Otolith trace element composition reveals spatial and ontogenetic structuring in the glacier lanternfish *Benthosema glaciale* (Reinhardt, 1837)

Francesco Saltalamacchia[1,2], Natalya D. Gallo[1,2,3], Arild Folkvord[1,4], Anne Gro Vea Salvanes[1,2], Karin Limburg[5,6]

[1]Department of Biological Sciences, University of Bergen, Bergen, Norway
[2]Bjerknes Centre for Climate Research, Bergen, Norway
[3]Norwegian Research Centre (NORCE), Bergen, Norway
[4]Institute of Marine Research (IMR), Bergen, Norway
[5]Department of Environmental Biology, State University of New York College of Environmental Science and Forestry, Syracuse (New York), US
[6]Department of Aquatic Resources, Swedish University of Agricultural Sciences, Uppsala, Sweden

Responses to Referee 2

General comments

R2_1

While the specific research questions are well addressed, I feel the authors fall somewhat short of fully achieving their broader aim of clarifying "how otolith chemistry can be used to infer habitat use in deep-water species," due to the lack of an in-depth multi-elemental analysis. Aside from the autocorrelation analysis, elements are treated separately throughout. I think this gap should at least be acknowledged as an avenue for future exploration, or the authors should justify why a multi-elemental approach was not pursued.

The present study was designed as a foundational characterisation of how individual elements vary through ontogeny before attempting an integrated reconstruction of habitat use. This complements our previous analysis of the same system, in which age-specific multielement signatures were used to discriminate spatial population components and revealed that their separation could change between ages classes (Saltalamacchia et al., 2026). The earlier study addressed geographic connectivity among sites, whereas the present analysis examines the trajectories and likely controls of the individual elements that constitute those multielement signatures, based on our knowledge of the study system and species ecology. This step is informative because the elements showed contrasting ontogenetic trajectories and potential differences in their environmental and physiological controls. A further step could be to combine these element-specific profiles into an integrated indicator of water-mass exposure and validate it against independent environmental and vertical-distribution data.

We have clarified this analytical scope and added the development and validation of multielement approaches to habitat reconstruction as an avenue for future work in the Discussion (Lines 612-619): “As the first age-resolved characterisation of otolith elemental trajectories in *B. glaciale*, this study provides a foundation for future multielement analyses of habitat use. Integrating complementary elemental signals may provide a more comprehensive representation of changes in water-mass exposure and habitat use than interpreting each element separately. Age-specific multielement signatures have already revealed spatial differentiation among

population components of *B. glaciale*, with evidence that the degree of separation among some neighbouring sites varied across age classes (Saltalamacchia et al. 2026). Future work could build on these results by examining how multielement signatures change along individual otolith profiles and by validating the resulting patterns against concurrent environmental and vertical-distribution data. Although experimental validation remains challenging for mesopelagic species, comparative studies across systems with contrasting hydrography offer a feasible approach to further constraining the mechanisms underlying otolith elemental variation."

Following the suggestion, we also moderated the broader wording of the study aim and conclusions. We replaced "how otolith chemistry can be used to infer habitat use" with "how otolith chemistry can support inference about habitat use" (Lines 139-140). We also replaced "This study shows that otolith chemistry provides a time-integrated record of ontogenetic habitat use" (Conclusions section) with "This study shows that otolith chemistry provides time-integrated evidence consistent with ontogenetic changes in habitat use…" (Line 621).

R2_2

Given how frequently environmental conditions are discussed throughout the manuscript, I think it would be worth moving a figure from the supplementary material into the main text, so readers can more easily grasp the environmental variation across sampling sites. Figure A4 could be a good candidate for this.

As suggested, we have moved the former Figure A4 from the Appendix into the main text (@line 202) as "Figure 2" (now referenced in-text @line 180). The naming of former figures 2-5 (and respective cross-references throughout the text) have been updated consequently.

Detailed comments

Abstract

R2_3

L17: Consider listing the six trace elements in parentheses after first mention (e.g., Sr, Ba, Mg, Mn, P, B).

Added "(Sr, Ba, Mg, Mn, P, B)" at Line 17.

R2_4

L110: Previous paragraphs on Sr and Ba explained how they were expected to react. Why was this not done for B as well? Are changes in pH expected among habitats?

The mechanisms controlling B incorporation into fish otoliths remain poorly understood. In other calcifying organisms, B incorporation has been positively associated with environmental pH, whereas evidence from fish otoliths suggests that B may also reflect physiological and biomineralisation processes. Limburg et al. (2023) identified long-term covariation between B:Ca, environmental conditions and the physiologically mediated element P, suggesting that both environmental and internal controls may be relevant. We included B in our study because its incorporation had not previously been investigated in *B. glaciale*, and comparable age-

resolved information remains scarce for myctophids more generally. These measurements therefore provide new evidence from a species and family for which otolith B:Ca is poorly documented.

We have clarified this rationale and the uncertainty surrounding the interpretation of B:Ca in the paragraph highlighted by the Referee. Following also R1_15, the revised text (Lines 113-116) now states: “Although multidecadal variation in otolith B:Ca has been associated with long-term changes in pH (Limburg et al., 2023), the extent to which B:Ca records environmental pH throughout an individual’s lifetime remains unresolved. Given the limited information on B incorporation in fish otoliths, particularly for myctophids, we included B as an exploratory tracer to characterise its ontogenetic and spatial patterns in *B. glaciale*.“

R2_5

L121-122: “In otoliths, Mn is influenced not only by ambient availability but also by growth and metabolism-related processes, which complicates its interpretation as a purely environmental tracer (Cavole et al., 2023; Turner and Limburg, 2015).” This sentence makes it sound like it’s a particular case for Mn, while it is also true for most elements used in microchemistry.

The original wording was not intended to suggest that combined environmental and physiological regulation is unique to Mn. We have rephrased the sentence (Lines 124-127) to make clear that this interpretive complexity applies broadly to elements used in otolith microchemistry: “In otoliths, Mn content reflects both ambient availability and physiological processes associated with growth and metabolism, as is the case for many other elements used in otolith microchemistry (Cavole et al., 2023; Hüssy et al., 2020; Turner and Limburg, 2015). Its interpretation therefore requires consideration of both environmental and physiological influences.”

R2_6

L158-161: Could it be worth mentioning why the slow growth period is happening in summer and the fast growth in winter, as it is usually the opposite?

Gjøsæter (1973) carried extensive research in the region and suggested that slower spring and summer growth may be associated with the May–July spawning period, while faster autumn and winter growth may partly reflect the “reversed” seasonal temperature cycle at the depths occupied by the species.

We have added this explanation to the revised manuscript (Lines 167-169): “This seasonal growth cycle is thought to reflect both the allocation of energy to reproduction, and the seasonal temperature regime at the depths inhabited by *B. glaciale*, where temperatures are generally higher in winter (Gjøsæter, 1973).”

R2_7

L186: Is there a grit size associated with the grinding paper? It might be worth mentioning.

Added grit size (“1000 to 2500-grit”) at Line 195.

R2_8

L195: I am confused by the mention of the ablation points accompanying the ablation transect here: "we ran edge-to-edge transects (ablation points) along the major otolith growth axis and passing through the core", as you mention a constant travel speed of the laser beam. Is it a series of points or a continuous ablation? Or are the ablation points referring to the output data points? Consider clarifying.

The laser moved continuously along each edge-to-edge transect at a constant travel speed. "Ablation points" referred to the individual output measurements recorded along that continuous path.

We removed "ablation points" and added "continuous" before "edge-to-edge transects" at Line 212 (it now reads "we ran continuous edge-to-edge transects along the major otolith growth axis and passing through the core"). We also replaced references to "ablation points" with "measurements" throughout the manuscript (including the header and caption of Table 1).

R2_9

L198: How much time does it take, approximately, to run 7–10 otoliths? Is it roughly every hour? It would be helpful to add that information.

Added "(Corresponding to approximately 1.5 hours)" at Lines 215-216.

R2_10

L309-310: I am wondering if this is the clearest way to write it: "The average number of laser ablation points per translucent and opaque increments was, respectively, 4-9 at age 0; 6-23 at age 1; 6-10 at age 2; 4-6 at age 3; 4-4 310 at age 4; and 3-4 at age 5." You could consider a small table instead.

Thank you for the suggestion. We considered presenting these values in a table. However, because the information covers only six age classes and the manuscript already contains several tables, we have retained it in the text to avoid adding another table for this short summary.

R2_11

L329: I am unsure of the added value of setting a reference age of 2. When it is mentioned again at L358, I would prefer the age to be mentioned directly.

Following the suggestion, we replaced "reference age" with "age 2" at any occurrence in the revised manuscript.