.2 % for intestines, 5 % for caeca, 14 % for cephalons and 77.5 %
224 for the remaining tissues (Tables S4 and S5).

225

226 *2.5 Gamma-spectrometry: ^{109}Cd and ^{65}Zn detection*

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

238

239 *2.6 One-compartment toxicokinetic modelling*

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

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

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

$$249 \quad \frac{dC_i(t)}{dt} = \begin{cases} k_{u,i} \times C_w(t) - k_{e,i} \times C_i(t) & \text{for } 0 \leq t \leq t_c \quad (1) \\ -k_{e,i} \times C_i(t) & \text{for } t > t_c \quad (2) \end{cases}$$

250 where $C_i(t)$ is the internal concentration ($\mu\text{g}\cdot\text{g}^{-1}$ dry weight) in the organ i ($i=1..5$) at time t
 251 (days), $k_{u,i}$ the accumulation rate from water (day^{-1}) for the organ i , $C_w(t)$ the external
 252 concentration in water ($\text{ng}\cdot\text{L}^{-1}$) at time t , $k_{e,i}$ the elimination rate (day^{-1}) for the organ i and t_c
 253 the duration of the accumulation phase (7 days). $i = 1$ corresponds to intestines, $i = 2$ to caeca,
 254 $i = 3$ to cephalon, $i = 4$ to remaining tissues and $i = 5$ to gills.

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

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

$$260 \quad C_{obs,i}(t) \sim \mathcal{N}(C_i(t), \sigma_i) \quad (3)$$

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

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

273 3. Results

274 3.1 Experimental conditions

275 The dissolved concentrations of ^{109}Cd and ^{65}Zn in water were variable during the accumulation
276 phase (Tables S2 and S3). A loss of $60 \pm 33\%$ of Cd concentration and $67 \pm 22\%$ of Zn
277 concentration in water was measured between two re-adjustments, each one occurring at
278 approximately 12 ± 5.7 h (Tables S2 and S3). The bioaccumulation of metals by gammarids
279 could explain only 8% of the Cd and 22% of the Zn losses, suggesting a strong adsorption of
280 elements on the polypropylene beaker walls and plastic baskets. Gammarids were exposed to
281 52.1 ± 27.3 ng.L⁻¹ of Cd and 416 ± 264 ng.L⁻¹ of Zn in stable equivalent. To simplify the model
282 implementation during the accumulation phase, we considered C_w as a constant exposure of
283 52.1 ng.L⁻¹ for Cd and 416 ng.L⁻¹ for Zn. During the depuration phase, the concentration of
284 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
285 negligible.

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

288

289 3.2 Uptake and elimination kinetics of Cd and Zn in gammarid organs

290 **Cadmium (Fig. 1, left panel).** During the accumulation phase, the concentration of Cd in each
291 organ reached maximal values on day 7 (Fig. 1 and Table S4). The rank of organs from the
292 highest to the lowest concentrations of Cd was similar throughout the period, reaching the
293 following values on day 7: gills (1.5 ± 0.97 $\mu\text{g.g}^{-1}$) \gg intestines (0.39 ± 0.24 $\mu\text{g.g}^{-1}$) \approx caeca
294 (0.33 ± 0.071 $\mu\text{g.g}^{-1}$) \gg cephalons (0.035 ± 0.018 $\mu\text{g.g}^{-1}$) $>$ remaining tissues
295 (0.013 ± 0.0044 $\mu\text{g.g}^{-1}$). This rank did not change at the end of the depuration phase (28th day).
296 However, although the intestines and caeca still had higher concentrations than the cephalons
297 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 ($5.0 \pm 7.75 \mu\text{g}\cdot\text{g}^{-1}$) \approx caeca
307 ($3.6 \pm 3.3 \mu\text{g}\cdot\text{g}^{-1}$) > gills ($0.85 \pm 0.49 \mu\text{g}\cdot\text{g}^{-1}$) > cephalons ($0.34 \pm 0.14 \mu\text{g}\cdot\text{g}^{-1}$) \approx remaining
308 tissues ($0.21 \pm 0.11 \mu\text{g}\cdot\text{g}^{-1}$). 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 $0.13 \pm 0.054 \mu\text{g}\cdot\text{g}^{-1}$, caeca at $0.10 \pm 0.0051 \mu\text{g}\cdot\text{g}^{-1}$, gills at
312 $0.098 \pm 0.025 \mu\text{g}\cdot\text{g}^{-1}$, cephalons at $0.068 \pm 0.013 \mu\text{g}\cdot\text{g}^{-1}$, and remaining tissues at
313 $0.062 \pm 0.0091 \mu\text{g}\cdot\text{g}^{-1}$.

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

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

385

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

398 Concerning Zn, the amount of metal in the remaining tissues accounted for one third of the total
399 Zn body burden (Fig. 2) during the exposure phase. In the literature, Nunez-Nogueira and
400 Rainbow (2005) reported that 40% of Zn is associated with the exoskeleton of decapods,
401 *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 $\mu\text{g}\cdot\text{L}^{-1}$) (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

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

432

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

442

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

452 tissue, even during depuration. Regarding the kinetics parameters, the k_u values of Cd in the
453 gills for *Mytilus galloprovincialis*, *Ruditapes decussatus*, and *Oncorhynchus mykiss* (Ju et al.,
454 2011; Rocha et al., 2015) are, respectively, 14, 22, and 326 times lower than the values for
455 *G. fossarum*. This suggests that the gills of gammarids accumulate Cd more rapidly than those
456 of bivalves and fish. However, regarding the elimination rate (k_e) of Cd by the gills, the values
457 were null or very low ($k_e = 6.93 \cdot 10^{-7} \text{ d}^{-1}$) in the bivalves *M. galloprovincialis* and *R. decussatus*,
458 respectively, suggesting that Cd is not eliminated from the gills as observed in gammarids. On
459 the contrary, the trout *O. mykiss* eliminates Cd more rapidly, with a significantly higher k_e value
460 of 0.32 d^{-1} . Cresswell et al. (2017) showed that after 6 h of exposure to $0.56 \pm 0.14 \mu\text{g.L}^{-1}$ of
461 Cd, the concentration in the gills of *M. australiense* decreases rapidly during the depuration
462 phase, whereas it decreases much more slowly when the shrimp were exposed for 7 days.
463 Nevertheless, for gammarids, bioaccumulation data at the organ scale are still lacking for
464 determining whether the absence or very low depuration of Cd by the gills: i) is characteristic
465 of the invertebrate group as opposed to fish; and/or ii) is instead related to the duration of
466 exposure, as already discussed for the crustacean *M. australiense* (Cresswell et al., 2017). Some
467 authors make the assumption that the gills of crustaceans store Cd for a later elimination by the
468 exuviae (Amyot et al., 1994; Nunez-Nogueira et al., 2006). However, there is no consensus on
469 this topic, as other authors have determined that the molting phenomenon has no influence on
470 Cd efflux (Cresswell et al., 2014a). The amount of Cd contained in the exuviae was not
471 measured in this study. Nevertheless, this absence of apparent Cd depuration from the highly
472 concentrated gills leads to two hypotheses: i) the accumulated metal is tightly bound to the
473 cellular components of gills resulting in a long-term storage of Cd (Table 1); and
474 ii) alternatively, stable Cd concentrations during the depuration phase could result from a
475 dynamic balance between the influx rate from the other organs into the gills and the efflux rate
476 from the gills toward the medium. Considering that the other organs showed very low levels of

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

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

483

484 This study provides the base for understanding the organotropism and toxicokinetic of essential
485 and non-essential metals in a sentinel species. Firstly, the measurements obtained for Zn
486 confirmed its good regulation by all organs of gammarids and consistent with the essential
487 character of this element whose accumulation has to meet the metabolomic needs (Amyot et
488 al., 1996; Rainbow and Luoma, 2011). Moreover, this regulation is very rapid, with 65% of the
489 Zn lost in the first 24 h of the depuration phase for all organs. This implies that in the context
490 of biomonitoring, the duration of exposure will not have an impact on the Zn concentrations
491 measured in gammarids. Instead, these concentrations will tend to reflect a constant or a very
492 recent contamination in the environment. On the contrary, the absence of Cd elimination in the
493 gills during the 21 days of the depuration phase shows that this tissue integrates the
494 contamination changes to which the organism is exposed. Indeed, at the end of the depuration
495 phase, it appears that the concentration in the gills is still the same as that measured at the end
496 of the accumulation phase and represents $55 \pm 8.0\%$ of the total dissolved Cd of the whole
497 body. The fact that gills are an organ of Cd accumulation in gammarids is consistent with
498 previous work conducted on *G. pulex* and *G. fossarum* (Felten et al., 2008; Issartel et al.,
499 2010c). The gills can therefore be considered a very good indicator of aqueous Cd
500 contamination, assuming no loss of Cd during the molting event. This storage function makes
501 them an organ of great interest in biomonitoring, but their low mass and difficult extraction

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

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

522 **5. Conclusions**

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

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

534

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542

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545

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755

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.

Organs	Parameters	Priors	[Cd] = 52.1 ± 27.3 ng.L ⁻¹			[Zn] = 416 ± 264 ng.L ⁻¹		
			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}		1249	833	1733	6901	3733	52770
Cephalons	k _{u,3}	log10.Unif (-5, 5)	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}		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}	log10.Unif (-5, 5)	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	σ ₁		0.12	0.10	0.16	3.5	2.9	4.4
Caeca	σ ₂		0.13	0.09	0.16	1.4	1.2	1.7
Cephalons	σ ₃	Gamma (0.001, 0.001)	0.009	0.007	0.011	0.12	0.10	0.15
Remaining tissues	σ ₄		0.003	0.003	0.004	0.08	0.06	0.10
Gills	σ 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); σ_{*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.

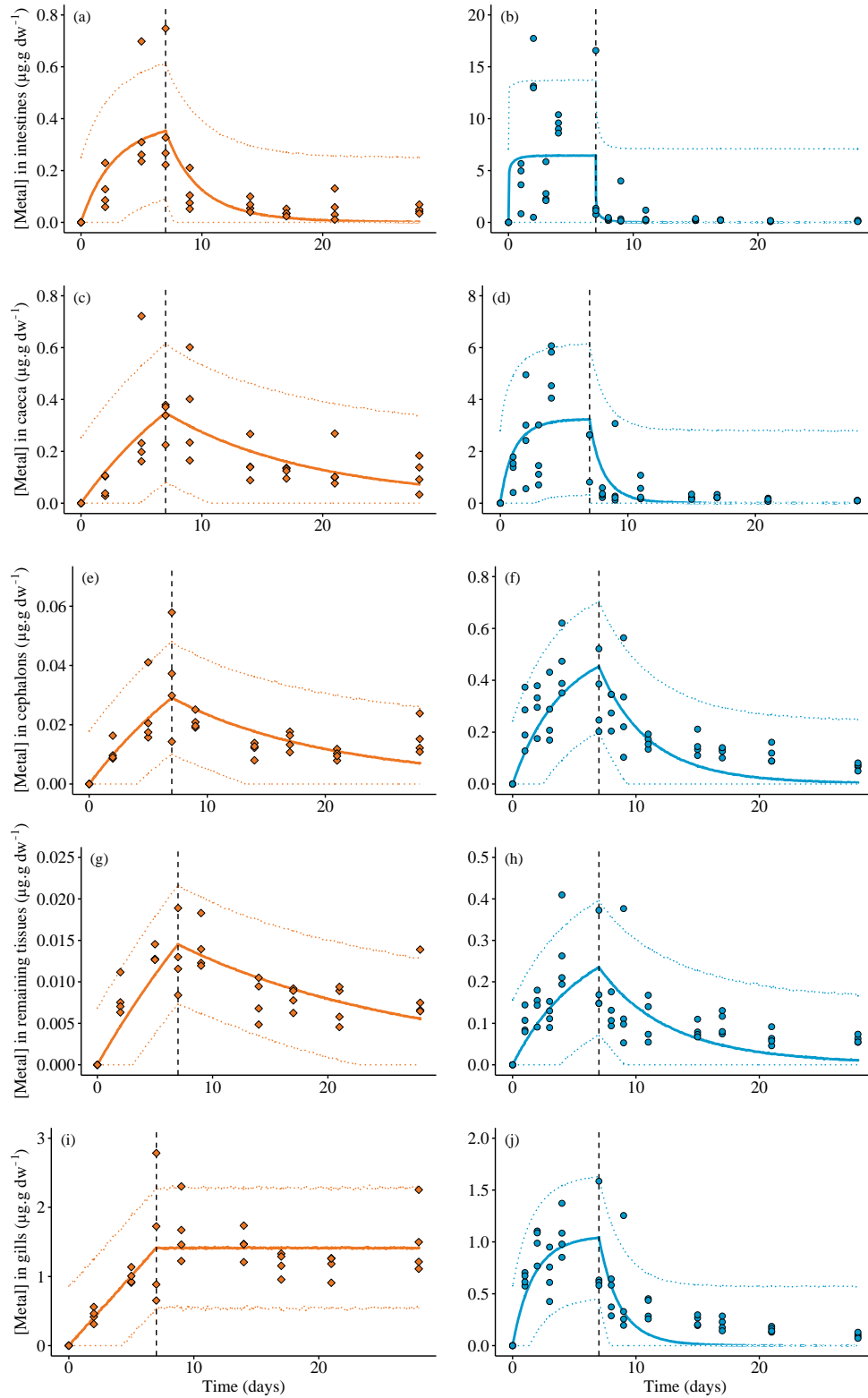


Figure 1. Measured (diamonds for Cd; dots for Zn) and predicted (solid line for the median and dashed lines for the 95% credible interval) concentrations of metals with the one-compartment model (Eqs. (1) and (2)) for a) and b) intestines; c) and d) caeca; e) and f) cephalons; g) and h) remaining tissues; and i) and j) gills of gammarids exposed to $52.1 \pm 27.3 \text{ ng}\cdot\text{L}^{-1}$ of Cd (left column in orange) and $416 \pm 264 \text{ ng}\cdot\text{L}^{-1}$ 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.

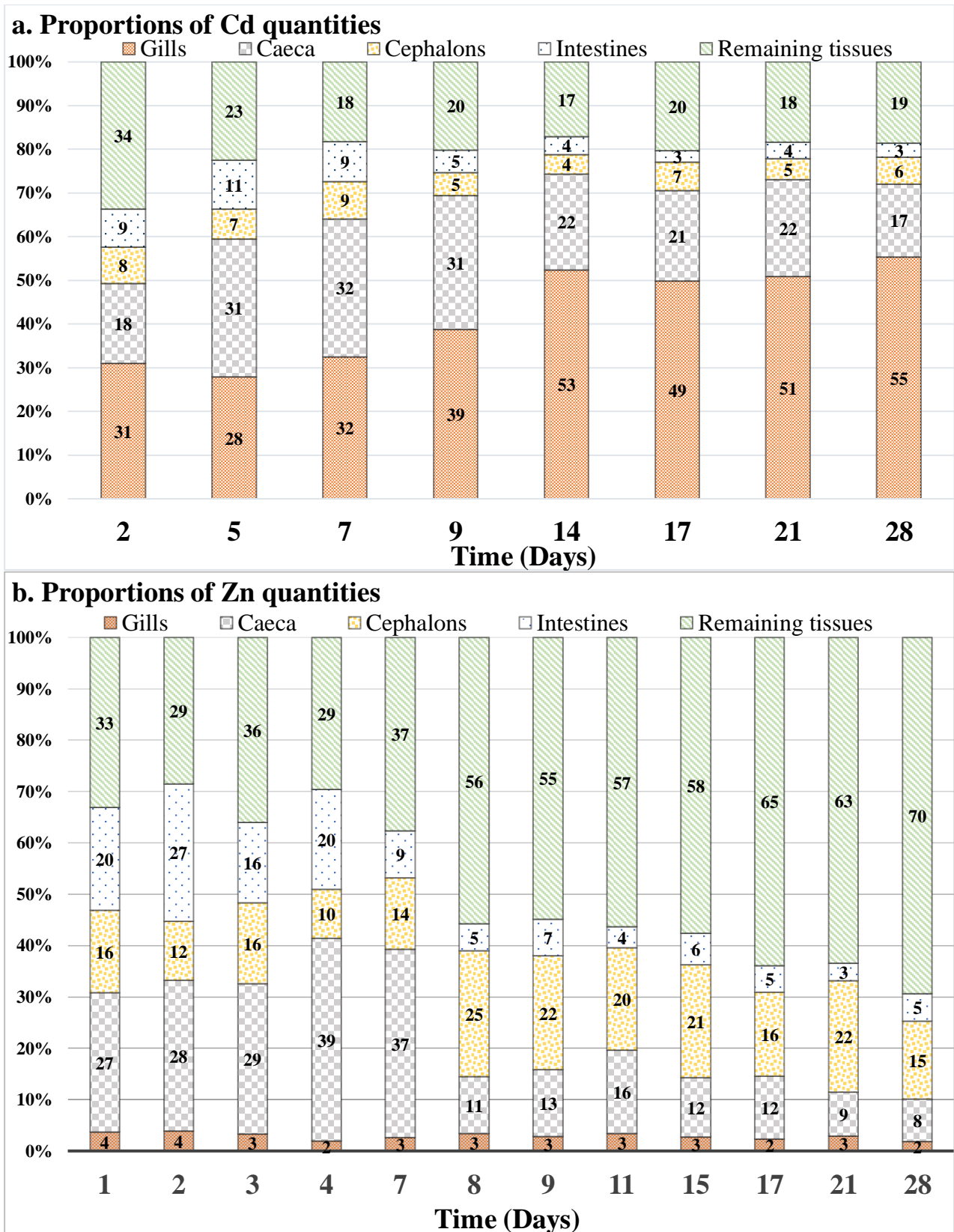


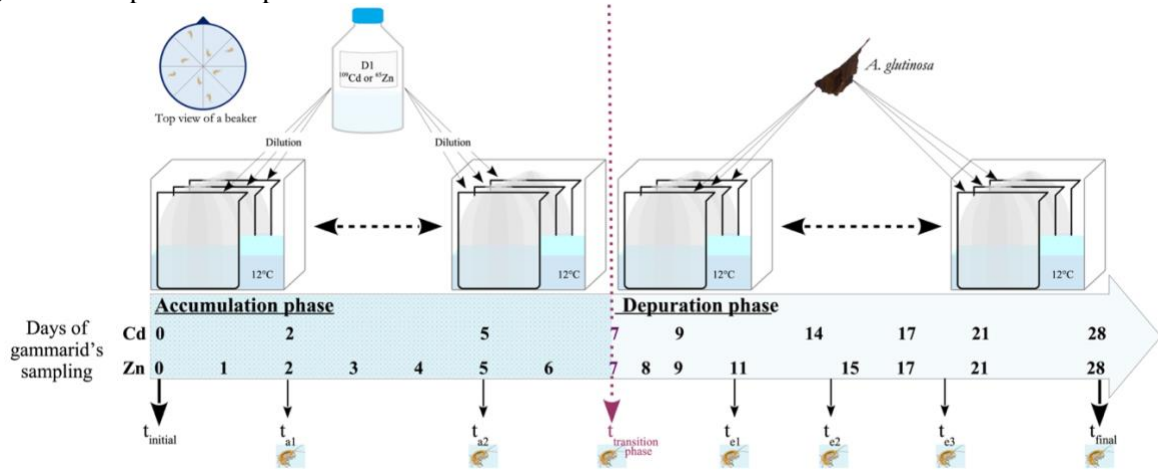
Figure 2. Mean of the relative proportions (%) of metal burdens (figure a. for Cd and b. for Zn) per organ (gills, caeca, cephalons, intestines, and remaining tissues) with respect to the whole-body burden at each sampling time (days) for accumulation (days 0–7) and depuration phases (days 8–28).

Supplementary data

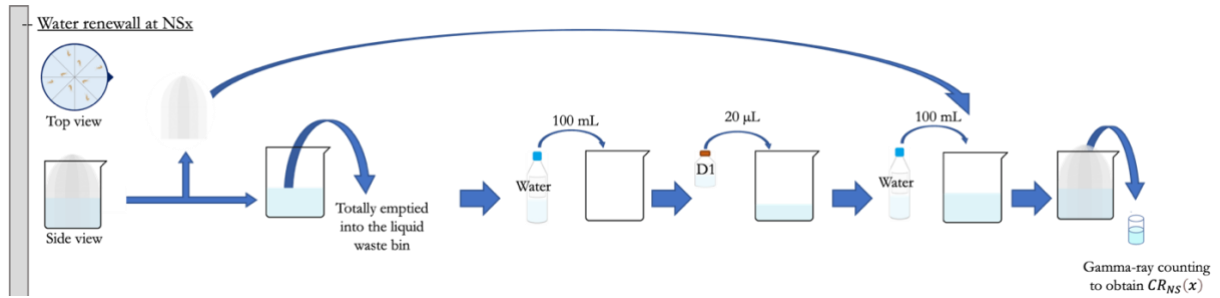
Table S1. Characteristics of the Évian® water used for the experiments.

	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

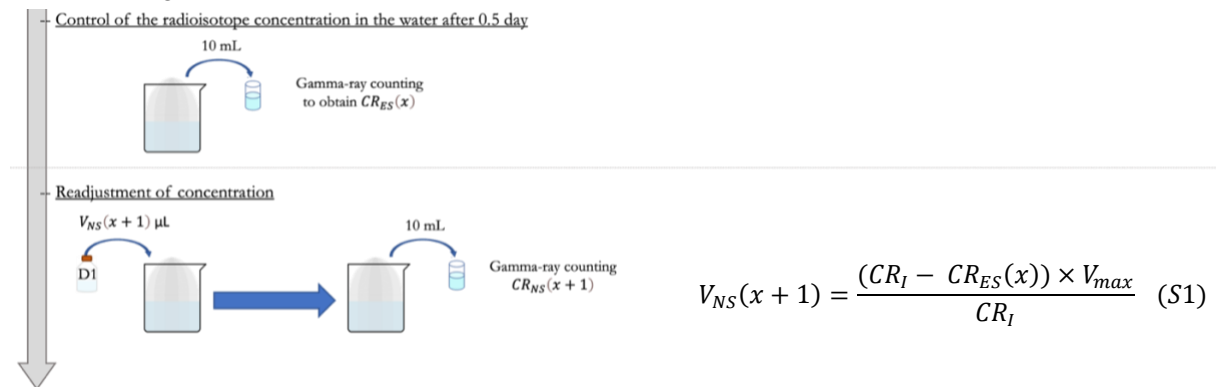


Figure S1. Main parts of the experimental plan. a) Global experimental plan, where $t_{\text{initial}}=0$ is the beginning of the experiment, t_{a1} and t_{a2} are sampling timesteps during the accumulation phase, t_c is the duration of the accumulation phases ($t_c = 7$ days), t_{e1} , t_{e2} and t_{e3} are sampling timesteps during the depuration phase and t_{final} is the total duration of the experiment ($t_{\text{final}} = 28$ 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 gammarids 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 $x+1^{\text{th}}$ 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 $x+1^{\text{th}}$ spike); and V_{max} is the maximum volume that can be spiked to reach the CR_I (i.e. $V_{max} = 0.02$ mL).

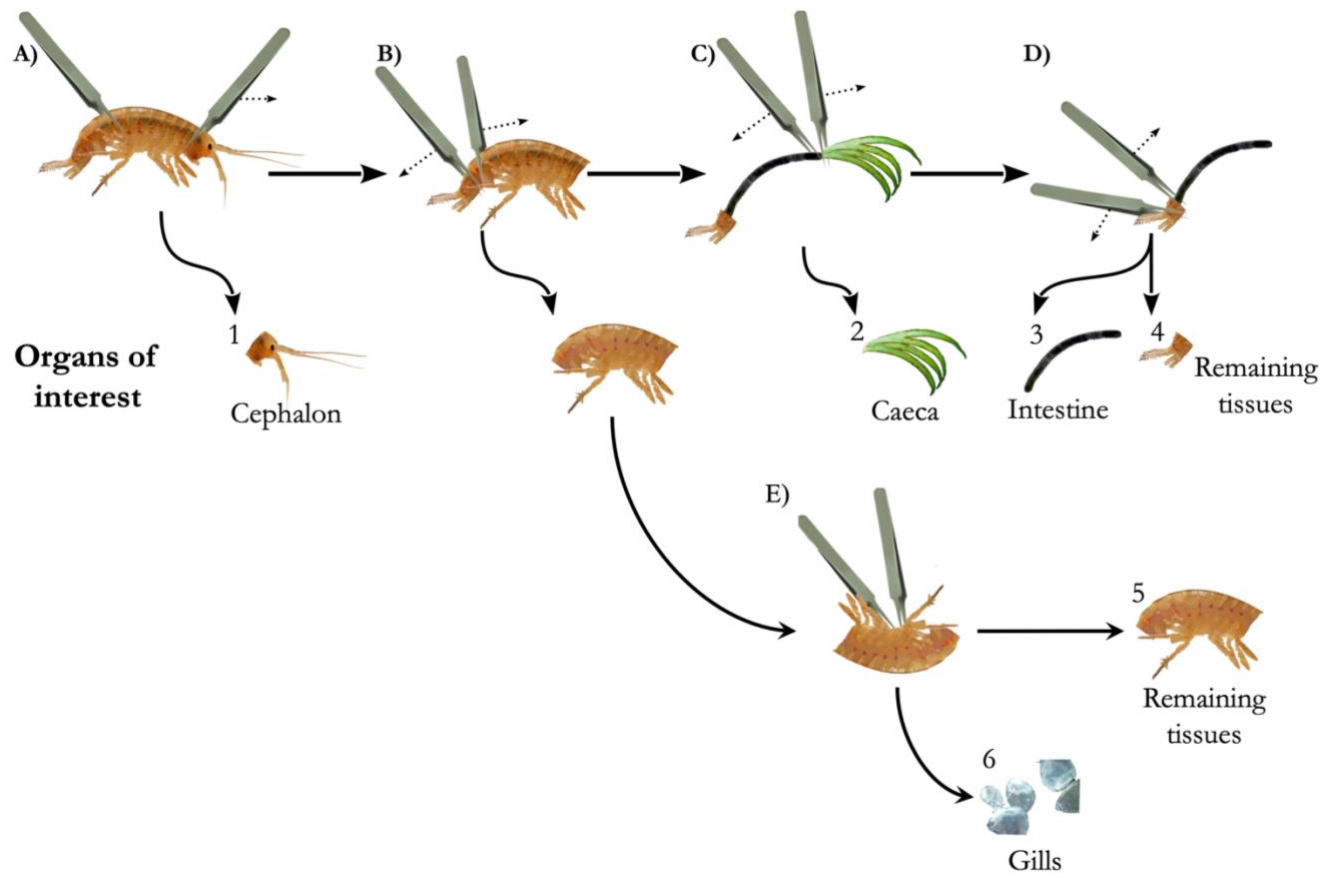


Figure S2. Dissection steps. A) separation of the cephalon from the rest of the organism; B) separation of the thorax and the abdomen from the urosome; C) separation of the caeca from the intestine; D) separation between the urosome and the intestine; and E) separation of the gills from the thorax and 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 \pm SD; Bq.mL^{-1}) and calculated concentrations of Cd in stable equivalent (Mean \pm 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})		Mean measured	Cd in stable equivalent (ng.L^{-1})		
		Nominale	Measured			Nominale	Calculated
-60.0	New spike 1		22.1 \pm 2.26			53.3 \pm 5.65	
0	End spike 1		2.27 \pm 2.38			5.67 \pm 5.96	
0	New spike 2		17.8 \pm 2.49			44.5 \pm 6.22	
7.3	End spike 2		11.8 \pm 3.72			29.4 \pm 9.29	
7.3	New spike 3		18.9 \pm 1.13			47.3 \pm 2.82	
23.2	Middle spike 3		17.1 \pm 5.01			42.8 \pm 12.5	
29.2	End spike 3		12.1 \pm 2.46			30.3 \pm 6.14	
29.2	New spike 4		30.5 \pm 5.85			76.2 \pm 14.6	
48.5	Middle spike 4		23.1 \pm 1.94			57.7 \pm 14.8	
53.0	Middle spike 4 bis		29.6 \pm 9.09			74.0 \pm 22.7	
71.8	Middle spike 4 ter		33.5 \pm 16.6			83.8 \pm 41.5	
80.3	End spike 4	15	16.2 \pm 6.12	18.6 \pm 11.0	50	40.4 \pm 15.3	52.1 \pm 27.3
80.3	New spike 5		34.5 \pm 5.17			86.2 \pm 12.9	
101.5	End spike 5		23.6 \pm 11.9			58.9 \pm 29.6	
101.5	New spike 6		23.2 \pm 2.13			58.1 \pm 5.34	
120.5	End spike 6		7.83 \pm 6.32			19.6 \pm 15.8	
120.5	New spike 7		28.9 \pm 5.65			72.3 \pm 14.1	
127.5	End spike 7		5.52 \pm 8.82			13.8 \pm 22.0	
127.5	New spike 8		25.5 \pm 6.26			63.9 \pm 15.6	
144.8	End spike 8		5.44 \pm 5.77			13.6 \pm 14.4	
144.8	New spike 9		19.1 \pm 4.87			47.7 \pm 12.2	
151.8	End spike 9		7.04 \pm 5.26			17.6 \pm 13.2	
151.8	New spike 10		24.7 \pm 5.24			61.8 \pm 13.1	
167.0	End spike 10		6.89 \pm 5.45			17.2 \pm 13.6	

Table S3. Measured concentrations of ^{65}Zn (Mean \pm SD; Bq.mL^{-1}) and calculated concentrations of Zn in stable equivalent (Mean \pm 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 \pm 1.250			408.7 \pm 34.94	
-60.0	End spike 1		10.21 \pm 1.283			285.6 \pm 35.88	
-60.0	New spike 2		23.47 \pm 3.029			656.7 \pm 84.68	
0.0	End spike 2		1.214 \pm 0.4714			33.9 \pm 13.18	
0.0	New spike 3		14.53 \pm 0.9808			406.2 \pm 27.42	
7.2	End spike 3		7.397 \pm 0.8870			206.8 \pm 24.80	
7.2	New spike 4		19.42 \pm 2.362			543.0 \pm 66.04	
23.4	End spike 4		8.665 \pm 1.157			242.2 \pm 32.35	
23.4	New spike 5		24.79 \pm 5.110			693.1 \pm 142.9	
29.8	Middle spike 5		14.90 \pm 2.281			416.6 \pm 63.78	
48.6	End spike 5		10.25 \pm 1.757			286.6 \pm 49.12	
48.6	New spike 6		16.24 \pm 1.133			454.0 \pm 31.69	
55.0	End spike 6		7.432 \pm 3.969			207.8 \pm 111.0	
55.0	New spike 7		22.44 \pm 6.707			627.3 \pm 187.5	
72.1	End spike 7		6.276 \pm 6.937			175.5 \pm 193.9	
72.1	New spike 8	15	20.81 \pm 9.351	14.89 \pm 9.3	420	581.8 \pm 261.4	416 \pm 263.8
78.7	End spike 8		10.52 \pm 9.721			294.1 \pm 271.8	
78.7	New spike 9		23.47 \pm 11.07			656.2 \pm 309.5	
96.0	End spike 9		10.55 \pm 10.57			295.0 \pm 295.6	
96.0	New spike 10		25.68 \pm 15.14			717.9 \pm 423.2	
102.7	End spike 10		16.45 \pm 13.26			459.9 \pm 370.6	
102.7	New spike 11		22.47 \pm 15.19			628.2 \pm 424.8	
121.3	End spike 11		15.83 \pm 11.51			442.6 \pm 321.8	
121.3	New spike 12		18.15 \pm 5.009			507.5 \pm 140.0	
126.8	End spike 12		6.210 \pm 6.576			173.6 \pm 183.8	
126.8	New spike 13		19.42 \pm 7.644			542.8 \pm 213.7	
143.9	End spike 13		3.710 \pm 3.171			103.7 \pm 88.64	
143.9	New spike 14		18.24 \pm 7.702			510.0 \pm 215.3	
150.8	End spike 14		10.46 \pm 6.144			292.5 \pm 171.8	
150.8	New spike 15		18.89 \pm 7.179			528.2 \pm 200.7	
167.9	End spike 15		11.39 \pm 7.945			318.5 \pm 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 \pm SD; Bq) in organs of gammarids; b) weight of the gammarids organs sampled from dissections estimated for each organ (Mean \pm SD; mg) from the total weights weighed; and c) concentrations of Cd in organs calculated in stable equivalent (Mean \pm SD; $\mu\text{g Cd.g organ in dry weight}^{-1}$).

a)

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

b)

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

c)

Day of sampling	Mean concentration of Cd in organs, calculated in stable equivalent ($\mu\text{g Cd.g organ dw}^{-1}$)				
	Caeca	Cephalons	Gills	Intestines	Remaining tissues
2	0.06899 \pm 0.04162	0.01089 \pm 0.003644	0.4377 \pm 0.1035	0.1255 \pm 0.07469	0.008012 \pm 0.002159
5	0.3282 \pm 0.2639	0.02369 \pm 0.01176	0.9942 \pm 0.1044	0.3761 \pm 0.2169	0.01317 \pm 0.0009170
7	0.3284 \pm 0.07127	0.03484 \pm 0.01813	1.512 \pm 0.9656	0.3910 \pm 0.2419	0.01296 \pm 0.004395
9	0.3505 \pm 0.1946	0.02115 \pm 0.002753	1.665 \pm 0.4629	0.1106 \pm 0.06998	0.01412 \pm 0.002916
14	0.1587 \pm 0.07610	0.01169 \pm 0.002566	1.470 \pm 0.2160	0.06559 \pm 0.02560	0.007901 \pm 0.002561
17	0.1223 \pm 0.01872	0.01451 \pm 0.003128	1.183 \pm 0.1686	0.03641 \pm 0.01203	0.008032 \pm 0.001342
21	0.1364 \pm 0.08885	0.009873 \pm 0.001634	1.154 \pm 0.1677	0.05729 \pm 0.05270	0.007162 \pm 0.002362
28	0.1117 \pm 0.06423	0.01551 \pm 0.005840	1.519 \pm 0.5172	0.04859 \pm 0.01483	0.008611 \pm 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 ⁶⁵Zn (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 ($\mu\text{g Zn.g organ dw}^{-1}$)				
	Caeca	Cephalons	Gills	Intestines	Remaining tissues
1	1.277 \pm 0.6006	0.2440 \pm 0.1081	0.6416 \pm 0.05790	3.773 \pm 2.136	0.1038 \pm 0.02955
2	2.735 \pm 1.810	0.2956 \pm 0.08714	0.9871 \pm 0.1551	11.09 \pm 7.401	0.1423 \pm 0.03749
3	1.573 \pm 1.011	0.2741 \pm 0.1159	0.6857 \pm 0.2227	3.245 \pm 1.761	0.1209 \pm 0.02689
4	5.120 \pm 0.9813	0.4584 \pm 0.1198	1.072 \pm 0.2219	9.396 \pm 0.7698	0.2692 \pm 0.09812
7	3.638 \pm 3.332	0.3392 \pm 0.1444	0.8526 \pm 0.4893	4.961 \pm 7.748	0.2095 \pm 0.1092
8	0.3749 \pm 0.1577	0.2922 \pm 0.06764	0.4718 \pm 0.1688	0.3357 \pm 0.1261	0.1271 \pm 0.03616
9	0.9188 \pm 1.437	0.3059 \pm 0.1966	0.5029 \pm 0.5053	1.027 \pm 1.684	0.1595 \pm 0.1468
11	0.5129 \pm 0.4173	0.1632 \pm 0.02506	0.3577 \pm 0.1011	0.4300 \pm 0.4148	0.1091 \pm 0.05364
15	0.2264 \pm 0.08451	0.1498 \pm 0.04349	0.2403 \pm 0.04987	0.2348 \pm 0.06989	0.08235 \pm 0.01900
17	0.2471 \pm 0.06142	0.1253 \pm 0.01755	0.2077 \pm 0.06268	0.2361 \pm 0.03012	0.1006 \pm 0.02757
21	0.1248 \pm 0.05742	0.1143 \pm 0.03446	0.1553 \pm 0.02518	0.1199 \pm 0.03754	0.06480 \pm 0.01943
28	0.1010 \pm 0.005138	0.06804 \pm 0.01321	0.09776 \pm 0.02527	0.1280 \pm 0.05441	0.06160 \pm 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:

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

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

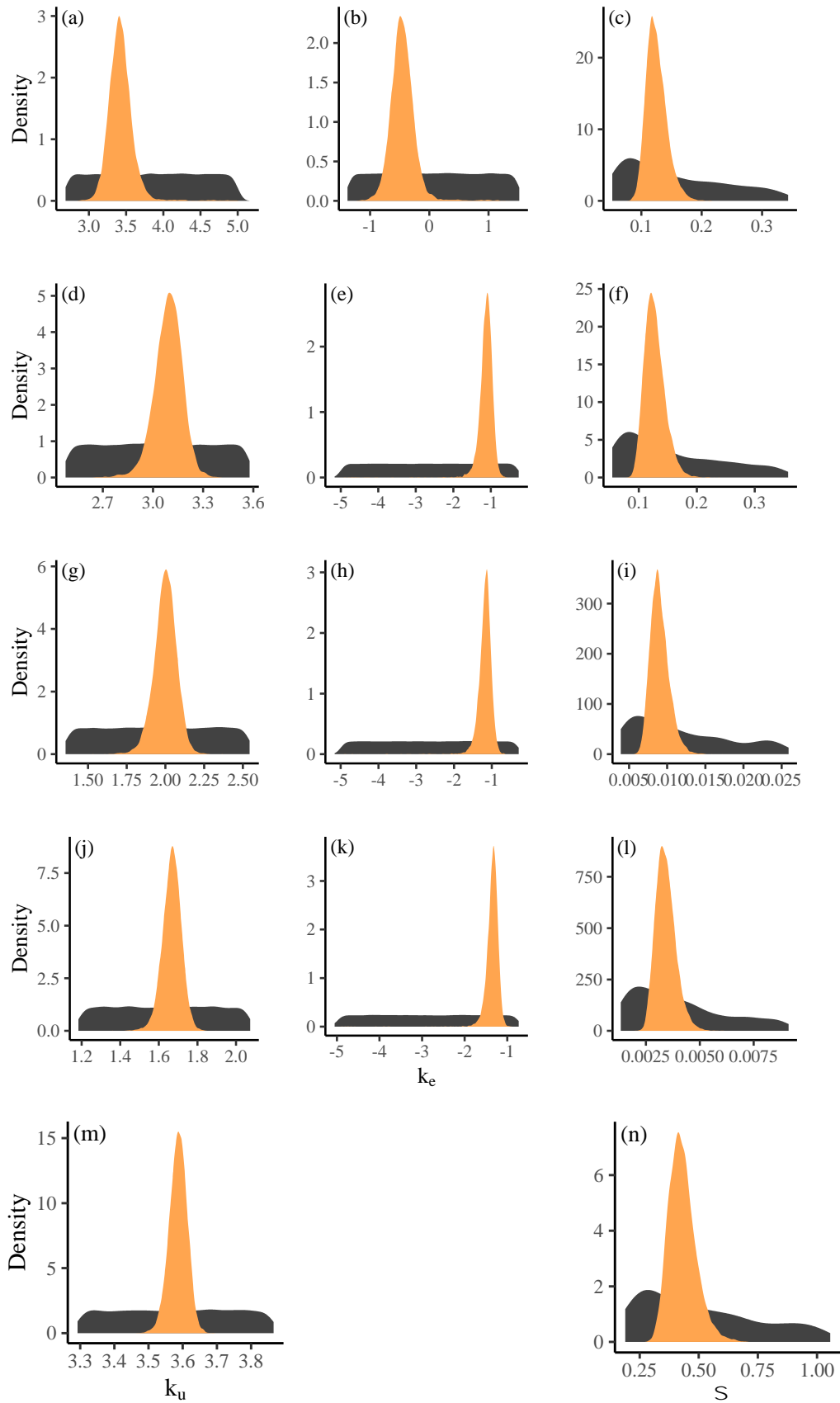


Figure S3. Representation of prior (dark grey) and posterior (orange) distributions of each parameter of the one-compartment model (Eqs. (1) and (2)) fitted to each organ for Cd data set: first line with a), b) and c) for intestines; second line with d) e) and f) for caeca; third line with g), h) and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

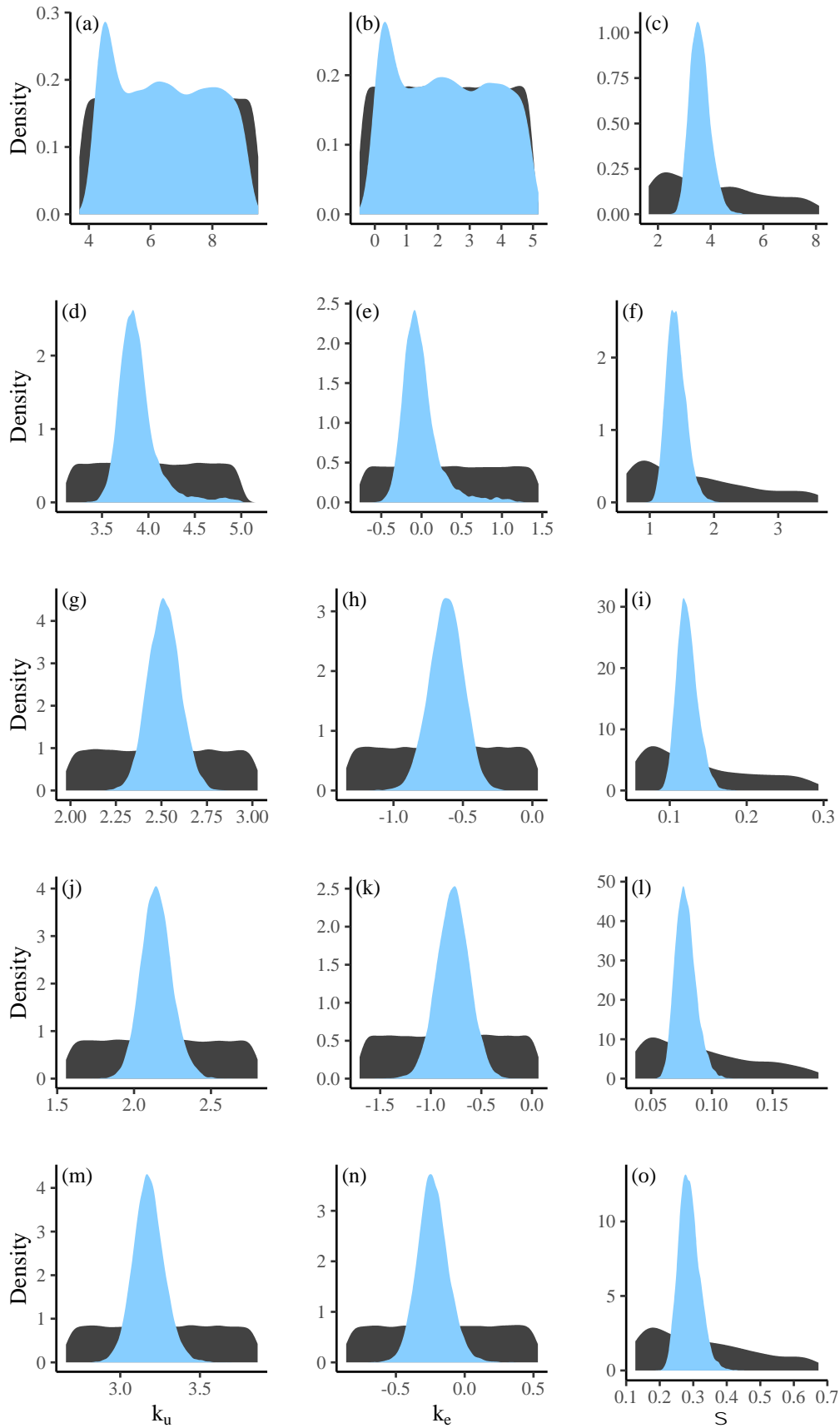


Figure S4. Representation of prior (dark grey) and posterior (blue) distributions of each parameter of the one-compartment model (Eqs. (X) and (Y)) fitted to each organ for Zn data set: first line with a), b) and c) for intestines; second line with d) e) and f) for caeca; third line with g), h) and i)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