Reviewer 2

Line by line comments

Title: for clumped isotope equilibrium it doesn't matter what the ambient fluid is

Re: We agree and have changed the title to acknowledge this.

"A well-constrained, coccolith-specific clumped isotope calibration of cultured coccolithophorids for marine temperature reconstructions"

L8: "all follow": disagree, biogenic carbonates such as brachiopods and corals do show disequilibrium effects documented in many publications

Re: Substituted "all" with "most".

L15: The literature is saturated with calibration studies. To catch the attention of the reader, the authors may want to make it clear in the abstract what is new in their study compared to previous studies, e.g., higher precision, more species analysed?

Re: We thank the reviewer for this excellent comment about the novelty of our study. Our study has well-constrained temperatures and carbonate system conditions, within 0.1°C precision. The precious culture study also was before the Intercarb standardisation and thus not as relevant. We have included more strong wording regarding our novelty.

L10-12: "Biogenic calibrations such as those based on foraminifera from seafloor sediments suffer from uncertainties in the determination of the calcification temperatures. Therefore, well-constrained laboratory cultures without temperature uncertainty can help resolve these discrepancies."

L21: Strongly disagree. No one would even consider using, for example, a foraminifera calibration for a mollusk.

Re: We have rewritten this section to better reflect what we meant with the statement and our conclusions. Recent clumped isotope calibration studies (Daëron & Gray, 2023; Daëron & Vermeesch, 2024) have found that biogenic carbonates, in particular foraminifera and bivalves, are indistinguishable from the inorganic carbonate calibration and formulated a general calibration. We acknowledge that every type of biogenic carbonate must first be evaluated if it does follow this general calibration, which is indeed what we do in this study. Following the logic of the reviewer, one would require a specific calibration for every type of organism and carbonate, which in itself has many difficulties as we discuss in section 4.5.

L30-32: "Thus, we suggest the use of our coccolith-specific calibration for further coccolith palaeoceanographic studies and that calibrations derived from laboratory-grown biogenic carbonates, in particular foraminifera, are desirable to reinforce the confidence of clumped isotope-based temperature reconstructions in palaeoceanography."

L40: Consider adding (Thiagarajan et al., 2011), (Kimball et al., 2016), (Spooner et al., 2016), and (Davies et al., 2022) for corals, and (Bajnai et al., 2018) and (Davies et al., 2023) for brachiopods. Also, even if some biogenic carbonates fall within the confidence interval of a compilation-based (inorganic) calibration, they may still exhibit disequilibrium effects, that are e.g., not resolved.

Re: We included the suggested references. We agree that there might still be disequilibrium effects and have reworded this section and discussed further in 4.5.

L50-60: "Further empirical studies on biogenic carbonates, such as for foraminifera, coccoliths, gastropods, and bivalves, have found similar relationships between Δ_{47} and calcification temperature

(Katz et al., 2017; Peral et al., 2018; Leutert et al., 2019; Piasecki et al., 2019; de Winter et al., 2022; Huyghe et al., 2022), although specific types of biogenic carbonates such as shallow-water corals (Spooner et al., 2016; Davies et al., 2022), juvenile bivalves (Huyghe et al., 2022), and brachiopods (Bajnai et al., 2018; Davies et al., 2023; Letulle et al., 2023) do not. However, there are clear discrepancies between most inorganic calibrations (Swart et al. 2019; Jautzy et al. 2020; Anderson et al., 2021; Fiebig et al., 2021) and an often used, generalised biogenic calibration (Meinicke et al., 2020). One interpretation is that this discrepancy results from uncertainties in the calculation of calcification temperatures for planktonic foraminifera and is resolved with alternate approach to calcification temperature estimation (Daëron and Gray, 2023). With this study using cultured coccoliths we generate biogenic carbonate under well-constrained temperature conditions, so there is little uncertainty in the calcification temperatures."

L51: This statement is only true for mollusks if one wants to do a high-resolution study.

Re: We have rewritten this sentence to provide further detail:

L67-70: "Both limited abundance and time requirements for picking limits the availability of planktonic foraminifera, and the need for sampling precise seasonal increments in slow-growing molluscs can also restrict the mass of carbonate available for analysis (Leutert et al., 2019; de Winter et al., 2022; Huyghe et al., 2022)."

L60: What are coccolith vital effects?

Re: We have reworded and clarified this statement. We now state:

L76-80: "Biogenic carbonates often feature carbon and oxygen isotopic compositions that differ from those expected for abiogenic carbonates near equilibrium, offsets informally called "vital effects". Such offsets have been described for coccolith calcite (Ziveri et al., 2003; Rickaby et al., 2010; Ziveri et al., 2012; Candelier et al., 2013; Hermoso et al., 2014; Stevenson et al., 2014; Hermoso et al., 2016, Katz et al., 2017) and the contributing processes simulated in models (Langer et al., 2012; Ziveri et al., 2012; Bolton and Stoll, 2013; Holtz et al., 2017; McClelland et al., 2017)."

L69: Do the two investigated genera have distinct calcification mechanisms that made the authors expect genus-specific effects?

Re: We have added a sentence to clarify that the two *Gephyrocapsa* strains were chosen to cover a broad temperature range and that these were contrasted with *Calcidiscus*, which was previously shown to exhibit distinct oxygen and carbon isotopic vital effects. We now write:

L89-93: "Coccolithophores from the Gephyrocapsa genus were cultured between 6°C and 27°C, using the warm-adapted G. oceanica and the cold-adapted G. muellerae. Inter-genus vital effects were tested through comparison with Calcidiscus leptoporus, which features distinct carbon and oxygen isotopic vital effects compared to Gephyrocapsa in previous studies (Ziveri et al., 2003; Hermoso et al., 2014; Katz et al., 2017)."

L75: Did the authors observe any culture stress-related effects e.g., in morphology? Are there signs of carbonate dissolution?

Re: We have added a brief sentence at the end of the section on cleaning and SEM evaluation, to indicate that we did not find evidence of malformation, and that the oxidative cleaning causes some coccolith dissolution.

L176-177: "As harvested from the cultures, all coccoliths exhibited regular morphology with no evidence of coccolith malformation. The cleaning protocol causes slight dissolution, fragmentation, and breakage of some of the coccoliths."

L149: “reacted” may not be the correct word choice here

Re: Agreed and replaced in L167: *“suspended”.*

L185: To avoid the excessive use of abbreviations (e.g., CRM, CRLS, ETF, TLE …), the authors may consider the rule of thumb to only introduce an abbreviation that appears three or more times in the text.

Re: We thank the reviewer for this excellent suggestion and will be implemented.

L198: The correct notation is either δ^2H or δD, but not δ^2D

Re: We changed the notation in L226: *“…δ^2H…”*.

L203: Can you give a mean value here for the reproducibility?

Re: We included the mean uncertainties for both the $\delta^{18}O_{sw}$ in L230*: “…average $\delta^{18}O_{sw}$ and uncertainty (mean σ=0.32‰)…*” and $\Delta^{18}O_{c\text{-}sw}$ in L233: *“…in subsequent figures (mean σ=0.29‰).”* in the text.

L265: Consider adding “no ‘resolvable’ difference”

Re: Agreed and included in L293: *“There is no resolvable difference…”*.

Additionally, the authors show prominent oxygen isotope disequilibrium effects. Irrespective of how the clumped data compares to other calibrations, what carbonate growth model makes it possible that oxygen isotopes are in disequilibrium while clumped isotopes are not?

Re: At the moment there are no models for coccolithophore biomineralization that simulate both oxygen isotopes and clumped isotopes in coccoliths, although this is work in progress (pers. comm. H. Zhang, J. Watkins). Indeed, there is not even a model simulating both carbon and oxygen isotopes in coccoliths. Here, we focus on providing complete and well constrained data, which could be used to parameterise quantitative models employed in the future.

L349: Does this mean that your regression errors are likely underestimated?

Re: For our study this is not necessarily the case as we made sure to include enough replicates and a wide temperature range.

L381: (!!) The authors wrote that the previous coccolith clumped studies were published before interlab standardization. What is the basis for a direct comparison of the values published in those studies and here? Did the authors recalculate the values by normalizing them to common standards?

Re: We acknowledge the confusion this might cause and have shortened this section. We included this study as their culture data was relevant for our study, in particular regarding the carbon and oxygen isotope offsets.

L431-436: “The similar culturing study of three coccolithophore species by Katz et al. (2017) also found no species-specific vital effects affecting the Δ_{47} values and a consistent Δ_{47}-temperature correlation. However, the study was conducted before the introduction of the I-CDES standardisation methodology using carbonates and used gas-based standardization, consequently the data could have a systematic difference that cannot be resolved with certainty. Thus, when comparing to other calibration studies we will not include Katz et al. (2017) in the dataset and use Eq. 4 as a coccolith Δ_{47}-temperature calibration, which is only based on our culture data in the I-CDES frame.”

L393: Why is it an “MIT” calibration?

Re: We have reformulated this section. This calibration is taken directly from the Daëron and Gray (2023) paper, the measurements and analyses were performed at MIT and only include inorganic carbonate measurements.

L440-442: "We focus on five biogenic (Peral et al., 2018; Meinicke et al., 2020; Caldarescu et al., 2021; de Winter et al., 2022; Huyghe et al., 2022) and one inorganic ("MIT calibration"; Anderson et al., 2021; Daëron and Gray, 2023) carbonate studies."

L443: The study did not attempt to fit coccolith data into a calcification model, which is probably why no physiological conclusions could be drawn.

Re: We agree with the reviewer and have included a statement regarding this.

L550-551: "A calcification model would aid in describing the sources of the vital effects."

Figure 8: (#1) This figure is overcrowded and difficult to decipher. Suggest not displaying sample data from other studies but only the regression lines. (#2) Please consider plotting the calibration lines only within the temperature range they were made for.

Re: We agree and have updated the figure to be clearer.

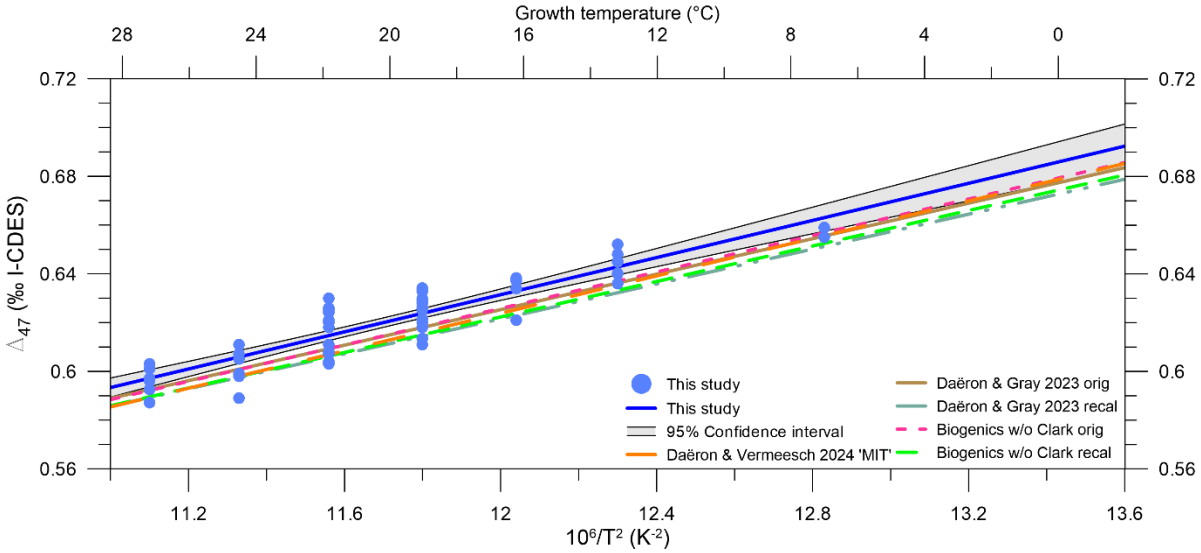

Figure 3: The kinetic limit in the (Watkins et al., 2014) paper relates to the pH-dependent incorporation of the DIC species in the carbonate. However, the simple comparison presented here implies that this is the only "vital effect" that is relevant for coccoliths. However, it may as well be that some fractionation effects drive the O isotope composition of the carbonate in one direction, whereas others in the other direction, and they cancel out. The point is that this is not known without detailing the calcification model and the possibly occurring fractionation effects.

Re: We have updated the section discussing our findings to better clarify and discuss the potential fractionation effects.

L316-324: "Here, a pH of 8.3 at the crystallisation-site and the fastest growth rate is assumed in the model of Watkins et al. (2013, 2014) and Watkins and Devriendt (2022), with which the 'kinetic limit' is derived and illustrated in Fig. 3. This gives an approximate 2‰ offset and incorporates a large range of experimentally derived and modelled inorganic calcites precipitated in presence of CA. Additionally, numerous experiments and potentially many natural biogenic and abiogenic systems may precipitate calcite from a solution in which equilibrium between DIC and H_2O is not maintained due to a lack of CA or fast calcification rates (Devriendt et al., 2017; Daëron et al. 2019). Rayleigh fractionation of oxygen isotopes in the internal DIC pool occurs as a result, which is transferred to the isotopic composition of the calcite and leads to lower $\Delta^{18}O_{c\text{-}w}$ values, thus exacerbating the disequilibrium fractionation potentially present between the DIC and calcite as described above."