

# ***Interactive comment on “Freshwater pearl mussels from northern Sweden serve as long-term, high-resolution stream water isotope recorders” by Bernd R. Schöne et al.***

**Bernd R. Schöne et al.**

schoeneb@uni-mainz.de

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First of all, we wish to thank the reviewer for his/her thoughtful comments and suggestions. In particular, the requested critical statistical re-assessment further substantiated the main conclusions of our study. Re-calculations were complex and simulations quite time-consuming, but it was certainly worth the effort. We have added the revised version of the manuscript with all changes highlighted red as a supplementary file to this author comment.

RC2: I have some broader concerns about some of the assumptions made when transforming the data, and some concerns about the statistical approaches employed. I

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anticipate that addressing these concerns will not have major impacts on the conclusions reached, but I cannot be sure at this point. [...] The authors provide a fairly straightforward equation (1) linking air temperature to water temperature, basically with a damping effect, based on the authors' previous research. This seems reasonable, but as this is an empirical relationship (that looks pretty good in original manuscript), it should really have some form of uncertainty estimates on the slope and intercept, and these uncertainties should be propagated to later equations. Otherwise it is difficult for the reader to assess whether the nature of this relationship is important later. Another assumption the authors make (first stated in Line 69) is the use of annual increment width to determine weighted annual water temperature. On its face, this approach also seems reasonable, but again, the importance of the choices made here (with respect to weighting) is not evaluated, nor are uncertainties in SGI measurements. The equation in question (3) is also presented with no uncertainty estimates, as above – this could be important, especially given the fair amount of scatter in Figure 3b. There is clearly a meaningful relationship, but that does not imply that using SGI for predicting  $T_w^*$  can be done without uncertainty, and there is certainly more scatter than the  $T_a$ - $T_w$  relationship.

AC2: As suggested, error estimates of the slope and intercept of the relationship between stream water and air temperature were provided (L149, Eq. 1). The same was provided for the relationship between shell growth (SGI) and stream water temperature (L186, Eq. 3). All errors were propagated and used in complex simulation experiments (see below).

RC2: The use of annual weighting scheme (detailed in Table 2) is also reasonable, but is of course a necessary simplification that includes several other assumptions. Does the exact 143 day growing window matter? How much does the exact weighting of samples matter? Maybe a comparison to an unweighted calculation may be appropriate as a test for the importance of this step?

AC2: Since it has not been evaluated so far, how much the seasonal growth traits

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change among specimens, we cannot provide an error estimate for the weights, which have been used to compute weighted annual averages. However, in a previous study (Dunca et al., 2005), it has been demonstrated that the seasonal timing of shell growth in this species remains notably invariant across large distances. We therefore assume that the seasonal growth traits are genetically controlled (biological clocks). Another implication for a nearly unchanged duration of seasonal shell growth comes from cross-dating SGI chronologies. If the seasonal growth traits would change, it would be impossible to combine SGI chronologies of young and old specimens. As the second part of the comment is concerned: Considering differences in time represented by each isotope sample can improve the results. For example, without weighted calculations the R2 value for the correlation between SGI and water temperature was 0.60, with the weighting scheme it increased to 0.64 (section 3.1). The larger the isotopic differences among samples of a given year are, the more does weighting matter.

RC2: Basically –  $T_w$  is determined from  $T_a$  without uncertainty.  $\delta^{18}O_s^*$  is calculated using assumptions outlined in the point above. Then  $T_w$  is used along with  $\delta^{18}O_s^*$  to calculate  $\delta^{18}O_{wr}^*$  via an equation with no uncertainty (though none is presented in the original text to be fair). I'd like to see some effort to either propagate uncertainty, or to at least test the sensitivity of their final result to these assumptions.

AC2: We have followed this recommendation, propagated all uncertainties and evaluated the effect on the regression analyses. For this purpose, we ran Monte Carlo simulations and randomly generated isotope chronologies (1,000 per set) that were subsequently compared to climate indices etc. A whole new section was added in the Discussion (section 4.7) and a new table with the results of these simulations tests added (Table 5). As anticipated by Rev#2, the propagation of errors did not change the core interpretation: the winter NAO leaves an imprint in the oxygen isotope value of winter precipitation, which is transferred into the river isotope values and eventually the shells during snowmelt in late spring and early summer. The bivalve shells thus serve as an archive for past changes of the wNAO.

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RC2: Statistics – I have some issues with the way statistics are handled in the manuscript. Like the assumptions above, it is quite possible that improving them will not change the ultimate conclusions of the manuscript, but there is certainly a possibility that some ideas will have to be revisited. Specifically: One principle for using p-values is that it is not appropriate to run a large number of p-value tests and the use 0.05 as a threshold for significance. There a number of ways to account for this (like a Bonferroni correction) that provide a more appropriate significant threshold when running multiple tests. That said, there are likely other multivariate statistical approaches that would be more appropriate to use for a variety of the questions the authors propose. They can probably get by without them here, but it might something to explore. The comparison of  $\delta^{18}\text{O}_w$  and  $\delta^{18}\text{O}_w^*$  (Fig. 6a) is determined to be “good” but by what standard. Can this comparison be improved/quantified statistically?

AC2: We have applied the Bonferroni adjustment for all p-values. The majority of regression analyses remained statistically significant at  $p < 0.05$ . As expected, regression analyses with a very low number of data points (Table 3) no longer reached the significance threshold when the Bonferroni adjustment was applied. However, the main conclusions were not affected. Since the main conclusion (wNAO affects  $\delta^{18}\text{O}_w$ ) is already obvious from the raw shell data ( $\delta^{18}\text{O}_s$ ), we refrained from applying even more complex multivariate statistical approaches. Future studies using specimens from a closely monitored river system should certainly consider this recommendation. Following non-parametric t-tests, the water isotope data sets are statistically not different. The caption of Fig. 6 was rephrased accordingly, and text added in section 3.3.

RC2: Broadly – if space isn't a concern, a table of all the various  $\delta$ asterisk/subscript variations (and potentially other abbreviations/nomenclature) and ideally even their method of calculation might be useful as a reference for the reader. I found myself constantly flipping back and forth to remind myself what each permutation meant.

AC2: Great suggestion! We have added such a list (new section: “Overview of abbreviations used in the manuscript.”).

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RC2: Line 35: “required” – could a quantitative measure of the required resolution be listed here? Also, this sentence would be better written a required resolution for ... what? added.

AC2: We have specified this further: “the required, i.e., at least annual temporal resolution”

RC2: Line 41: “short”, “small” both could also have a quantitative measure added.

AC2: Quantification added, “small” deleted

RC2: Line 42: “signatures” at least according to Sharp (Principles of Stable Isotope Geochemistry) the word ‘signatures’ should be reserved for large reservoirs with consistent isotopic values (e.g. the ocean, the mantle). There are few other places below I will flag the isotopic terminology as a place to make slight (possibly pedantic) improvements.

AC2: We feel that “signatures” is a valid alternative to value or data and thus did not change this term.

RC2: Line 88: I believe that typically for endangered species, if there was an approved permit for collection, that the permit should be referenced here and/or in the acknowledgements, even if they were originally collected as part of another project. I am not sure if the journal has a policy on this issue though.

AC2: The permit does not have a number.

RC2: Line 94: should this be “flow-through” lakes?

AC2: Yes, changed.

RC2: Line 95: “dried from air” would be better written as “air-dried,” or “dried in ambient air,” or something similar; “from air” is awkward.

AC2: Changed as requested.

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RC2: Line 101-102: This mirrored idea is intriguing, but I am having trouble visualizing it. Given its potentially utility to others, a supplementary image of this set up would be nice.

AC2: Instead of adding another figure, we specified the text as follows: “. . . mirroring sides (= the portions that were located to the left and right of the saw blade during the cutting process)”

RC2: Lines 114-117: Given the importance of this method to this manuscript, it might be worth providing a few more details here, despite the previous publications.

AC2: Although we think that this method is fairly well described in the literature (many review articles), we have added the following: “Briefly, for detrending, measured annual increment widths were divided by the data predicted by the cubic spline fit. From each resulting growth index, we subtracted the mean of all growth indices and divided the result by the standard deviation of all growth indices of the respective bivalve specimen. This transformation resulted in SGI chronologies.”

RC2: Lines 122-125: The text here seems to indicate they micromilled shell material by hand while maintaining consistent sample spacing. That’s really quite challenging in my experience (though not impossible). It would be useful in Figure 2 to include an image of the actual drilled sample pathways. Was any attempt made to control (or later check) the depth of drilling? This would could be a significant source of variability.

AC2: We have added this information schematically in Fig. 2c. We have long-term experience with such kind of micromilling and obtained much better results than with the stiff NewWave/Merchantec micromill robot, which cannot really follow shapes in three dimensions. It would certainly be a useful undertaking to quantify how the two methods compare to each other and which produces the largest (= most realistic) seasonal amplitude. We are ready for a test! A depth control would make sense if one simultaneously monitors the shape of the growth front. Most likely, this is not routinely done when the micromill robot is used. However, when the sample is watched under the

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binocular microscope during sampling, it is possible to track the growth front in three dimensions.

RC2: Lines 135+: I couldn't find access to a full table of data, but such a table must be provided with the final submission. That table (or tables) should include both the uncorrected  $\delta^{18}\text{O}$  value, but also the corrected  $\delta^{18}\text{O}$  for aragonite.

AC2: As written in section "Data and code availability", everything will be made available on request. We would be more than happy to submit a package with EXCEL files as a supplement if permitted by the journal.

RC2: Line 227: Why do we care that omitting GJ samples slightly increases R2?

AC2: Agreed. Deleted.

RC2: Line 228: I feel like a reference was omitted at the end of this line?

AC2: Missing reference was added.

RC2: Line 240: "Major common period" is not a calculation I am familiar with. Is this a formal calculation or a general observation by the authors?

AC2: "common" deleted

RC2: Line 281: "isotopes" should probably be "isotope values" or just " $\delta^{18}\text{O}$ "

AC2: As requested by Rev#1, we added "value(s)" or "data" behind all deltas

RC2: Figure 1: Potentially color/symbol differently for bivalve vs. water vs. temperature measuring sites? I'd propose removing the lines on 1b that presumably indicate districts within the province? They are not mentioned and visually complicate the figure. Moving Lat/Long markers outside 1b (especially the internal tickmarks) would also help simplify the figure. Potentially indicate the precipitation direction for NAO+/- on 1a if possible?

AC2: As requested, different symbols were used for shell sites and measuring sites.

We cannot remove the grey lines in the figure, because the base map that we used is not a vector graphic. We prefer leaving the lat/lon markers in the figure, because otherwise the curvature of the lat/lon would not be apparent any more. Precipitation directions would further complicate the figure.

RC2: Figure 2: Possibly include an image of the sample after drilling here as well?

AC2: We are not sure how would this help the reader.

RC2: Figure 3: if more space is available, making 3a longer (extending x-axis) would be helpful in visually comparing the two records. Uncertainty on 3b best fit equation should be calculated and used (see comments above).

AC2: A similar figure was previously used in two articles by the lead author. The new Fig. 3 is just a version with more chronologies. Even if we expanded the x-axis in Fig. 3a, we think that the cross-plot is more informative and better reveals the strength of the relationship between SGI and  $T_w^*$ . In the final version, we will request this figure to be plotted to page width. As requested, uncertainties were added and the errors propagated and used in the simulation experiments.

RC2: Figure 4: Are these four just the best selections, or are they the most highly resolved? What was selection criteria? Like Figure 3a, extending these laterally would make viewing easier.

AC2: These four specimens were sampled with very high spatial resolution. And the majority of isotope data came from these shells (Table 1). Text in figure caption was amended accordingly. In the final version, we will request this figure to be plotted on a single page.

RC2: Figure 4: Are these four just the best selections, or are they the most highly resolved? What was selection criteria? Like Figure 3a, extending these laterally would make viewing easier.

AC2: We will ensure that this figure is plotted on a single page in the final version.

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RC2: Figure 6: Line 866- is there a statistical way to evaluate the “good agreement” observed here. Visually here, I do not find this as convincing as some of the other comparisons made.

AC2: See reply to comment further above: We have conducted non-parametric t-test and verified that the measured and reconstructed data are statistically invariant.

RC2: Figure 7: “ $\delta^{18}\text{C}$ ” typo, also pretty hard to see at presented size.

AC2: Thanks for spotting this. As in other figures, we will request this figure be plotted on a single page in the final version.

RC2: Table 1: Weird formatting issue (floating 800 in top right), line 799 – I believe the second “L” here should be an “i”?

AC2: Formatting issue is not our fault. “i” vs “L”: indeed misspelled. Thanks.

RC2: Table 3: see comments above about statistics and p-values. Also, probably don’t need to report R and R2 – if R is reported just to indicate is relationship is positive, why not also instead provide the slope, which is more informative? I am a little torn here – I expect these relationships are pretty solid, but is running a regression on  $n=4$ , appropriate? + Table 4: See comments above about p-values, and R and R2.

AC2: We prefer leaving both the R and R2 values for completeness. All p-values are now Bonferroni-adjusted.

Please also note the supplement to this comment:

<https://www.hydrol-earth-syst-sci-discuss.net/hess-2019-337/hess-2019-337-AC2-supplement.pdf>

Interactive comment on Hydrol. Earth Syst. Sci. Discuss., <https://doi.org/10.5194/hess-2019-337>, 2019.

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