Peer Community In Ecotoxicology & Environmental Chemistry

# Molecular-level responses highlight physiological stress in muscle and liver tissue of apparently healthy European sardine specimen

***Davide Anselmo Luigi Vignati*** *and* ***Abdulsamie HANANO*** *based on peer reviews by* ***Sophie Prud'homme***, ***Roberta Bettinetti*** *and 1 anonymous reviewer*

Anaïs Beauvieux, Jean-Marc Fromentin, Claire Saraux, Diego Romero, Nathan Couffin, Adrien Brown, Luisa Metral, Fabrice Bertile, Quentin Schull (2024) Molecular response to multiple trace element contamination of the European sardine. bioRxiv, ver. 4, peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry. https://doi.org/10.1101/2024.02.16.580673

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Fish is an essential component of healthy human diets and the preservation of fish stocks and other marine resources is included as a target of Sustainable Development Goal 14 'Conserve and sustainably use the Oceans, Sea and Marine Resources for Sustainable Development' (UNEP). However, several fish stocks remain in sub-optimal (or worse) conditions due to overfishing and a range of stressors including chemical pollution. Chemical pollution can result in high level of chemicals in fish making it unsuitable for human consumption. Furthermore, the occurrence of chemical-related physiological stress in otherwise apparently healthy fish requires additional research efforts. In natural environments, further complexity arises from fish being simultaneously exposed to multiple contaminants/stressors as opposed to laboratory investigation usually dealing with one or very few contaminants/stressors at a time (Schäfer et al., 2023).

Beauvieux et al. (2024) examined the possible role of accumulation of multiple elements on the physiological status of first-year-of-life specimen of European sardine collected in the Gulf of Lions (northeastern Mediterranean Sea) as a contributing factor to the declining sardine population observed in the region since 2008. The ultimate objective of the paper was to identify potential biomarkers of stress in fish otherwise not

exhibiting any anomalies in body condition, in agreement with the principles of chemical stress ecology put forward by van der Brink (2008).

Out of a total of 105 specimen, individuals were selected according to the lowest (n = 14) or highest (n = 15) levels of contamination and subject to proteomic analysis of liver and red muscle tissues. A first Principal component analysis on all specimen highlighted the possible influence of the Rhone river as a source of geogenic and anthropogenic elements to the Gulf of Lions.

A second PCA performed only on specimen selected from proteomics analysis allowed to identify three elemental mixtures possibly responsible for the observed physiological effects. Proteomic analysis in liver and muscle tissue identified both similarities and differences in the pathways involved in response to stress. More in detail, the expression patterns of Myosin and Myomesin were downregulated in red muscle for highly exposed specimen, which suggests possible effects of elemental accumulation on the locomotion abilities of Mediterranean sardines. Pathways involved in lipid metabolism and immune processes were up-regulated in liver, pointing to increased energetic costs for maintaining the overall fish homeostasis in presence of metal contamination. It is interesting to note that these effects were observed at concentrations below the legal thresholds for human consumption (except for As), albeit such thresholds are available only for a limited number of elements (Cd, Pb, Cd, As and inorganic Sn) (EU, 2023).

Although stressors other than trace elements could contribute to the observed molecular responses, as acknowledged by the authors themselves, Beauvieux et al. (2024) show that biological responses at lower levels of biological organization can provide both early-warning indications of potential adverse effects in the long term and better understanding of drivers of population decline. By advancing our knowledge of the physiological responses to trace elements and identifying potential biomarkers, this study lays the groundwork for more effective monitoring and conservation strategies. Further studies addressing the combined effects of multiple environmental stressors remain essential to develop holistic approaches to marine ecosystem management and species conservation.

***References:***


Beauvieux A., Fromentin J.-M., Saraux C., Romero D., Couffin N., Brown A., Metral L., Bertile F., Schull Q. (2024). Molecular response to multiple trace element contamination of the European sardine. bioRxiv, ver. 4 peer-reviewed and recommended by Peer Community in Ecotoxicology and Environmental Chemistry. https://doi.org/10.1101/2024.02.16.580673

EU (2023). Commission Regulation (EU) 2023/915. https://eur-lex.europa.eu/eli/reg/2023/915/oj/eng

Schäfer R. B., Jackson M., Juvigny-Khenafou N., Osakpolor S. E., Posthuma L., Schneeweiss A., Spaak J., & Vinebrooke R. (2023). Chemical Mixtures and Multiple Stressors: Same but Different? Environmental Toxicology and Chemistry, 42(9), 1915-1936, https://doi.org/https://doi.org/10.1002/etc.5629

UNEP: https://sdgs.un.org/goals

Van den Brink P. J. (2008). Ecological Risk Assessment: From Book-Keeping to Chemical Stress Ecology. Environmental Science & Technology, 42(24), 8999-9004. https://doi.org/10.1021/es801991c


## Reviews

## Evaluation round #3


DOI or URL of the preprint: https://doi.org/10.1101/2024.02.16.580673
Version of the preprint: 4

### Authors' reply, 29 July 2024

Dear Dr. Vignati,

Thank you very much for your reply. Indeed Fig 5 is central and already available in high resolution on Zenodo as specified in the "Data, Script, Code and Supplementary Information Availability" section of our manuscript:

"Scripts, Figures 5 and 7, and other datasets are available online: https://doi.org/10.5281/zenodo.10683281."

Let us know if we can provide any more information,

Bests,

Anaïs Beauvieux

### Decision by Davide Anselmo Luigi Vignati and Abdulsamie HANANO, posted 29 July 2024, validated 29 July 2024

**Thank you for version 4. Is there any way to make Figure 5 more readable?**

Dear Dr. Beauvieux,

I am comfortable with the revised version #4, except for one detail that I overlooked (please accept my apologies for this).

In her review, Sophie Prud'homme wrote:

Figure 5: Term names are illegible in the PDF deposited as a preprint. It would be appreciable to find a solution to insert this figure with a greater resolution.

Is there any way to solve this issue?

Adding a high-resolution figure in the supporting information? Or provide a high resolution figure in figshare or some institutional online repository?

Indeed, Figure 5 is a central piece of the work and it would be a real added value to make the term names visible to readers.

Do not hesitate to contact us for any assistance you may need on this point.

Thank you very much.

Sincerely yours,

Davide A.L. Vignati

## Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2024.02.16.580673

Version of the preprint: 3

### Authors' reply, 15 July 2024

Thank you for your suggestions. We reviewed the suggested editorial changes and check the supporting information file.

### Decision by Abdulsamie HANANO and Davide Anselmo Luigi Vignati, posted 10 July 2024, validated 10 July 2024

**Investigating Trace Element Contamination in European Sardines**

Dear Dr. Beauvieux,

as anticipated by external mail on July, 4th, I would appreciate if you could verify some suggested editorial change to submission PCI Ecotox Env Chem #210.

My suggestions are included for you consideration as track changes in the attached word file provided by you on the same date.

Please also check the following in the supporting information file accompanying the preprint:

ESM5. The figure caption says that PCA was performed on 28 individuals. However, the main text (line 184) says that 14 (low contamination) + 15 (high contamination) individuals were used; giving a total of 29 individuals. Which is correct?

ESM10. In the figure caption, reference is made to 'Table S4'. Does this refer to ESM11? If so, please use "...(see ESM11 for further details). ..."

Thank you very much for your collaboration.

Sincerely yours,

Davide A.L. Vignati **Download recommender's annotations**

## Evaluation round #1

DOI or URL of the preprint: https://doi.org/10.1101/2024.02.16.580673

Version of the preprint: 2

### Authors' reply, 18 June 2024

**Download author's reply**

**Download tracked changes file**

### Decision by Abdulsamie HANANO and Davide Anselmo Luigi Vignati, posted 09 May 2024, validated 13 May 2024

**Molecular response to multiple trace-element contamination of the European sardine**

Dear Dr. Anaïs Beauvieux,

We have completed the review process for your manuscript titled "Molecular response to multiple trace-element contamination of the European sardine," which involved evaluation by three peer reviewers. Enclosed in this message are the detailed reports provided by the reviewers.

While your manuscript demonstrates several strengths both linguistically and scientifically, the reviewers have raised some points for revision that should be addressed to enhance the final version of the manuscript.

Consequently, I kindly request that you revise your manuscript in accordance with the reviewers' comments and resubmit it to the PCI.

Kindest regrads,

Dr. A. Hanano

PCI recommender

### Reviewed by Sophie Prud'homme, 09 May 2024

This article is remarkably well written and argued . The title is appropriate, and the abstract presents well the objectives and main finding of the article. The background and questions are clearly and synthetically presented, and the methods are precisely and clearly outlined. The exploration and interpretation of proteomic

data and their relation to trace metal contamination data is thorough and well argued, making the approach convincing, which is not always the case in literature. It however lacks an information concerning the functional enrichment execution, that make for now impossible to judge about the relevance of the functional enrichment (see below, comment on L273).

The discussion of the data is enriched by a comparison with the available bibliography through an additional figure and table in supplementary materials, which provides transparency to the discussion.

I only have some recommendations and points of discussion on the manuscript :

> L114-115 : Summarizing liver function in terms of detoxification is a bit simplistic, and all liver functions should be listed.

> L119 : Please define "LFQ"

> L273 – **"Functional enrichment analysis and pathway network" sectio**n: The background used to perform the functional enrichment on clusters have to be specified. Indeed, using a generic background (not organ specific) could lead to incorrect/biased pathway enrichment and lead to biased biological interpretation. (for example, discussed in Wijesooriya K, Jadaan SA, Perera KL, Kaur T, Ziemann M (2022) Urgent need for consistent standards in functional enrichment analysis. PLoS Comput Biol 18(3): e1009935. https://doi.org/10.1371/journal.pcbi.1009935)

According to the answer to this question, there may be some adjustments to perform to data analysis that may influence the content of the article.

> L280 : Can some arguments can be provided to justify the choice ton consider the top 10 enriched biological processes ?

> Results section "Physiological response to inorganic contamination": It would be important to provide, for each tissue, the total number of protein detected (not formally provided for muscle) and the proportion of this background included in significantly correlated modules.

> Figure 5: Term names are illegible in the PDF deposited as a preprint. It would be appreciable to find a solution to insert this figure with a greater resolution.

> L489: I'm not convinced that the number of overexpressed pathways can be used to compare the intensity of organ response. It's more a reflection of the diversity or heterogeneity of the response. The absolute number of proteins included in clusters correlated with mixtures, or the part of detected proteins that are included in clusters correlated with mixtures in each tissue seems to be a better metric to compare the intensity of organ response – and should be considered by the authors.

>L513 - "Red muscle proteome response to mixture 1 was less marked than that of the liver proteome": It is unclear on what criteria the categorization of "less marked" is based, and it should be specified.

>L548-549: Given that mTOR activity is dependent on several post-translational modifications, considering only its abundance as a biomarker of metals contamination may not fully respond the objectives - Authors should consider the characterization of mTOR post-translational modifications in addition to its abundance. (see for example Yin et al, Int. J. Mol. Sci. 2021, 22(4), 1784; https://doi.org/10.3390/ijms22041784)

## Reviewed by Roberta Bettinetti, 03 April 2024

my comments

Title and abstract

Does the title clearly reflect the content of the article? Yes,

Does the abstract present the main findings of the study? Yes

Introduction Are the research questions/hypotheses/predictions clearly presented? Yes,

Does the introduction build on relevant research in the field? Not at all. There are several points that you can explain better. Just in the last two years a new campaign on Mediterranean see has been condcted and more data are now available on metals and legacy pesticides. Pelase check and add also for comparisons.You have also to consider the existence of legacy and emergent POP's and the cocktail is made also by them, not only by trace elements. Please discuss in this section also the "problem" connnected with the realism of the

use of biomarkers which is quite controversial. I would consider also the role of Temperature in time, during the last years as a probable factor causing the decreaase of number of fish (less or diverse preys as it is hppening in freshwater environments). You should explain better this aspect, since of course (specify it better in the introduction) contamination of trace elements can't be the only cause (other fish? other equilibrium conditions?)

Materials and methods Are the methods and analyses sufficiently detailed to allow replication by other researchers? not at all: please explain how you measured wet weight of fish - pleas esplain the method by Fold since it is quite old and not easy to find - explain why you did not take into account methyl mercury

I'm not an expert of proteomi, so I've nothing to say about.

Are the methods and statistical analyses appropriate and well described? Yes

Results In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? I don't know

Are the results described and interpreted correctly? Yes, - why did you look for the relationshiè between lenght and size and you corrected the calues? It is not clear to me

Discussion Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argumer Yes, even if you did not consider the importance of temperature variations. I wouldn't compare concentrations in seas which are so different and concentrations are to low and methods probably too different to be comparable.

please consider the size of the preys, thanks

## Reviewed by anonymous reviewer 1, 15 April 2024

Beauvieux et al. provided data on the contamination of the European sardine and the relationship between the level of pollution with potential physiological responses in the Mediterranean sea. Considering that this basin has been longed reported as one of the most polluted sea at wordwide level, it is surely important to provides new updated data on marine ecotoxicology.

Despite its potential, this manuscript would surely benefict from an exhaustive bibliographic search as it is keepen very superficial throughtout its introduction and discussion as well. For instance:

line 44-45 need a reference;

line 55-57: the authors mentioned multiple TE stating they can cause several adverse effects, however the cited work is only one and it is reffered to only copper. The authors need to cite proper references.

line 457-460: the levels of TE are potentially higher in the Med compared to other basins is likely linked to multiple sources of contamination, both natural and human's. It is much more complicated than just stating "higher levels because the Mediterranean throphic webs show enhanced abilities to better accumulate TE pollutants".

Other observations:

- authors need to be consistent throughout the manuscript. They need to use the acronym the first time the written about TE and then use either the acronym or the full name. However, in the discussion they still refer to Hg (mercury) etc. (Line 476).

- References need to be uniformed in style (e.g. something is "et al.," other time "et al.")

Line 154: No need for "Trace-element", the authors could simply say "Trace element"

Line 159 and 170 etc.: be careful in writing correctly (in style) the chemical formula as well as the unit

Line 208: Latin name goes italics, moreover, if authors decide to use the common name or the Latin name is ok, but they need to be consistent.