Peer Community In
Ecotoxicology & Environmental Chemistry

Food type influences dietary metal uptake and elimination in Gammarus fossarum

Patrice Couture based on peer reviews by **Valentin Geslin** and **Davide Anselmo Luigi Vignati**

Ophélia Gestin, Christelle Lopes, Nicolas Delorme, Laura Garnero, Olivier Geffard and Thomas Lacoue-Labarthe (2024) Assimilation efficiencies and elimination rates of silver, cadmium and zinc accumulated by trophic pathway in *Gammarus fossarum*. bioRxiv, ver. 4, peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry. https://doi.org/10.1101/2023.07.14.549054

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Given their narrow associations with human civilization, including urban, agricultural and industrial settings, freshwater systems worldwide are primary recipients of contaminants from anthropogenic origins, threatening biodiversity (Dudgeon 2019). Freshwater invertebrates are typically abundant in these environments. They are easily sampled, and several species can also be raised in the laboratory. Furthermore, they have the propensity to accumulate contaminants from their environments through both aqueous and dietary routes. These traits make them ideally suited as bioindicators of environmental contamination and for the study of the mechanisms of contaminant uptake and effects. Therefore, over the last decades, several studies have investigated the bioaccumulation and toxicity of a wide range of organic and inorganic contaminants. Knowledge of the relative importance of the aqueous and dietary exposure routes is key to understanding the processes involved in contaminant uptake and organismal and ecological consequences. Although the mechanisms of aqueous uptake have received much attention in recent literature, those associated with dietary uptake are far less known. This is the case for species commonly used for biomonitoring environmental contamination such as the amphipod *Gammarus fossarum*, and for metals of major concern for the Water Framework Directive (WFD) such as Ag, Cd and Zn.

To address these knowledge gaps, Gestin et al (2024) investigated the assimilation efficiency (AE) of Ag, Cd and Zn from two contrasting types of food, one plant (alder leaves) and one invertebrate (*Chironomus riparius* larvae) for gammarids using a pulse-chase-feeding method in a laboratory setting. Food was radiolabeled and fed for a short period to gammarids (3 to 5 hours for alder leaves and 1 hour for chironomid larvae), after which they were left to depurate for 14 days, during which period they were fed with uncontaminated alder leaves.

During the depuration period, gammarids were monitored to follow radioactivity using a gamma counter. A nonlinear least squares modelling approach was used to estimate assimilation efficiencies and elimination rates of the metals from each food source.
From this data, the authors concluded that Cd was assimilated with a higher efficiency, followed by Zn, with Ag showing the lowest AE. Their data also showed that the AE of Cd and Zn was higher when gammarids were fed alder leaves compared to chironomid larvae. In contrast, elimination rates were not different among metals but varied between food types, with metals from chironomids being eliminated more slowly than those from alder leaves. Elimination rate and AE of Ag could not be determined for gammarids fed chironomid larvae, due to undetectable radioactivity. This study highlights that the assimilation and elimination rates of metals ingested from food depend on their chemical properties and on the way the metals are stored in prey. The data needs to be interpreted by taking into consideration that since chironomid larvae were live prey, they could internalize the metals and make it more difficult to accumulate for the gammarid consumer, compared to the inert matrix of dead alder leaves. This study will contribute to improving toxicokinetic models needed to improve regulatory guidelines for metals in freshwater systems.

References:

Dudgeon, D. (2019). Multiple threats imperil freshwater biodiversity in the Anthropocene. Current Biology 29(19):R960-R967. https://doi.org/10.1016/j.cub.2019.08.002

Gestin, O., Lopes, C., Delorme, N., Garnero, L., Geffard, O., Lacoue-Labarthe, T. (2024). Assimilation efficiencies and elimination rates of silver, cadmium and zinc accumulated by trophic pathway in Gammarus fossarum. bioRxiv, 2023.07.14.549054, ver.4 peer-reviewed and recommended by Peer Community In Ecotoxicology and Environmental Chemistry. https://doi.org/10.1101/2023.07.14.549054

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2023.07.14.549054
Version of the preprint: 3

Authors' reply, 16 May 2024

To the editors of PCI Ecotox. Env. Chem.,
Thank you for your careful review of our manuscript. The changes made in this second revised version have been highlighted in green and the responses have been made directly in the document attached, in response to the comments.
Thank you for your consideration of our manuscript.
Sincerely,
Ophélia GESTIN
Download tracked changes file

Decision by Patrice Couture, posted 10 May 2024, validated 13 May 2024

Revisions required

Dear Ophélia Gestin,

I have reviewed your revised manuscript and your replies to the reviewer's comments and suggestions. While I find your replies generally correct, there is one that needs further attention. I pasted it in a comment in your manuscript. I also thoroughly reviewed your revised manuscript. I identified a number of grammatical errors, issues with decimals and other minor details.

Please make the requested corrections and resubmit your manuscript. I attach the annotated document for your consideration.

Once again, thank you for considering PCI EcotoxEncChem for this interesting work. **Download recommender's annotations**

Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2023.07.14.549054

Version of the preprint: 2

Authors' reply, 02 May 2024

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Decision by Patrice Couture, posted 08 March 2024, validated 08 March 2024

Revisions requested

Dear author,

Thank you for submitting your manuscript to PCI EcotoxEnvChem. We are fully aware of the excessively long period it took to review this manuscript and we apologize for this.

We have now received two reviews. Based on these reviews, we encourage you to submit a revised manuscript, along with a documents listing each of the suggestions of the reviewers and the actions you took to address them, or why you chose to ignore them.

Do not hesitate to contact us for any question regarding the reviewing process.

Reviewed by Valentin Geslin, 06 September 2023

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Reviewed by Davide Anselmo Luigi Vignati, 04 March 2024

Dear members of the managing board of PCI Ecotox Env Chem,

the contribution by Gestin et al. studies the assimilation efficiencies and elemination rates of Ag, Cd and Zn in gammarids via the trophic pathway using radio-labelling approaches. The is of interest considering that much of current knowledge and hazard/risk assessment on trace element accumulation is based on uptake or exposure via the aqueous route.

I have three general comments and a few specific ones that are detailed below.

In the introduction, the specific aims of the paper can be stated more precisely to better explain how the present study specifically advances current knowledge. Lines 112-113 state that the aim of this study was to determine AE and elimination rates of Ag, Cd and Zn in G. fossarum. I am perfectly comfortable with the new data produced by the study, but I would appreciate more information on why this aim was chosen (see also specific comments).

In the results and discussion section, the implications and ramifications of this research also deserve some explanations. At lines 311-312, the authors state that "...there is no general rule for predicting AE among metals and biological species". However, our ultimate objective remains precisely to find those general rules rather than go on performing experiments on a metal- and organism-specific basis. In my opinion, it would be very interesting to use the results from the present study to put forward hypotheses on how we can work toward that ultimate objective. Do we need to change methods/approaches in labelling studies so that activity in exposed organisms can be quantified in specific cellular compartments? Do we need a combination of pulse-and chase experiments with other techniques? A little bit less ambitious, but equally important, how does the present study advances the specific knowledge on gammarids exposed to Cd, Ag and Zn?

The document is sufficiently well writter and generally easy to read. Figures and tables are clear (and Figure 1 is commendable in its clarity). The authors may still want to proofread the text for a number of minor typos and minor errors in English usage such as subject in the plural and verb in the singular.

SPECIFIC COMMENTS

Title. Please consider if "Assimilation efficiencies and elimination rates of Ag, Cd and Zn accumulated...." would not be more appropriate. We are dealing with these three specific elements. Unless some general principles for other elements can be derived from the study, I would prefer the more specific title.

Abstract. The abstract adequately reflect the content of the article. Verify if changes may be needed after adressing the general comments.

Keywords. Do we need both "trophic transfer" and "dietary pathway' in key-words? The two imply different concepts, but are practically the same within the purposes of the present article.

Introduction

Lines 59-60 and line 64. Can you please cross-check the statements in these portions of the text? Lines 59-60 state that G. fossarum is known to accumulate diet-borne metals, while line 64 states that less is known regarding the metal diet-borne assimilation and elimination. The two statements are not mutually exclusive. However, is it possible to be more specific with regard to what is actually less-known? Are available studies limited to elements other than those considered here? Do we lack clear numerical values for assimilation efficiencies and elimination rates for the metals considered in the study, despite having the corresponding values for uptake for aqueous route?

Line 73. Se is a metalloid. Consider using the term 'elements' instead of 'metals'.

Lines 72-77. Please verify if the references cited in this part of the text are those you actually wanted to use. I screened their abstracts, figures and conclusions. Borgmann et al. (1989) seems to be about methodological developments to optimize ecotoxicity testing in invertebrates, but does not mention exposure routes. Xu and Pascoe (1994) worked with Gammarus pulex, not with Daphnia magna. Pellet et al. (2014) is appropriate except that they could determine an AE only in the case of Cd.

Lines 75-77. I agree that that metal handling by organisms and metal toxicity vary according to the exposure route. However, the reference cited at lines 72-75 do not fully support this statement. None of those references actually studies AE and toxicity at the same time (or I missed it). If this is the case, it would be better to say that "previous results suggest" or "may imply that"...rather than the strong statement of a causal relationship used in the original text.

Line 84: crosses

Lines 112-113. Is it possible to have some more details on the scientific reasons for choosing these elements? Do we have a lot of information on uptake via dissolved pathway and need reliable data on the dietary pathway for comparison? Do we already have data on uptake via the dietary pathway, but data are highly uncertain and this study tries to understand why?

Lines 114-120. This part is really an excellent way to conclude the introduction. May I just ask for a little bit more information on the actual specific aims of the study? Determing AE and other parameters is a perfectly acceptable scientific objective and so is the study of the influence of food type on diet-borne accumulation. On the other hand, the introduction does not really tell us what we already know on the three elements that are considered in the present study nor on the specific influence of food type on AE of these elements for G. fossarum.

If nothing is known on the selected elements in terms of diet-borne AE, please say so in an explicit way. You are filling a knowledge gap for fundamental information, which is a worthy objective in itself. If previous but incomplete information on these elements exists, please be sure to precisely mention somwhere in the introduction what is already known on them and then explain at the end of the introduction how the present work advances current knowledge

Line 131. Could you please be more specific? Does 'regular renewal' means 'daily renewal' or other intervals?

Lines 146-147. I am not sure to understand properly. I would say that Cd and Zn solutions were provided in a 0.1M HCl matrix. Saying that Cd and Zn were provided in their chloride form means, in my opinion, that CdCl2 and ZnCl2 were dissolved into the matrix. Analogous observations apply to Ag. Please consider if changes are necessary.

Line 153-154. Can you please explain how the conversions were performed (maybe in the supporting information) or provide a reference with the details of the procedure?

Line 157. Does this mean about 108 leaf discs for each element or 108 discs in total for the three elements?

Was there any specific reason for choosing the levels of activity used to label leaf discs? Is it possible (useful?) to provide the corresponding values in µg/L?

Line 162. Please cross-check with line 139. At line 139, we say that chironomids were fed till the second larval stage. At this point, we have third instar larvae. If this is not a typo, please explain what happened while larve grew from the second to the third instar.

Line164. Same remark as line 157 for choosing activity levels.

Line 167. Please specify the rinsing procedure. Was it a 5-day soaking as for leaf discs?

Line 178. What procedure was used to assess which larve had eaten the most? Were the remains of leaves dried and weighed? Were pictures taken to assess the extension of the eaten surface?

Figure 1. Thank you for providing this clear overview of the experimental procedure. In the uppermost part of the figure, on the right-hand side, is it correct to indicate that gammarids were fed chironomids contaminated with 110Ag? At lines 168-169, the text says that no activity was detected in this case. Line 172 also seem to suggest that, in the end, gammarids were not fed chironomids labeled with Ag. Can you please clarify and decide if the figure needs to be modified?

Lines 222-223. Was a specific R package used or did the authors write the code? Please specify.

Lines 226-234. Please consider if this part of the text can be moved elsewhere in section 3. I do not have objections to the concepts put forward in the text. However, I would expect being shown the results of the present study before reading this kind of comments.

Line 236. May I suggest to modify the title of this subsection? What is described in section 3.1 is actually "data quality evaluation and selection".

Section 3.2. I am missing a brief discussion of the kinetics models obtained from feeding gammarids with labeled chironomids. Modelling data for Cd and Zn are presented in Table 1, but only data for gammarids fed with alder leaves are discussed in the text.

Furthermore, in the case of Cd, more than half of the gammarids fed with chirnomids had body activities below 150Bq and were excluded from the model. Excluding low-activity specimen was a sensible choice to avoid working with unreliable raw data, but please discuss if and how the exclusion of these low-activity specimen could have affected model calculations.

Figure 2. In the figure caption, please specify that graphs on the left refer to gammarids fed with alder leaves and graphs on the right to gammarids fed with chironomids.

Table 1. Raw data in Tables S1 and S2 (and figure S1) show a large variability in the activities measured in each exposed gammarid. On the other hand, the errors on the estimates presented in table 1 rarely exceed 10%. Is it possible to comment on this?

I would also be curious to see a comparison (in the supporting information) between the experimentally measured values and the corresponding modelled values to get a glimpse in the overall predictive strength of the model.

Line 295. Correct 'infinitely' to 'indefinitely'.

Line 311-313. This remains indeed a big challenge. On the other hand, what can the community do to overcome this obstacle? While discussing kinetics of Ag elimination (lines 285-295), the authors correctly stressed the importance of intracellular compartimentalization to understand how gammarids handle Ag. What are the implications of these phenomena in relation to the applicability of pulse and chase experiments to determine AE? More specifically, how do the results from the present study advance knolwedge on gammarids exposed to Ag, Cd and Zn? Which questions are answered and which new ones are brought about? See also introductory comments.

Lines 320-321. Lam and Wang (2006) and Pellet et al. (2014) do not deal with D. polymorpha. Is it appropriate to cite them here?

Line 327. Please define the acronym TAM (Trophically Available Metals) here. Otherwise, define it at line 330.

Line 331. Do similar consideration apply to organism fed with alder leaves?

Lines 339-341. I am not sure that these lines support the statement at lines 336-338. An AE of 42% from algae is not higher than an AE 74% from squid. Please check and consider revision for clarity. Or am I misunderstanding the statement?

Lines 345-347. Was this bias not corrected by the rinse step after the labelling?

Lines 349-354. I do not fully understand if the authors are actually recommending that TAM fractions should be determined in living food sources. If yes, what would be the methodological procedure to be followed or the methodological bottlenecks to be solved?

Furthermore, Chironomids were labelled only via the aqueous pathways, while in natural conditions metal uptake via the dietary pathway may be as important as the aqueous one. This does not invalidate the experiments. However, consider if this aspect deserves to be mentioned in the discussion as a recommendation for future studies.

Lines 355-374. No objections on the information provided here. However, I would appreciate an explicit explanation on how the results from the present study advance current knowledge on the assimilation and elimination of Ag, Cd, Zn in gammarids and, if applicable, in invertebrates. In the same vein, based on the available results, what should be the priorities of future studies to advance knowledge in this research field?