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

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Responses to Referee 1

General comments

R1_1

The potential for ontogenetic changes in growth rate to affect the temporal resolution of the LA-ICP-MS record deserves discussion. Because ablation proceeds at a constant travel speed (line 196), a single laser spot integrates over a longer period of otolith formation in slower-growing, older fish than in faster-growing juveniles (Fig. 3). This raises the possibility that finer-scale chemical variation is smoothed out in older age classes, which could be important when interpreting the flattening of trajectories in later life (Fig. 4).

We have added this limitation to Section 4.5 (Lines 594-598): "Temporal resolution also varied across each transect because laser ablation progressed at a constant speed. As increments narrow with age, each measurement integrates progressively longer periods than during juvenile life. Fine-scale chemical variation is therefore increasingly averaged, which may contribute to the attenuation of sub-annual oscillations at older ages alongside increasingly stable use of deeper habitats".

R1_2

Average increment widths are shown pooled across all sites in Fig. 3, and Fig. A3 gives site-specific cumulative otolith distance at each age landmark. It would be useful to report site-specific increment widths by age class, likely in the supplement given the probable size of the table. This would clarify whether the differences already visible in Fig. A3 translate into differences in age-specific increment width, and whether such differences could themselves contribute to the spatial patterns in E:Ca.

We added a supplementary figure showing age-specific increment widths for each site (new Figure A4, referenced in text at Line 518). The figure shows raw values, means and SDs. We chose a figure rather than the suggested large table because we believe the figure provides the same information in a more intuitive way while also displaying the underlying distributions.

R1_3

AIC values are not reported in Table A1, only the qualitative rationale for each candidate formulation. Since model selection by ΔAIC < 2 is described in the text (line 260), the AIC or ΔAIC values should be added as a column so readers can evaluate model support directly.

We have retained Table A1 as a description of the candidate model formulations and their rationale, and added a separate table in the Appendix (new Table A2) reporting the AIC and ΔAIC values for all elements and model-selection stages. The text now refers to this table at line 298. The former Table A2 (multivariate correlations) is now Table A3, and all in-text references to it have been updated accordingly.

R1_4

The models do not appear to explicitly account for autocorrelation along the ablation transect. Adjacent points on the same transect are likely more similar to each other than the mean structure (shared smooth plus random slope) predicts, beyond what the per-fish random intercept and random age slope can capture. The authors could clarify whether this was tested, for example via a residual autocorrelation diagnostic per fish, and if structure remains, whether an AR1 term would be appropriate, or explain why this is unlikely to affect the reported trends.

We thank the Referee for identifying this issue. Following the suggestion, we assessed residual dependence separately within each fish after fitting the age and individual-level structure. The median fish-specific lag-1 correlation of the Pearson residuals was then used as an element-specific AR1 term and held constant throughout the subsequent spatial and temporal model comparisons. We then verified the adequacy of this structure by examining residual autocorrelation in the final models.

Compared with the original submission, including the AR1 structure resulted in different spatial formulations being selected for most elements. However, the predicted age trajectories remained stable and similar to those originally reported, with changes mostly affecting fine-scale features. Where necessary, we updated the relevant paragraphs of Materials and Methods, Results and Discussion accordingly. Details below.

<u>Material and Methods</u>

We added details about the autocorrelation procedure at Lines 272-275:

“Residual dependence between consecutive measurements was then assessed within each fish. For each element, the median fish-specific lag-1 correlation of the Pearson residuals was used as a fixed AR1 parameter in all subsequent models. Models were fitted using discrete estimation, with the first measurement from each fish marking the start of an independent series.”

We removed the statement that the basis dimension was “reduced when needed for convergence” (line 294) because this is no longer relevant, as all models now converge with k = 15. We also added “and residual autocorrelation” to the sentence about final-model diagnostics (line 296).

We updated Table 2 based on the new models selected for each element.

Results

We replaced the former Figure 4 (Figure 5 in the revised version), Table 3 and Table 4 with outputs from the AR1 models. The changes to the main text were as follows:

Sr (lines 356-360)

[The patterns identified in Figure 5 are the same as in the original submission, but due to the parametric part of Table 3 being no longer relevant for Sr (a different spatial formulation was selected after accounting for autocorrelation), we rephrased the first few sentences of this paragraph.]

From: "At the reference age of 2.00, Sr:Ca was lower at the coastal site (COA) than in Fensfjord (FEN), Masfjord (MAS), Osterfjord (OST) and Sørfjord (SØR); Sogndalsfjord (SOG) was intermediate and not clearly different from COA (Table 3). Fjord trajectories generally reached higher adult Sr:Ca than the coastal site."

To: "In early life, Sr:Ca appeared to be lower at the coastal site (COA) than in Fensfjord (FEN), Masfjord (MAS), Osterfjord (OST) and Sørfjord (SØR)".

Also, the sentence "For Sr:Ca, the most supported model supported site-specific age trajectories of varying complexity (m2e; Table 3)" was changed to "For Sr:Ca, the most supported model included site-specific age trajectories with shared complexity (m2c; Table 3)."

Ba (lines 363-364)

Changed "The selected model for Ba:Ca retained a shared age trajectory with site-specific deviations (m2d; Table 3)." To: "The selected model for Ba:Ca retained site-specific age trajectories of varying complexity (m2e; Table 3)."

Mg (lines 381-383)

Removed the sentence "At the reference age, average Mg:Ca in the fjords was not different than that at COA, except for OST, where it was lower (Table 4)" because the parametric part of Table 4 is no longer relevant for Mg. Also "site-specific age trajectories of varying complexity (m2e; Table 4)" is now "site-specific age trajectories with shared complexity (m2c; Table 4)."

Mn (lines 384-387)

Changed "Mn:Ca also declined with age, broadly matching the Mg:Ca pattern (Fig. 5). The coastal site had high early-life Mn:Ca, followed by a steep decline. Except FEN and OST, fjord trajectories tended to lower initial levels and slower declines. The selected model supported site-specific age trajectories with shared complexity (m2c, Table 4)."

To: "Mn:Ca also declined with age, broadly matching the Mg:Ca pattern (Fig. 5). Values were generally higher and more variable during the first two years, followed by lower and flatter trajectories in adulthood. Early-life

Mn:Ca was comparatively low at MAS, but adult values overlapped more strongly among sites. The selected model supported a shared age trajectory and site-specific deviations with shared complexity (m2b, Table 4)."

P (lines 388-390)

[This element includes the panel which changed the most from the original submission. The updated AR1 model retained a shared age trajectory with site-specific deviations. The broad increase in P:Ca from approximately age 2 remained, but the previously flatter coastal trajectory was no longer apparent.]

We therefore changed the paragraph: "P:Ca was relatively flat during early life and increased from about age 2 in the fjords (Fig. 5). This increase was less evident at the coastal site, where P:Ca remained comparatively flat and reached lower values in older fish. Among fjords, trajectories were broadly similar in shape (i.e. showing increases with age). The selected model supported site-specific age trajectories with shared complexity (m2c, Table 4)."

To: "P:Ca was relatively flat during early life and increased from about age 2 (Fig. **Error! Reference source not found.**). Although some site-specific deviations remained, the trajectories shared the same broad ontogenetic pattern. The selected model supported a shared age trajectory and site-specific deviations with shared complexity (m2b, Table 4)."

Discussion

Mn (lines 531-534)

From: "In our otoliths, however, although some slight differences (especially at ages 4-5) might be observed between the well-ventilated COA/FEN and the inner fjords, adult Mn:Ca did not show strong enrichment in fjords with lower deep-water oxygen (MAS, SOG, SØR; Fig. A2, Fig. A4). Under the conditions sampled here, physiological control and declining exposure to shallow/coastal Mn sources appear to dominate the Mn:Ca trajectory."

To: "In our otoliths, however, adult Mn:Ca did not show strong enrichment in fjords with lower deep-water oxygen (MAS, SOG, SØR; Fig. 2, Fig. A2). Under the conditions sampled here, the shared ontogenetic decline appears more consistent with physiological control and declining exposure to shallow/coastal Mn sources than with persistent enrichment from low-oxygen deep waters."

P (lines 545-552)

We replaced "This pattern fits a maturation-related change in otolith biomineralisation or energy allocation." with: "The broadly shared timing and direction of this change suggest a common ontogenetic process, potentially involving changes in otolith biomineralisation or energy allocation around maturation, rather than a predominantly site-specific environmental response. " (lines 545-547)

We also changed the section discussing the COA difference, from: "In marine teleosts, elevated P:Ca has been associated with periods of active growth and ontogenetic transitions (Borelli et al., 2003). The flatter, lower

adult P:Ca trajectory at COA, despite larger body size, indicates that P:Ca does not simply scale with somatic growth. One possible explanation is that coastal and fjord fish differ in adult energy allocation. Spatial differences in reproductive investment have been reported in the co-occurring mesopelagic fish *Maurolicus muelleri*, with lower gonadosomatic index at the coast than in a fjord site (Salvanes and Stockley, 1996), and broader fjord–ocean differences in gonad weight and condition (Kristoffersen and Salvanes, 1998)."

To: "In marine teleosts, elevated P:Ca has been associated with periods of active growth and ontogenetic transitions (Borelli et al., 2003), but the increase observed here occurred as annual increments generally became narrower. Together with the negative association between P:Ca and annual increment width in the exploratory analysis (Fig. A5), this indicates that P:Ca does not simply increase with local otolith growth. A maturation-related change in physiological allocation remains plausible, although this association cannot be isolated from the present data". (lines 548-552)

Appendix A

In Table A1, the site formulations highlighted in bold (model formulations selected for at least one element) have been edited to reflect the updated analyses.

R1_5

The rationale for interpolating rather than removing below-LOD values is only briefly addressed in the Limitations section (lines 542 to 544), where it is described as conservative and unlikely to bias results. This reasoning should be expanded and justified.

We have expanded the justification in Section 4.5 (Lines 584-588): "Because interpolation was restricted to a few sparse points bounded by neighbouring valid measurements within the same transect, it was unlikely to generate the broad age-structured or spatial patterns reported here. This approach was considered more conservative than removing these measurements and introducing gaps in otherwise continuous transects. Nevertheless, interpolation may locally smooth fine-scale variability, particularly in later-life increments represented by fewer measurements".

R1_6

As some sites there is a small sample size (e.g. n is between 6 and 8 at three sites), while I understand the logistics and the problems that can arise with obtaining appropriate sample sizes for this type of study I think that there should at least be some discussion of the implications of this and the impacts that it could have on the overall results.

The uneven and sometimes small site-level sample sizes are now acknowledged more explicitly in Section 4.5 (Lines 599-604): "Finally, estimates for the sites with limited sample size (COA, SOG and SØR) may have been more sensitive to individual variation than those of the better-represented sites. This limits confidence in fine-scale differences among site-specific trajectories, particularly at older ages where observations were additionally sparse. Repeated measurements across ontogeny nevertheless supported estimation of individual trajectories, while the hierarchical models accounted for persistent among-individual differences in both average elemental

levels and age-related change. We therefore emphasise broad ontogenetic patterns and contrasts supported across multiple sites rather than fine-scale features unique to sites with smaller sample sizes."

R1_7

Otolith weighing is mentioned as part of the extraction method, but no otolith size or weight data are reported in the Results. Was this compared among sites? This is distinct from the increment-width data in Fig. 3 and Fig. A3 and could add useful context for interpreting site differences.

Otolith weight was recorded during sample processing but later shown to have been done inconsistently across different surveys. The information was therefore not used in the present analyses, and the reference to weighing was retained inadvertently from an earlier draft. We have now removed it from the methods (line 193) to avoid confusing readers.

R1_8

I appreciate figure 3 showing the differences in mean otolith size. What is driving the larger size of the opaque section in year 1 – I see that the SD is also much larger here, are there one or two individuals that are driving this? How might this much larger mean increment width impact results and what the LA-ICP-MS results show?

The comparatively wide age-1 increments and their large SD mostly reflect broad individual and spatial variation in otolith growth, with no extreme values. This can now be seen in the new Figure A4 (added in response to R1_2). Since juveniles occupy shallower waters than older fish (Dypvik et al., 2012), variation among individuals may reflect spatial and temporal differences in the environmental and feeding conditions experienced during the fast-growth season in more variable water masses. Additional variation may arise from differences in spawning time and subsequent early growth history. In a previous paper we also documented compensatory growth in the species (Saltalamacchia et al., 2025). The subsequent narrowing of increments coincides with the approach to maturation, which begins during age 2 (Gjøsæter, 1981), and the shifting towards deeper, more stable habitats.

Although we do not discuss individual increment-width patterns in detail because they are outside the principal scope of the study, we now report and acknowledge possible growth effects on element incorporation as detailed in our answer to R1_26 (and its related new Figure A5).

R1_9

Are there site-specific differences in vertical habitat use, and if so, what are the ecological and methodological implications of this?

Site-specific differences in vertical habitat use are plausible, but comparable data on depth distribution and migration behaviour are currently unavailable in the region. Any such differences are unlikely to fully explain the broad spatial patterns observed here, but they could modify the water masses and environmental conditions experienced by fish and therefore contribute to finer-scale variation among sites. Methodologically, this introduces some uncertainty when attributing spatial variation in otolith chemistry to differences in water chemistry.

We have elaborated on this point at Lines 460–465: “The interpretation of this broader spatial pattern is complicated by the possibility of site-specific differences in vertical habitat use. Differences in water clarity, hydrography, sill and basin depth, and prey distribution could alter fish depth distributions and DVM behaviour among systems (Aksnes et al., 2017; Dypvik et al., 2012b; Langbehn et al., 2019), thereby exposing fish to different water masses. Because independent vertical-distribution data were unavailable across sites, differences in vertical behaviour could not be evaluated as a potential additional source of the observed spatial variation in otolith chemistry.”

R1_10

Could the authors use the full multi-elemental suite together, rather than each element on its own, to better parameterise movement across water masses? This might dampen the impact of physiological effects on any single element and give a more robust proxy of habitat use. This is different from the covariation analysis in section 3.2, which looks at whether pairs of elements move together, not whether the elements can be combined into a single index. While I am not suggesting that the data is reanalysed it might be worth touching on this in the discussion.

A combined multi-elemental approach represents a valuable next step towards reconstructing movement across water masses. In our previous study (Saltalamacchia et al., 2026), age-specific multielement signatures were analysed using redundancy analysis to discriminate spatial population components of *B. glaciale*, with the degree of separation among some neighbouring sites changing between ages 1 and 3. That analysis focused on geographic connectivity, while the present study addresses the complementary question of how the individual elemental signals vary continuously through ontogeny. The two studies provide the spatial and element-specific ontogenetic context needed to interpret how individual elemental signals may contribute to a future integrated indicator of vertical habitat use.

We have added this perspective to 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.”

Specific comments

R1_11

Line 19: It would be good to include the specific elements measured here, since only those with results are mentioned in the abstract.

All the elements used in the manuscript (Sr, Ba, Mg, Mn, B, P) are mentioned in the Abstract. We now added the element list at Line 17, also addressing R2_3.

R1_12

Line 50: Is it just size, or also ontogenetic variation in the intensity or direction of DVM? The two are closely linked, but it might be worth mentioning the ontogenetic aspect here too, given how heavily this manuscript leans on age-based differences.

We have replaced “size” with the more general “ontogenetic” in the sentence “size or season-dependent variation in the intensity or direction of DVM” (Line 50).

R1_13

Line 53: Is having quotation marks around "voyage recorders" necessary? Worth checking with the copy editor.

We have retained the quotation marks because “voyage recorders” (line 53) is used here as a metaphor rather than as established terminology. The quotation marks signal this figurative use and help convey the concept that otoliths preserve chronological information about the environments and habitats experienced throughout a fish’s life. We will nevertheless defer to the journal’s copy-editing conventions during production.

R1_14

Line 58-59: Minor rewording could make this clearer. It's not immediately clear whether they mean the amount of new material deposited is proportional to activity, or that the elements incorporated are proportional to activity.

We changed “with new material deposited proportionally to the individual’s physiological and metabolic activity” to “with the rate of new material deposition influenced by the individual’s physiological and metabolic activity” (Lines 58-59).

R1_15

Line 112: The evidence for pH impacting boron levels is a bit mixed. While Limburg et al. (2023) found that the overall decrease in pH over time has been associated with changes in B, this might not always hold at the individual or population level under controlled experimental conditions, particularly over the course of an individual's life. This pattern could be weaker within individuals.

We have revised the statement “recent studies in cod have linked lower otolith B to lower pH and lower dissolved oxygen (Limburg et al., 2023)” to “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" (Lines 113-115). The revision aims to distinguish the long-term population-level association from the less well-established relationship within individual life histories.

R1_16

Line 127: P is far less frequently used as part of the elemental suite in otolith studies; it would be great to have a sentence or two here just providing a little more reasoning for its inclusion.

P was included specifically because its role in otolith chemistry remains poorly characterised despite its proposed association with the organic matrix and physiologically regulated biomineralisation.

We have expanded the Introduction to add that "Its ontogenetic trajectory may help assess whether elevated incorporation is associated with rapid juvenile growth or with later changes in energy allocation around maturation and reproduction" (Lines 136-137).

R1_17

Figure 1: The labelling of Sognefjord twice on the map confused me. It took me a while to understand that one label refers to the fjord itself while the second is pointing to the sampling site. None of the other fjords have this dual labelling, so it might be worth removing the general fjord label or making it visually distinct from the sampling site labels.

The sampling site is in Sogn*dal*sfjord, which is a branch of Sognefjord. We agree that the Sognefjord label was probably unnecessary and confusing, so we removed it as suggested.

R1_18

Line ~205: While most of the LA-ICP-MS detail is reported in Saltalamacchia et al. (2026), I think the authors still need to provide at least the precision and accuracy here from the standards.

We have now added information on analytical precision and cross-validated standard recovery to the Methods. Because calibration and cross-validation used the same reference material (MAPS-4), the recovery values describe internal calibration performance rather than independent validation against a second matrix-matched standard.

The Methods section @lines 219-224 was expanded to: "Analytical precision was evaluated from repeated MAPS-4 measurements distributed throughout the sessions and expressed as relative standard deviation (RSD). Precision ranged from 3.6% to 5.8% RSD across the selected elements (Sr, Ba, B, Mg, Mn, P). As an internal assessment of calibration performance, individual MAPS-4 runs were withheld in turn and quantified using time-dependent response factors estimated from the remaining runs within the same session. Cross-validated mean recoveries ranged from 99.3% to 99.6%, with mean absolute errors of 2.7% to 3.9% across elements."

The list of retained elements "(Sr, Ba, B, Mg, Mn, P)" was removed from the paragraph immediately following (line 225), as it is now included in this new paragraph.

R1_19

Line 216: Were measurements taken across the otolith before sectioning? This might help with understanding how accurately the sections were cut.

No direct dimensional measurements were taken before sectioning. Instead, images of the intact otolith and resulting section were aligned using the otolith outline, edges and core position, similarly to what illustrated in Figure 3. This allowed us to assess the realised cutting plane, and sections that deviated substantially from the intended longitudinal orientation were excluded. This led us to realise that the narrow core was not intersected consistently even among otherwise satisfactory sections. We therefore excluded all measurements assigned to this region and restricted the analysis to subsequent growth increments on the dorsal side, where increment identification and alignment were more consistent among individuals.

We have clarified this quality-control procedure by revising the corresponding Methods paragraph (Lines 236-240): “Sectioning quality was assessed by aligning and comparing images of each intact otolith with the resulting section using the otolith outline, edges and core position; sections that deviated substantially from the intended longitudinal orientation were excluded. Since the otolith core was not intersected consistently among retained sections, we excluded all measurements assigned to the core and restricted the analysis to subsequent growth increments.”

R1_20

Figure 2: It might be worth changing the colours used to highlight the sectional plane and the laser transect. It took me a little while to realise the figure annotations were representing different things.

We revised the figure (Figure 3 in the revised submission) to distinguish the sectional plane from the laser-ablation transect more clearly. The sectional area in the top panel is now delimited by a polygon rather than shaded as the laser path in the bottom panel. The figure caption (lines 243-247) has been updated accordingly.

R1_21

Line 290: The abbreviation MCMC hasn't been defined in full yet, so it should be added here.

We changed “MCMC” to “Markov chain Monte Carlo (MCMC)” (Line 317).

R1_22

Line 392: What if sexual maturation itself is driving the change in elemental composition, rather than, or in addition to, the habitat shift?

Sexual maturation may contribute to the shifts in elemental composition observed around ages 2–3, alongside the inferred ontogenetic change in habitat use. We have expanded section 4.5 (Lines 605-610) to acknowledge this additional mechanism and the difficulty of separating physiological changes associated with maturation from accompanying behavioural and environmental changes: “Changes in some elemental trajectories around the expected onset of sexual maturation may reflect shifts in energy allocation associated with maturity, alongside accompanying changes in habitat use. Because maturity stage was not recorded, these physiological and

behavioural influences could not be separated. Several avenues therefore remain for strengthening inference from otolith chemistry in mesopelagic fishes. Targeted sampling of otolith edge composition across seasons, age classes and maturity stages would improve resolution of sub-annual variability and clarify the role of reproductive processes in temporal changes."

R1_23

Line 407: Is this a habitat thing or an incorporation thing?

Both external and internal processes may contribute, as discussed at Lines 427-430 and 437-439 (where we talk about growth-mediated dilution), and also later at lines 445-449 (negative within-individual associations between Sr:Ca and Mg:Ca-Mn:Ca). Sr and Ba are environmentally responsive elements, so their ontogenetic trajectories are expected to reflect changing exposure to depth-structured water masses. However, as observed in previous studies, growth and physiological changes can also modify their incorporation, particularly for Sr. Because age, body size, growth and depth distribution change concurrently in *B. glaciale*, the present observational data cannot fully separate changes in environmental exposure from changes in incorporation.

Following the suggestion, we revised the sentence from: "In parallel, concurrent time-series measurements of water chemistry would allow more explicit environmental matching and refine the interpretation of elemental incorporation".

To: "Concurrent time-series measurements of water chemistry would allow more explicit matching of otolith and environmental chemistry and help distinguish variation in elemental exposure from age- and growth-related changes in incorporation". (lines 610-612)

The addition following comment R1_24 (lines 443-444) also partially contributes to this.

R1_24

Line 425: I think this section is well structured and relates well to the previous paragraph discussing the physiological modulation of Sr. It might be worth adding a caveat around the fact that depth use stabilises but Sr levels still change, and flagging that physiological effects may be contributing to this later-life pattern.

We have added this qualification to the Discussion (Lines 443-444): "Sr:Ca also continued to increase at older ages, when vertical habitat use is expected to become more stable, further indicating that changes in depth exposure alone cannot explain the later-life trajectory."

R1_25

Line 440-443: While reading this I made a note in the margins that said "this is good stuff."

Thank you for this positive feedback.

R1_26

Line 485: The authors discuss age a bit here, but what about the potential for fish size to impact these relationships between elements and physiological/environmental conditions? I know size and age are pretty confounded, but size independent of age has been shown to have an impact elsewhere.

This comment raises an important point about the potential influence of size and growth beyond the overall age-related patterns. We examined this relationship during exploratory analysis, and the results were broadly consistent with the growth-related interpretations emerging from the primary GAMMs and multivariate analysis. Because increment width was measured once per annual increment, whereas the primary GAMMs used multiple point-level measurements within each increment, we assessed this relationship separately using annual-increment summaries. Given the reviewer's comment and the informative element-specific pattern that emerged, we have now added a short account of this exploratory analysis to the Discussion (Lines 553–561, detailed below) and present the model estimates in the new Figure A5.

"It is also important to acknowledge that variation in growth may influence elemental incorporation alongside age and environmental dynamics (Hüssy et al., 2020). In an exploratory analysis using element-specific linear mixed-effects models, we observed positive associations between annual increment width and Mg:Ca and Mn:Ca, and negative associations with Sr:Ca and P:Ca, after accounting for age, sampling site and fish identity. Associations with B:Ca and Ba:Ca were not clearly supported (Fig. A5). These results support an element-specific contribution of growth to otolith chemistry. However, increment width changes systematically with age and represents local otolith growth rather than body size directly. Moreover, fish of different ages and sizes may differ in their vertical habitat use and consequent environmental exposure. The overlapping contributions of chronological age, body size, local otolith growth and depth-dependent habitat exposure therefore remain difficult to separate."

Further details about this output are provided in the Figure caption.

R1_27

Line 498: The fish sampled here are from mid-water rather than near the benthos. How far from the benthos could redox-influenced sediment Mn still be affecting E:Ca chemistry in these individuals?

Low oxygen can increase Mn release from sediments, but this would affect otolith chemistry only if dissolved Mn reached the water layers occupied by the fish. As we lack depth-resolved dissolved-Mn measurements, we cannot determine how far any sediment-derived Mn enrichment extended into the water column. However, oxygen depletion in the study systems was generally moderate, and the sampled fish may consequently have had limited exposure to any enrichment concentrated near the seafloor.

We have revised the paragraph highlighted by the Referee to clarify this point: "Oxygen depletion in our study system was generally moderate (Fig. A2), and sampling targeted mid-water habitats rather than waters immediately above the seabed. The sampled individuals may therefore have had limited exposure to near-seafloor Mn enrichment, which could explain the weak redox signal recorded in their otoliths." (Lines 538-541)

R1_28

Line 520: I would be interested in the potential future use of P:Ca as a tracer of metabolism.

This is a promising but still largely unexplored avenue for future research, as the mechanisms controlling P incorporation remain insufficiently understood for quantitative interpretation.