

Response to Referees' Comments

Referee #2

The manuscript “Mesopelagic fish responses to Pleistocene climatic variability in the Eastern Mediterranean and implications for the biological pump” by Agiadi et al. represents a potentially interesting contribution to the understanding of biological pump variability in relation to Pleistocene climate changes, using otoliths as a proxy. The methodological approach is innovative and still rarely applied to this fossil group. However, the methodology section is, in its current form, difficult to follow and lacks clarity. Moreover the entire manuscript is not enough focused on the central question it aims to address. I find a very dispersive methodologies and discussion that go far to the central question of the paper. The manuscript needs a substantial rewrite and restructuring.

The manuscript entitled “Mesopelagic Fish Responses to Pleistocene Climatic Variability in the Eastern Mediterranean and Implications for the Biological Pump” by Agiadi et al. represents a potentially valuable contribution to our understanding of biological pump variability in relation to Pleistocene climate change, using otoliths as a proxy. The methodological approach is innovative and still rarely applied to this fossil group. However, in its current form, the Methods section is difficult to follow and lacks clarity. Moreover, the manuscript is insufficiently focused on the central research question it aims to address. Both the methodological description and the Discussion are overly dispersive and frequently deviate from the core objectives of the study. In my opinion, the manuscript requires substantial rewriting and restructuring before it can be considered for publication.

Thank you for your time and effort in reviewing our manuscript and for your kind comments, which we address one-by-one below.

At line 655, the authors address a crucial point of the manuscript. They state that fish DVM is disrupted during MIS 21 based on a single data point that differs from the others within the same interglacial, where I instead observe a pattern very similar to that of the other interglacials in the investigated interval. Therefore, the generalized scheme presented in Fig. 7 does not appear to be fully supported by your data.

Line 655 is in the Conclusions, so doesn't fit with the comment. Likely the Referee means the results shown in Figure 6. The point in MIS 21 is at 858 kyr, where the modeled lifetime-average depth of the fish is 1502 m! This is drastically greater than the depths in our entire time series! It is indeed a single data point, but measurement corresponds to several individuals (10). Furthermore, as we clearly state in our Discussion, otolith proxies have the limitation that they will unlikely be present in such abundances in the sediment to provide the high resolution of other proxies such as foraminifera for example.

At line 621, the authors refer that the otoliths from MIS 21 derived from juvenile specimens. All the analyzed otoliths are from juvenile specimens? This point is crucial and should be clearly specified. For *Ceratospilus*, several data points are presented for the interglacial intervals, whereas only a single datum is available for the glacial interval, which makes any comparison statistically weak. During MIS 21, the isotope record of *C. maderensis* shows both heavier and lighter values, suggesting substantial variability that is not adequately

discussed. Could this variability be related to differences in otolith growth stage? At present, the dataset appears insufficient to support the proposed interpretation. Similarly, the isotope values of *Hygophum benoiti* are largely concentrated within interglacial intervals, while the single datum from MIS 22 falls within the same range as the interglacial values. On this basis, the inference of a distinct glacial–interglacial signal seems insufficiently supported.

In Figure 6, the size of the circle corresponds to the size of the fish (as stated in the caption). The two samples that we refer to in line 621 (approximately corresponding to the middle of MIS 21) come from juvenile individuals. No, not all the otoliths were from juveniles, only in these samples and in the one in late MIS 23, and this is because we could not find adult specimens in those levels to analyze. We will clarify.

We analyzed otoliths from all the levels that we could find them for the two target species. Unfortunately, we did not have enough in the glacials. We did not perform any statistical comparison in this case.

The time series does include values that are heavier and lighter. This is already clearly presented in the results and illustrated in the supplement. We do mention the high variability for *C. maderensis* in particular, but do not indeed discuss this further for each MIS separately. Thank you for highlighting this. We will add to the Discussion on this point.

It is possible that otolith growth rate changed within the study interval for both species. We did not test for this in the current study. Nevertheless, we do not believe that the observed isotopic ratio variability is related to otolith growth stage, because the very different isotopic values are observed for adults in most cases (except see above); this was assured based on the good development of the otolith morphology, which only happens at maturity. We will comment on this in the Discussion at this point and in the Limitations paragraph. Thank you.

Figure 8 is schematic aimed to illustrate our initial observation and what we believe happened in early MIS 21 at 858 kyr. We will make sure this is more clearly shown in the caption as well.

Moreover, there are aspects related to the references that I would like to clarify. In particular, several methodological concepts are referred to Agiadi et al. (2024), “Pelagic ecosystem responses to changes in seawater conditions during the Middle Pleistocene Transition in the Eastern Mediterranean” (DOI: <https://doi.org/10.1101/2024.12.28.630586>). However, this DOI redirects to a preprint version of the present manuscript submitted to Biogeosciences. This creates confusion, as essential methodological information appears to be delegated to a work that is effectively the same study or a very closely related preprint.

To clarify, Agiadi et al. (2024b) is the openly accessible dataset that we produced for this paper, which has been deposited in Zenodo. On the other hand, Agiadi et al. (2024c) is the preprint of this paper that we cite only in the Data and Code availability statement, as required by the journal. The title has changed from the previous version and we can correct that, but the doi is the same.

Moreover, at line 365 the manuscript cites Fuster-Alonso et al. (2024), which is listed in the references as a code repository developed for Agiadi et al., 2024. This latter work is a bioRxiv preprint addressing the same conceptual framework and methodological basis as the

manuscript under review. The distinction between these contributions and their respective roles in the methodological framework is therefore unclear.

[Fuster-Alonso et al. \(2024\)](#) is the code for the present paper that has been deposited in open access in Zenodo. It refers to the original preprint version ([Agiadi et al. 2024c](#)). We have now associated the code in Zenodo to the new preprint version to avoid this confusion.

I apologize for insisting on this point, but I find the procedure confusing and potentially problematic from the perspective of transparency and reproducibility. Even if this approach is considered acceptable, I strongly recommend that the authors revise the methodology section to make it more explicit, self-contained, and comprehensible, without referring readers to external manuscripts or preprints for concepts that are essential to understanding the results and discussion of the present study.

The only citations we have in the main text are to the code and the dataset. The preprint citation is only present in the Data and Code availability statement.