A modern snapshot of the isotopic composition of lacustrine biogenic carbonates – Records of seasonal water temperature variability

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RC2

The study by Labuhn et al. discusses an interesting issue of applicability of oxygen stable isotope measurements in specific lacustrine carbonates in reconstructions of past water temperatures. The study adds to the already existing knowledge as pointed out by authors.

I found the manuscript well written and interesting. The introduction is informative and points out the key information based on the available literature sources. The data are well presented with high-quality graphics. The authors discuss the possible mechanisms that control the stable isotope composition of the carbonates studied and explain the possible reasons for the differences in the stable isotope composition of encrustations and shells.

The study confirms the established knowledge that due to the differences in stable isotope composition $\delta_{18}O$ measurements should be performed on the specific types of carbonates instead of bulk carbonate samples of unknown and potentially time-variable composition. The most important outcome of the study is showing that by studying selected carbonates it is possible to estimate seasonal water temperature changes.

Despite the overall good quality of the study I suggest considering the specific comments listed below before the manuscript can be accepted for publication.

We thank Reviewer 2 for her evaluation of the manuscript and the constructive comments. Our replies and modifications in the manuscript are explained after each comment (in blue).

Specific comments:

Line 4: change ‘on’ to ‘by’

Corrected.

Line 98: delete double ‘the’

Corrected.

Lines 145-148: I would not limit the growth of Chara to May-July. What about August and possibly also September? You suggest that charophytes studied are perennial.

It is true that the charophytes can be perennial. The sentence was misleading, because it referred to the meteorological conditions in the year of sampling. The samples were taken in July, i.e. the August and September temperatures of that specific year do not have any influence. Since the reference to Chara growth is not necessary in this paragraph about meteorology, we have deleted the sentence.

Lines 156-157: What was the bottom area (cm2) where each of the surface sediment was sampled?

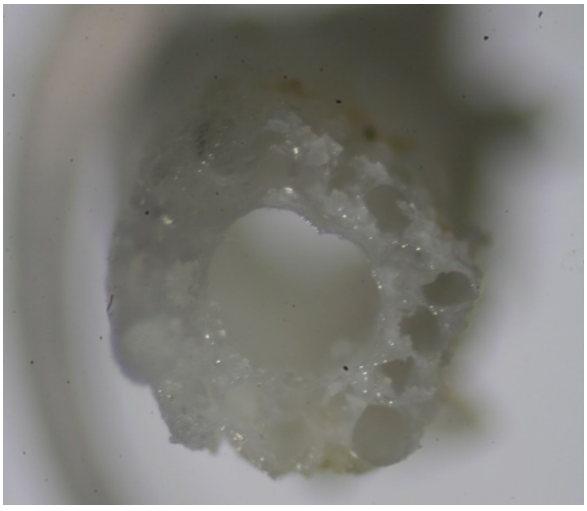
We thank the reviewer for pointing out the missing details on the sampling procedures. We have included the size of the bottom area (8x8 cm2) in Line 156.

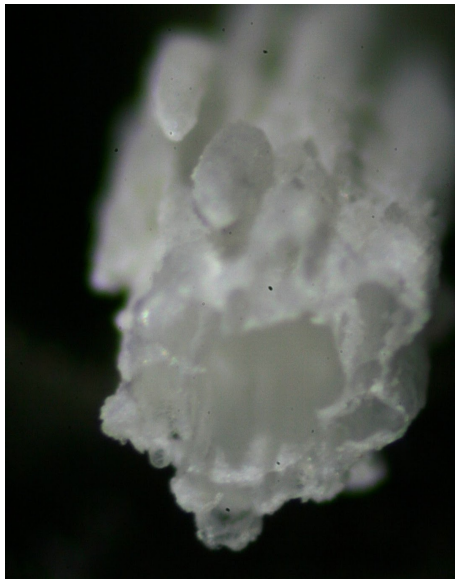
160-162: Information about the sampling of charophytes is lacking. Were the whole macroalgae taken? Cut at the water-surface sediment interface? How many individuals of each species were sampled?

These details on charophyte sampling have been added in Lines 158-159: the whole algae were sampled, up to six individuals which were cut off by hand by the divers.

Lines 168-169: Since I was involved in the studies of the isotopic composition of recent charophytes I have also tried to remove organics with H2O2. I have never managed to remove all. Part of the stem was always resilient and remained after the treatment.

This is an important issue. It is crucial that the carbonate is not contaminated by organic matter, as carbonate and organic matter differ in their isotopic composition. We cut off branchlets and internodes from the charophytes prior to the H2O2 treatment (see Line 162-163). These small parts were free from organic matter after the treatment. All samples were inspected under a binocular prior to isotope measurements. Examples of clean encrustations from our living charophyte samples after H2O2 treatment are shown in the images below. The inside of the encrustation, where the stem used to be, is clearly free from any organic matter.





Line 169: Please explain what the 'fine-grained calcite sticking to the encrustations' is. Why did you remove it? How do you know that you did not remove a fraction of encrustations at the same time?

We do not know the origin of this fine calcite. The material might originate from the encrustations themselves, or it could be inorganically precipitated calcite deposited on the encrustations. For this reason, we measured its isotopic composition separately from the encrustations, in order to evaluate whether it could represent a contamination and bias the results of the isotope measurements on encrustations. Our results indicate that the fine calcite actually is composed of disaggregated Chara encrustations, because the d13C values are very similar (see Lines 282-285).

To explain the reason for these measurements, we have added the following information (Lines 176-177):

"The removed fine calcite was also kept for isotope measurements in order to test if it is composed of inorganically precipitated calcite, or if it originates from the encrustations themselves."

Lines 255-257: I guess both living specimens and Pisidium shells taken from surface sediments in fact originate from the surface sediments therefore it is better to say: shells of living Pisidium specimens and empty Pisidium shells

Indeed, our previous terminology was imprecise, because both the "dead"/empty Pisidium shells as well as the living specimens are found in the sediment. In agreement with the comment of Reviewer 1 on Fig. 6, we have changed this paragraph to (Lines 268-270):

"There is no significant difference in isotope values between the valves of living specimens sampled in 2018 and the subfossil valves (i.e. which were formed and deposited during previous years), possibly because their time spans of carbonate

accumulation overlap. The mean d18O is the same, and the mean d13C differs by 0.19 permil."

Furthermore, we have revised the entire manuscript, figures and captions to keep a consistent terminology regarding the different types of samples.

Line 257: probably it would be good to change 'living and dead samples' to 'shells of living and dead mussels'

We agree with the reviewer and changed the terminology accordingly; see comment above.

Lines 263-264 and 380-382: Charophytes – you studied 'single stalks from an internode or branchlet'. Encrustations at one specimen are not formed at the same time but as charophytes grow. Therefore in the isotopic studies of charophytes, specific fragments were studied, e.g. apical fragments. The variation of stable isotope values of charophyte encrustations studied may result from the fact that different fragments had CaCO3 precipitated at slightly different times, i.e. as the charophyte grew. In my opinion, the larger isotopic range of Chara hispida is also due to the gradual seasonal growth and precipitation of encrustations in the changing ambient conditions.

We thank Reviewer 2 for this comment and the following ones related to Chara growth and the timing of calcification. These aspects are important to consider in the interpretation of the isotopic composition of the encrustations, but did not become sufficiently clear in the previous version of the manuscript. A number of changes have been made accordingly (see also the following two comments).

Lines 279-281: "Each analyzed sample consisted of only one internode or branchlet, which means that environmental variations within the growing season or between different years are not averaged in individual samples. The samples represent only the short period of time when the respective part of the Chara grew."

Lines 392-395: "Chara can be perennial, but the samples measured in this study, encrustations from single internodes or branchlets, were precipitated at different times as the Chara grew, i.e. individual samples may represent different times within a growing season and possibly different years, hence explaining their larger isotopic range."

Lines 264-265: Do you have confirmation that Chara hispida from the lake studied overwintered? Charophytes are not always perennial. Overwintering can occur but it is not a rule. I have observed this during the field studies I participated in. You can have a look at publications e.g. of Mariusz PeÅ echaty – an experienced charophyte scientist with extensive field experience, in which the issue is discussed.

We do not have any confirmation from our field observations that the studied Chara overwintered. We therefore have added the information that Chara can be perennial, but that this is not always the case, and added the suggested reference (Lines 276-277):

"Chara can be annual or perennial (Martin et al. 2003, Pełechaty et al. 2013). It is not known whether overwintering occurs in Locknesjön, but different parts of the algae are formed during different times of the growing season, or possibly during different years."

Lines 265-266: Whole new and several dozen cm high charophyte can grow within one season –personal field observations.

It is true that charophyte growth can be fast. The idea of this sentence was to say that the calcification occurs only around the part of the Chara which is growing, i.e. the apices. To be clearer, also considering the changes in the above comment related to annual/perennial growth, we have modified the sentence to (Lines 277-278):

"The growth of Chara is apical, i.e. calcification at a given point in time occurs only around these apices (Coletta et al. 2001)."

Line 266: Which internodes and branchlets were sampled? Apical ones or fragments from different parts of charophytes. Also, what were the sizes of charophytes? How tall were the macroalgae studied? This information is important in the context of the discussion. Thick and dense charophyte stands can form a specific microhabitat, they can also limit the extent of water mixing to the bottom.

We sampled fragments from different parts distributed throughout the charophytes. They were about 20 cm tall. The density of charophyte stands at the sampling locations in Locknesjön was relatively low, and we suppose that it does not have an effect on the isotopic composition of the encrustations. We do not observe any significant trend in d18O or d13C with depth (see Fig. 9), and the charophytes became very sparse at 7 m depth and absent at 8 m depth.

We have added information on the sampled fragments in the Material and Methods section:

Lines 173-174: "Internode and branchlet samples were cut off from different parts of the living Chara [...]."

Lines 158-159: "The density of Chara hispida stands at the sampling locations was relatively low. The Chara population became very sparse at 7 m depth and absent at 8 m depth."

Lines 279-282: This difference may result from the intensity of photosynthesis and density of charophyte patches.

We have added the intensity of photosynthesis (associated with fast calcite precipitation) as a reason for these offsets (Lines 295-297):

"Other studies, on the contrary, have found significant offsets from equilibrium due to kinetic effects during intense

photosynthesis associated with fast calcite precipitation (Apolinarska et al., 2016; Andrews et al., 2004)."

See comment above about the density of charophyte stands.

Lines 283-284: Here 'fine calcite' is mentioned once again, what kind of calcite is that? More explanation is needed.

This indeed needs an explanation, which we have added in Lines 176-177 (see our reply to the comment on Line 169).

Lines 317-319: Influence of stratification is mentioned here however, previously in the manuscript it was stated what waters within the epilimnion are well mixed and looking at the data one can see that thermocline is below the deepest site sampled. Also, see lines 345-348 and most important lines 352-357: These fragments confirm my concern about interpreting $\delta_{18}O$ values in Candona as related to water stratification.

We thank the Reviewer for pointing out this lack of clarity. We do suppose that the water in the epilimnion is well mixed, and that all our sampling locations down to 8 m water depth are above the summer thermocline. These assumptions are supported by the water temperature profiles (Fig. 3) and the isotopic composition of the water (Fig. 5, Fig. 9). Therefore, stratification is not an explanation for the differences in d18O between ostracod instars A-4/-3/-2 and instars A-1. They all form their valves during a time of stratification (in the epilimnion, above the thermocline), and the seasonally changing temperature within the epilimnion causes differences in d18O.

On the other hand, stratification - or the absence thereof - becomes important for adult ostracods, as they form when isotopically enriched surface water is mixed with deep water with a lower d18O (although the lower water temperature at this time is the dominant influence on the valve carbonate d18O). We have re-written this paragraph as follows (Lines 331-338):

"Juvenile ostracods form their valves when the lake is thermally stratified during the warm season. As the instars A-2 to A-4 form at the time of maximum water temperature, the mean d18O of their valves is significantly lower than in A-1 and adult samples. There is no significant difference between A-2, A-3 and A-4 instars. The average d18O of A-1 samples is higher than in younger instars because A-1 instars develop when water temperature starts to decrease in autumn. Moreover, by the end of the summer the water in the epilimnion is more enriched in 18O by evaporation compared to the time when the younger instars calcify their valves, leading to a further increase in the d18O of A-1 valves. The valves of adult ostracods start forming in late autumn. When overturning occurs in the lake, stratification ceases and the isotopically enriched surface water is mixed with deep water with a lower d18O."

Lines 324-329: Also, δ13C of adult ostracods is lowest because of water mixing and return of the 13C-depleted DIC of the waters from below the thermocline

This explanation has been added to the paragraph (Lines 345-347):

"When adult valves are formed, the lake is not stratified and the overturning brings DIC with a lower d13C from depths below the summer thermocline to the surface."

Lines 424-425: Which is an apparent drawback.

We agree with Reviewer 2, because the quantification of the temperature influence on carbonate d18O would require continuous measurements of water temperature throughout the period of carbonate precipitation in a sample. A part of the motivation for our study was to test this "snapshot approach" and evaluate whether relevant information to aid the interpretation of paleoclimate records from sediment cores can be obtained without long-term monitoring. Even if continuous water temperature measurements were available, it may be difficult to link the precise timing of calcification in biogenic carbonates to a specific water temperature, because the calcification does not occur continuously throughout the lifetime of the organism.

437-438: Temperatures absolutely unlikely to occur. In central Europe, even during days with temperatures > 30 during the day, water temperature in the epilimnion reaches 24-25oC.

We agree that these temperatures certainly do not occur in lake water in our study area. The sentence has been re-written replacing "unlikely" with "impossible" (Line 451).

Fig. 3a: Why don't you present the complete, i.e. whole year precipitation and temperature data for the year of sampling – 2018? This may differ from the long term data. In fact, the difference is already visible, especially in precipitation values.

We have added the monthly temperature and precipitation data of the year 2018 from January to July (which corresponds to the time of sampling in 2018), in Figure 3a.

Sincerely,

Karina Apolinarska