

Reply to anonymous Referee Comment

Page 1

Title

tropical/sub-tropical?

- The title will be changed. Detailed information on the climatic conditions of Florida will be given in section 2 of the manuscript.

I am not 100% convinced about this title, there is some info on tropical ostracodes that development is related to seasonal changes, so I do not see how this title/manuscript would show novel results...I could give a list of some papers but authors should as well do a better research on this. env variables related to seasonal changes are temperature, mixing of lakes, precipitation/begin of rain, please search for this.

- We did extensive research on life cycles and calcification of tropical freshwater ostracods and environmental parameters influencing them, without success. A second research including the headwords suggested by the referee was without success. It would have been helpful, if the referee would have given at least one or two concrete references on the topic.

I would really try to highlight the most important discovery of this manuscript in order to provide an improved title.

- The title will be changed.

Abstract

didn't see anything regarding d13C isotope results, as well as some background info as for d18O, please add. would be important to mention why did you run analysis on *C. ilosvayi* and not other species

- Additional information recommended from both referees will be added in the Abstract

Line 8 and larval stage? other factors? vital effect? reproduction, food sources, etc.?

- The abstract will be rewritten and this particular sentence will be deleted. However, larval stages are included in the term species. Vital effects are species specific and considered to be constant within one population and do not influence the maximum variation. The influence of vital effects is included in the discussion. Food sources are not relevant for the oxygen isotope composition of the shell. For carbon isotopes the dissolved inorganic carbon in the water is the main source for the formation of the shell. The influence of the ostracod diet is unclear and might be very complex. Missing background data don't allow a proper discussion on that. We are not sure what is meant with reproduction. If calcification time is meant, still the sum of environmental changes will define the isotopic variation of a population and this is restricted to the time in which the population calcifies.

Line 11 here you need to say when you took samples, since the focus of the paper is seasonal, you need to say when samples were taken, where, and months? years? because you mention something on seasonal changes at the end of the abstract, which is already too late.

- The seasonal approach is based on a model with different assumptions on the general seasonal development of rivers and their relation to ostracod isotopic signatures. The seasonal data are obtained from the literature and independent from our sampling. Thus, timing of sampling is not the key to the seasonality in this case. A clear separation of this will be given in the abstract.

what kind? only rivers? lagoons? lakes? rivers? try to give the reader as much info as possible

- This information will be added to the abstract.

what kind of instrumental data? physical? chemical? both? be more specific

- This information will be added to the abstract.

a wider region or as well south florida?

- The same region was meant, this sentence will be rephrased.

Line 17 only rivers were studied?

- In the first part of the study all sites are compared, while for the model only rivers are used. Respective sentence will be rephrased to make that clear.

Line 21 need to explain before when you took samples and that sampling was seasonal

- As stated above, the seasonal calcification period is based on the model that was applied using literature data on the seasonality.

Line 23 how? how does your study improve future studies, paleontology is too general, maybe it would make the manuscript stronger if you could give precise use of such studies, rather than mention a too general use.
related seasonal

- More precise use of this study for paleontology will be given in the manuscript. The investigation of the within sample-variability of modern ostracods in relation to environmental changes is the basis for the interpretation of high frequency climate variabilities in the ostracod record. Such paleontological studies have already been performed by Escobar et al. (2010) and Dixit et al. (2015).
- However, this sentence will be deleted

Page 2

Line 7 describe

- This sentence will be rephrased. Vital effects are discussed in section 5

Line 16 until now I do not know how often did authors collect samples, it seems that the temporal scale is so important but it is needed to describe this in the abstract

- The separation between the seasonal data for the model and our data will be described more clearly in the abstract.

Line 22 and d13C?

- The model only refers to oxygen isotopes.

but in the abstract you mention temperature, that is not chemical

- The temperature mentioned in the abstract refers to seasonal data revealed from the USGS climate viewer, not to our own measurements. However, as we also did temperature measurements this will be changed to physico-chemical in the abstract.

Line 25 years? same temporal scale/simultaneously to sample collection?

- Exact information on the seasonal data origin is given in the Methods and Table 3 and 4.

it is also important to mention in the abstract how you collected ostracodes (surface samples or hand net, since you say that they are "living ostracodes" then this is highly relevant. as well did you analyze only adults or each larval stage? or mixed?

- For the abstract the description "living ostracods" is in our opinion appropriate, more details on sampling are given in the methods.
- We analyzed mainly adults, some juveniles are also included, information will be given in the abstract and discussed later

Page 3

Line 4 in the abstract one can understand that you only studied rivers therefore include more info there

- As stated above, the separation between the two parts of the study will be given in the abstract

Line 5 are you sure that one can talk about seasonality only with two samplings?

- This comment probably also results from the unclear separation between the regional comparison of samples and the model approach which is based on monthly data from the literature.

a more robust study would include 2 years or 1 but more frequently sampled...justify it please. Why those months? are they really the two months in a year that are more significant to prove your hypothesis? I would as well try to include a sentence in the abstract what is the scientific question you are trying to answer, so that it does not sound as a methodological manuscript.

- Repeated sampling would be desirable, but, especially in regional studies it is too time consuming and expensive and in many cases not realizable. This is exactly the point of the study. When no temporal/seasonal information of the ostracod species is available, than the isotopic composition

and range of an ostracod population can reveal information on the calcification time and duration. The seasonality is not based on the two samplings, but on the model that was applied to calculate theoretical calcites and the comparison the isotopic ranges of *C. ilosvayi*.

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Line 31 Since you collected samples from a wide variety of aquatic ecosystems, displaying different chemical properties and probably physical, makes it more difficult to understand which env. parameter is/are responsible for ostracode seasonal changes...please discuss this later

- A paragraph will be added in section 5.1

Page 5

Line 5 I would clearly indicate which months are considered winter, summer, spring, fall and dry and wet season...all is important for your results and discussion, part of the info is here but not in a clear way

- This section will be restructured to point out important influences on the seasonality of surface waters

then why didn't you take samples in these months? just try to explain why do you believe that the months you sampled, should be the right ones, what was the criteria or how can you prove that your methods are correct/reliable.

- When no information on the life cycle of a species is available then the right time for sampling is uncertain. Sampling in the warmest or coldest month does not necessarily mean to be more successful. But, as stated before, the timing of sampling is not the important point for the study, but the within sample variation that reflects the conditions during calcification time of *C. ilosvayi*

Line 29 info on d13C, sometimes d13C show a more clear seasonal effect

- In this section we give information on the seasonal variation of isotopes in Florida obtained from the literature. As stated in the last paragraph of the section, data on the d13C composition in Florida are rare. But, general information on seasonal variation of different environments could be added.

Page 6

Line 24 I know that this is a benthic species, but using a ekman grab? did you use one? please describe which equipment you used so that authors do no need to go to Meyer et al. 2016 to see this. Living ostracodes or more reliable samples are those from hand nets, because generally when you use an ekman grab even if you say that one uses the top cm, those are always some years or even decades. Since this is not a paleoenvironmental study, it is not possible for you to know sedimentation rates, but if you are talking about living ostracodes, then the way to do it would have been to collect ostracodes either way, and directly see them under the stereoscope if they were actually living. If not, then I would trust hand net samples more than ekman samples. Please add information and justify methods used.

- We used a hand net scratching over the surface to get sediment material from the upper ~2cm and to sample living animal.

- To refer to former studies to get more detailed information on the methods is a common procedure, however, relevant information on sampling are added

adult, juveniles? all? male, female?

- We found female, male and juvenile stages. Information on that will be added in the methods and supplementary material and discussed in section 5.

Line 25 shortly mention in abstract

- Will be added.

Line 27 how?

these samples need to be fixed, what did you use? otherwise isotopic signatures are changed by biological activity

- This is described in Spötl (2005), which we also cited: "...Prior to going into the field, the exetainer is preloaded with five droplets of phosphoric acid (ca. 90%), capped and the headspace is flushed with He 6.0 in the autosampler by penetrating the butyl rubber septa of the exetainers' disposable caps..."
- Just in case the referee refers to the ostracod material and not to water samples: surface sediment samples containing ostracod material were stored in ethanol (90%) to preserve soft part material until picking. A respective sentence on that will be added

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Line 5 until now it was clear to me that d18O was measured, d13C as well but lots of information is missing, and dD is new to me. Did you as well determine this and are you using it for your discussion? as well as DIC? then you need to include this info in the abstract

- the correlation of dD and d18O was used to show the GMWL and the deviation of the water isotopes along a local evaporation line. dD will be added to the abstract.

Page 8

Line 4 is there more biological information for this species in the literature? if so then add in the introduction. maybe since this is a widely distributed species in the entire neotropical region.

- There are some information on the biology and geographical distribution available and will be added in the manuscript.

I think that in the introduction the authors needs to make a better job selling the topic to non-experts, or making it more interesting to a wider interest, more info on ostracodes, why this species and not others, why is this study new? what is different from other past studies?

- The introduction will be rewritten considering the critical and helpful points of the referee.

Line 8 how long does this species live? some info about that? what does the literature say?

- To our knowledge, there is no information available on the life span of *C. ilosvayi*. There is some information on seasonal abundances from different localities from Purper (1974), Higuti et al. (2007), Pérez et al. (2011). These information will be added in the introduction.

is this calculation meaningful? what if calcification is longer? and different per type of aquatic ecosystem,

- Our model approach has not been tested before. Climatic data are commonly given in monthly time intervals, and a variation in monthly precipitation and temperature data can be seen in Florida, so this seemed to be a realistic starting point for calculations.
- A longer duration of the calcification is already considered in the discussion.

you have until now, not said if this applies only for adults (what makes more sense) or all larval stages. How many samples/ostracodes did you use per analysis? I hope single valves, since the focus of this paper is isotopic...if you mix 2 or more then you have a slight error, and if different samples/analysis have different number of valves then it is hard to compare among analysis right?

- Most of our measurements were performed on adults, but some also on the A-1 juvenile stage. The latter once are excluded from the model. The number of measurements and number of valves differed between samples, depending on the material that was provided. The number of measurements is given in the supplementary material.
- The averaging caused by bulking will be added in the discussion.

Line 26 why a long period? and not the years where you took samples? what is the idea behind this? explain please.

- Measurements of precipitation are not distributed equally (see Table 4). We included all available data to get significant monthly values

Page 9

Line 4 did not mention this before, remember that all needs to be explained in methods and no surprises when you are in results. only present in results, things that you have mentioned before, no new things.

- The TDS is calculated from the ion concentrations, this will be added in the methods water?
- Yes, this should be already clear from the methods.

Line 30 I would use same number of decimals for isotopes and rest of environmental information...1 decimal is probably fine and please always add a minus or plus before

- According to the referee, numbers of environmental parameters are adapted to one decimal for all isotopic data and most chemical and physical data.
- Minus and plus marks will be placed in front of isotopic values, while isotopic ranges will not be marked with any sign

Page 11

Line 15 add plus throughout the manuscript

- Will be added.

Page 12

Line 8 I would first discuss your data and then complement with other references or give the interpretation/explanation supported by references later

- The section will be restructured according to the referee

Page 13

Line 5 total?

- Yes, this is the total amount of phosphate. Respective sentence will be changed.

Line 6 are these your own data? if not then cite

- Yes, they are. This will be indicated.

Page 14

Line 6 more sources in Leng et al.

- The referee probably suggests the addition of Leng and Marshall (2004), which will be added. If another reference is suggested, the year of the reference would have been helpful.

Line 7 use same nr of decimals throughout the manuscript

- Will be changed.

Line 31 showed

- Will be changed.

Page 15

Line 17 I would like that authors elaborate more this idea, because I do believe that it is highly important to consider larval stages and parallel run lab studies of species life cycle for a robust interpretation

- The idea will be extended in the introduction, also some more information on that are already given in section 5.2.1.
- Laboratory studies are hardly comparable to natural environments and are mostly designed to test changes of single factors. In addition, lab studies are not always possible to perform, as in our case, e.g., because transport of living individuals to our lab was not possible. But, our approach is based on single sampling, and juvenile material was limited to a few individuals and not enough to run isotopic measurements. Besides, there are still uncertainties concerning biomineralization of juvenile stages which may influence isotopic results.

Page 16

Line 21 sentences should not start with abbreviations in such cases full species name should be written

- This will be changed.

Page 17

Line 11 Pérez et al. (year)

- This will be changed.

Line 14 as well adults? female? I have not seen much information on larval stages in your study, which would have been a tremendous contribution

- information on juveniles are scattered, but will be added.

Line 21 why only rivers? what about the rest of aq. waterbodies?

- Rivers are mainly influenced by temperature and precipitation, while in other waterbodies evaporation is an additional important factor. As we have information on the d18O composition of rain, calculation of theoretical calcite in rivers is possible

Page 18

Line 34 not true...there is some information, however these topic is not the focus of the papers so one need to carefully read the scientific papers in order to get information on life cycles.

there is info on conc. dissolved oxygen, temp, rains, maybe some in spanish but one cannot generaliza that there is no published information. Ask directly the experts from middle and southamerica and you will find out more.

- As stated above, we did extensive literature research (also with the suggested headwords) without success. Some specific literature suggestions from the referee would have been very helpful.

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Line 10 write full name

- This will be changed.

Line 18 I find this part too long. Most information could go in abstract, since are the main results, and here authors should rather present 3-4 main conclusions, what did they discover that will benefit the community working with this discipline? what is new from other studies? this part should not be similar to the abstract

- Conclusions will be rewritten and some information will be included in the abstract.

Line 21 why is this important? how will this improve paleostudies?

- When calcification of a species is seasonally restricted the isotopic signatures that are reflected by that species will differ from a species which calcifies during another season or during the whole year. This information can be used for a better interpretation of high-frequency climate changes in paleontological studies (e.g., Escobar et al. 2010, Dixit et al. 2015)

Line 31 could be? or were? remember that these are conclusions...

- This will be changed to “were”.