

EGUsphere-2022-576 - Reply to comments reviewer 1

Dear editor,

We appreciate the time and effort spent by Reviewer 2 to comment on our manuscript. We are glad to read that the reviewer likes the design of our study overall and thinks the topic is worthwhile. Their main concern relates to the effective sampling resolution of our LAICPMS method and our ability to capture daily variability in the shells. Below, we provide a rebuttal to this major point below before listing our point-by-point replies to the other questions raised by the reviewer. We hope the changes we suggest will make our manuscript acceptable for revision and subsequent publication in Biogeosciences.

In general, this is a very interesting manuscript on a topic that has generated quite a few contributions in the past few years, not only in molluscs but also – even earlier – in foraminifera tests. The issue at stake is what controls the observed (sub)daily chemical variability, here specifically in mollusc shells. This is what De Winter et al. set out to resolve by comparing spatially-resolved chemical signals in molluscs with and without photosymbionts, namely tropical giant clams (*Tridacna*, *T*) vs scallops (*Pecten*, *P*) that also live in strongly contrasting tidal regimes. This is an interesting, actually quite nifty approach that has the potential of significantly contributing to the issue at stake.

We are glad to read that the reviewer appreciates our study design and will include some references to earlier studies describing high-resolution chemical variability in foraminifera tests (e.g. Eggins et al., 2003; Anand and Elderfield, 2005).

Anand, P. and Elderfield, H.: Variability of Mg/Ca and Sr/Ca between and within the planktonic foraminifers *Globigerina bulloides* and *Globorotalia truncatulinoides*, *Geochemistry, Geophysics, Geosystems*, 6, <https://doi.org/10.1029/2004GC000811>, 2005.

Eggins, S., De Deckker, P., and Marshall, J.: Mg/Ca variation in planktonic foraminifera tests: implications for reconstructing palaeo-seawater temperature and habitat migration, *Earth and Planetary Science Letters*, 212, 291–306, [https://doi.org/10.1016/S0012-821X\(03\)00283-8](https://doi.org/10.1016/S0012-821X(03)00283-8), 2003.

The manuscript is quite long, overall well written besides key issues identified below, and contains extensive, almost too extensive (several GB of data!) SI. It presents LA-ICP-MS data of 8 specimens (3x P, 5x T) plus corresponding age models, followed by spectral analysis - and overall aims to identify the nature and cause of high-resolution, i.e. sub-daily elemental variability.

We appreciate the feedback on the length of our manuscript and its supplements and will try to shorten it (especially the Results section) in the revision. However, we believe that the components in the supplement are necessary to ensure the reproducibility of our study, which is why we deposited them in an online repository where there is space for large files.

However, I'm afraid to say that without significant additional documentation, their current dataset – especially valid for slower growing giant clams *T* – is **not** capable of revealing sub-daily compositional signals. Thus, it is hard to see how their careful, elaborate evaluation via spectral analysis etc can be upheld. I'll detail this below.

So, a more detailed evaluation of the manuscript and its implications has to wait until this documentation has been provided or these issues have been clarified. Hence, this review does not necessarily cover all aspects, as it strikes me necessary to iron our input data first before making further, potentially far-reaching interpretations.

The main issues to be addressed include:

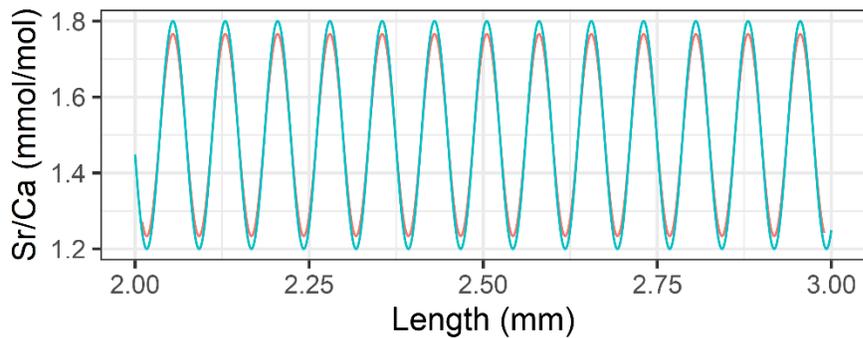
1) Spatial vs. temporal vs. sampling resolution of LA-ICP-MS data: The earlier papers on daily-resolved geochemical cycles in *Tridacna* by Sano and co-workers used a NanoSIMS at 2 μm spatial resolution, which subsequent LA-ICP-MS work at 3-4 μm by e.g. Warter et al tried to achieve as well. Previous work by De Winter et al (2020) used 10 μm spots (circular, rectangular). Thus using a rectangular slit of 100 x 20 μm , 20 μm in growth direction, hardly counts as ultra-high spatial resolution (L 107), and it crucially is insufficient to achieve hourly resolution (L107) in many of the samples investigated, chiefly the *Tridacnas*. According to their Tab. 1, *Pecten* grow $\sim 250 \mu\text{m}/\text{day}$ and thus 20 μm indeed nominally represent ~ 2 h. However, their subtropical counterparts, *Tridacna*, only grow between 22-40 $\mu\text{m}/\text{day}$ (Tab. 2). It's the comparison between the two groups that represents the overall aim of the authors, so the slowest growing ones do matter a lot. They address this issue on p25 (L415-419) and state that they achieve "...resolution of the LAICPMS data (0.4 μm)...". However, every LA spot averages over 20 μm or possibly more as there is also the lateral dimension of 100 μm to be considered - due to the laser-sampling at 20 μm (L417). The (nominal) 0.4 μm resolution (L415) comes from the interaction between sweep time (0.1 s) and laser scan speed (4 $\mu\text{m}/\text{s}$; all in Tab.11), which **at best is the sampling resolution of an individual data point, but NOT what can be resolved temporally in clams that grow 20-40 $\mu\text{m}/\text{day}$ and are being analyzed with a 20 μm laser spot.**

So, I'm sceptical that they can achieve 50-fold better ($=20/0.4$) temporal resolution, allowing them to claim (L412): [...] *average temporal resolution of the LA-ICP-MS line scans was 0.04h, 0.24h, 0.44h and 0.27h for [...].* But this is crucial for their spectral analysis where they require data at sub-daily time resolution.

We acknowledge that our explanation of the temporal resolution achieved with our LAICPMS method could be clarified, and we aim to do so in the revised version of our manuscript. We confirm the reviewer's analysis that every sample in our LAICPMS analysis averages an area with a width of 20 micrometer in scanning direction. The reported sampling resolution of 0.4 micrometer indeed comes from the combination of sweep time and scan speed, and this may have been confusing in the original manuscript. While we do achieve a roughly 0.4 micrometer resolution in terms of the number of datapoints per unit distance, we acknowledge that the use of a 20 micrometer wide spot causes smoothing of the record akin to applying a moving average. We hope to demonstrate that we can still pick up daily rhythms in our datasets using this approach by means of a virtual example:

Below, we provide some examples of how this smoothing would affect a theoretical banding consisting of daily cycles in El/Ca (e.g. Sr/Ca). We simulated this banding with a baseline Sr/Ca value of 1.5 mmol/mol and a diurnal amplitude of 0.6 mmol/mol (comparable to the amplitude found in e.g. Sano et al., 2012). The Sr/Ca record was projected on the distance domain using a Von Bertalanffy growth model of an artificial shell, which is constructed in the same way as the growth models for the tridacnid shells in this study (specifically specimen TM84 and SQSA1). We then virtually sampled from this dataset, simulating the full LA-ICP-MS procedure as follows: We sweep over the virtual record using our laser spot width of 20 micron, our scan speed of 4 micrometer/second and our cycle time of 109 ms (which includes ICP-MS dead time; see reply to comment below) and an integration time of Sr per ICP-MS sweep of 10 ms (see **S11**). We plotted the resulting record to demonstrate the smoothing that occurs due to our sampling method. We repeated this experiment with parameters for the growth of specimen TM84 (representing one of the faster-growing tridacnid specimens in our study) and SQSA1 (the slowest-growing specimen in our study and show the result in the plot below.

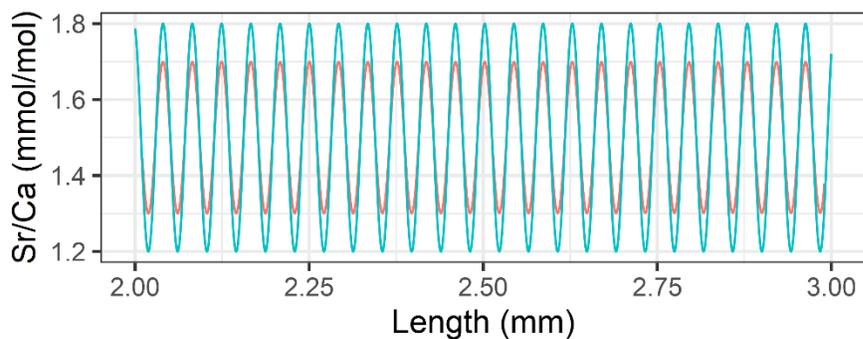
Smoothing of the record in *Tridacna maxima* 84



colour

- 20 micron spot size
- true data

Smoothing of the record in *Tridacna squamosina* SQSA1



This experiment shows that, even in the slowest-growing tridacnid specimen in our study (SQSA1), this combination of 20 micron spot size and scan speed can pick up the daily resolution in the record and recover two thirds of the amplitude of the Sr/Ca signal. A signal like this would be picked up easily by the spectral analysis methods in our study, albeit with a slight reduction of the power value in the power spectrum (which is proportional to the square root of the amplitude). For the faster-growing tridacnid species, the amount of smoothing of the artificial record is nearly negligible. We hope these plots clarify the difference between our scanning resolution and the spatial smoothing due to our spot size and will add them, together with the script we used to make them, to the appendix of our revised manuscript as well.

In addition, please note that the median increment widths listed in Table 2 sometimes refer to semi-diurnal increments, meaning that the actual daily growth rate is twice the value listed in the table. The values listed in Table 2 are median values over the entire record, which contains variability in growth rate. Analyzing the entire growth period of the shell for (sub-)daily growth patterns and chemical variability inherently causes smoothing of the chemical variability due to averaging of the signal in places in the shell with strong variability with places in the record where (sub-)daily variability is less prominent. This caveat of our methodology is acknowledged in the original manuscript near the end of section 4.3.1. The spot size smoothing issue explained above adds to this smoothing effect and this will be acknowledged in the revised Discussion section. Note that the consideration of (sub-)daily variability over the entire growth period distinguishes our sampling strategy from that of the previous studies cited by the reviewer (Sano et al., 2012 and Warter and Müller, 2017), where an excerpt of the record is used to demonstrate the

presence of this variability. We believe our analysis better captures the variability in expression of ultradian rhythms over the entire growth period.

Sano, Y., Kobayashi, S., Shirai, K., Takahata, N., Matsumoto, K., Watanabe, T., Sowa, K., and Iwai, K.: Past daily light cycle recorded in the strontium/calcium ratios of giant clam shells, *Nat Commun*, 3, 761, <https://doi.org/10.1038/ncomms1763>, 2012.

Warter, V. and Müller, W.: Daily growth and tidal rhythms in Miocene and modern giant clams revealed via ultra-high resolution LA-ICPMS analysis—A novel methodological approach towards improved sclerochemistry, *Palaeogeography, Palaeoclimatology, Palaeoecology*, 465, 362–375, 2017.

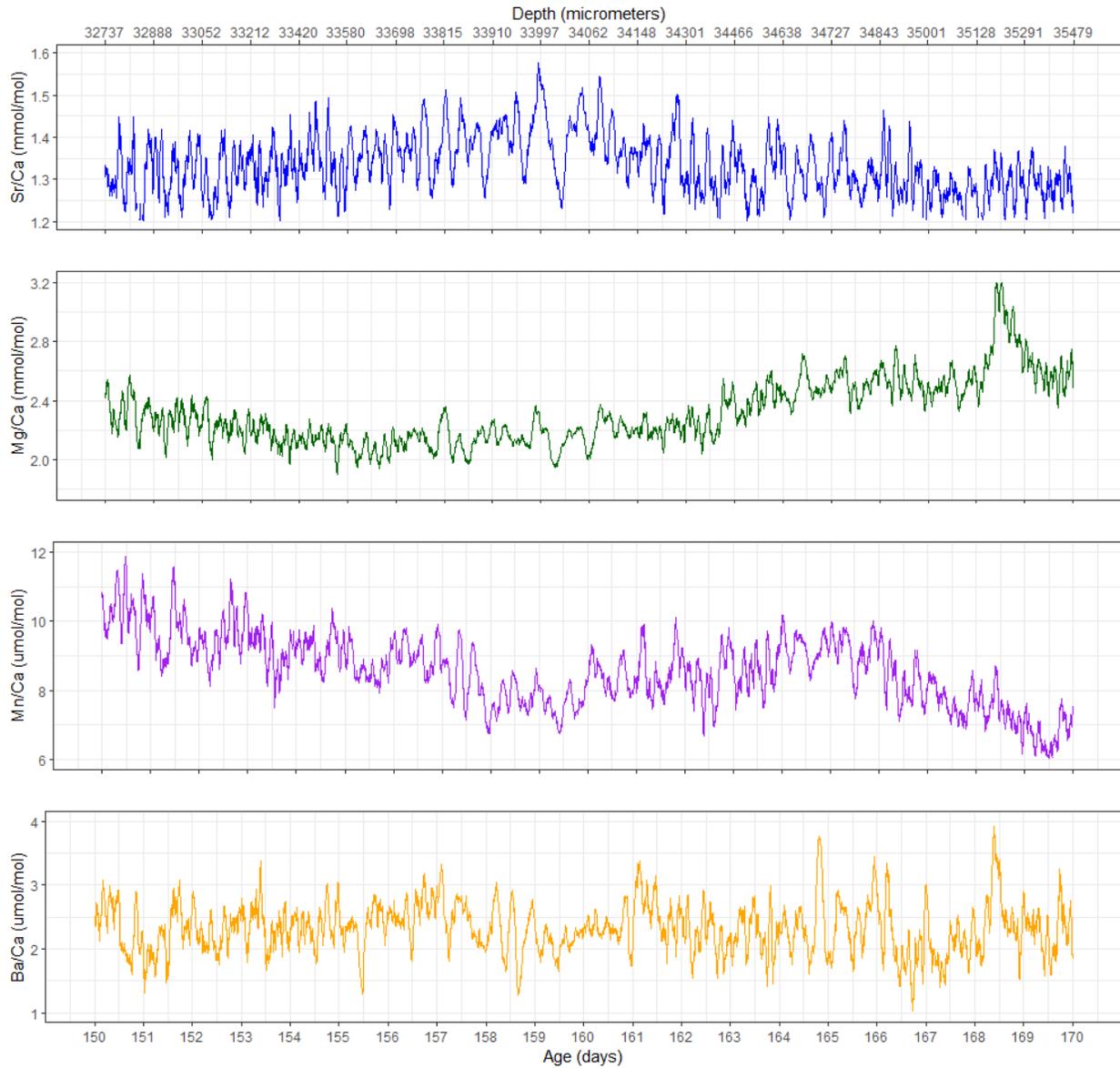
I thus invite the authors to show comparative plots, at very high spatial resolution, namely ~400 μm for *Tridacna* and ~2000 μm for *Pecten* - that reveal compositional cycles at sub-daily resolution (similar to the work by Sano or Warter etc.) based on their current dataset. The data shown in Fig. 2 do not provide this level of detail at all. I did not find a data table that would have allowed me to re-plot the data myself.

In the original manuscript, we decided not to add zoomed-in plots of the daily variability in the records because we aimed to discuss the expression of (sub-)daily variability in the entire growth period of the specimens rather than highlight parts of the shell where this variability is strongest (see reply above and discussion in section 4.3.1 of the original manuscript). In reply to this comment, we will add example plots of these high-resolution trace element rhythms in tridacnids and pectinids to show that this variability is present in our records. We also add two examples of such plots, one from *P. maximus* specimen 2 and one from *T. maxima* specimen 29, below to show what the (semi-)diurnal variability looks like when plotted in detail

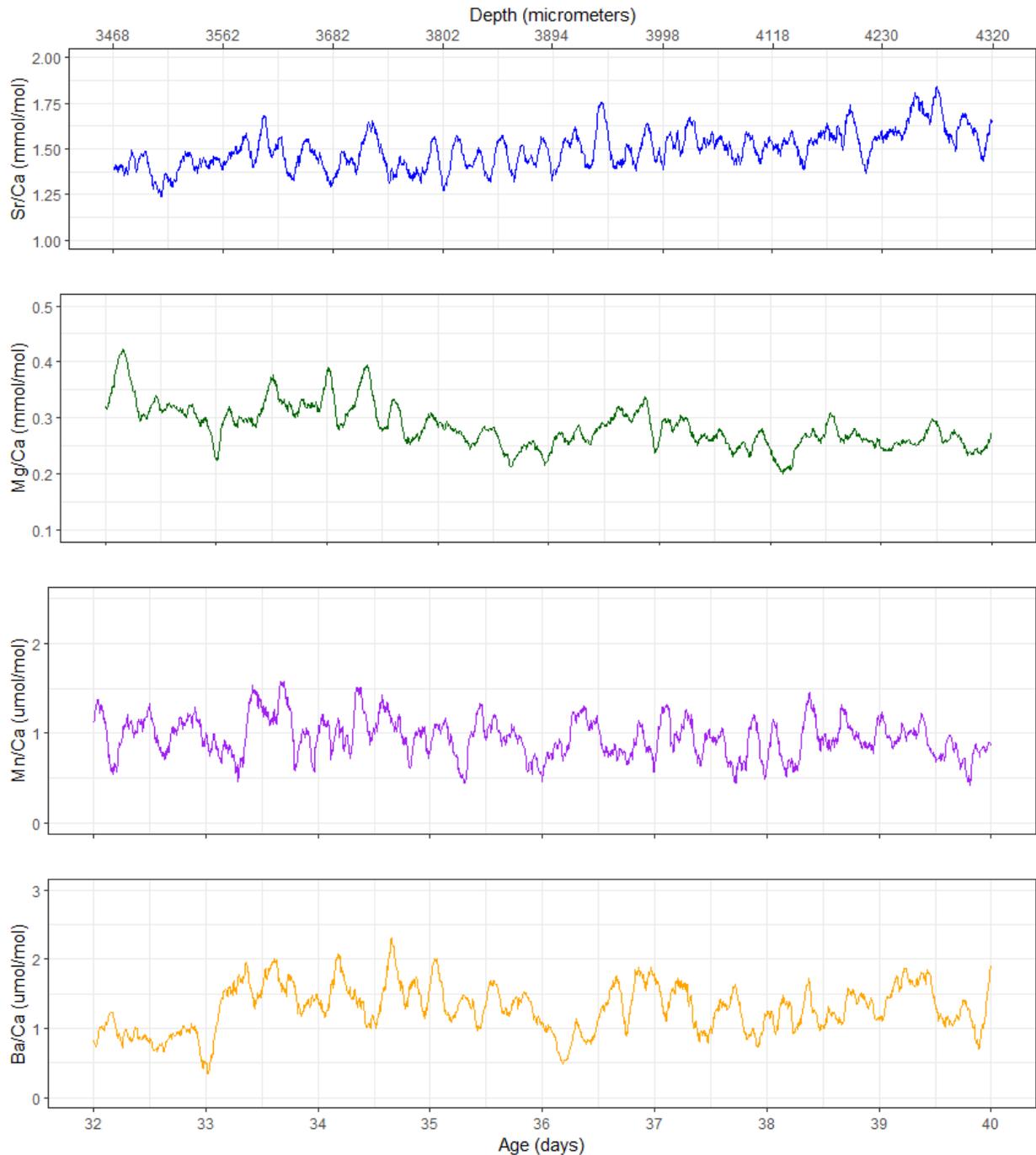
In *Tridacna* shell 29, which prior work from Killam et al. (2021) suggested formed twice daily growth lines, data which we incorporated into our growth model, many of the days show “doublets,” possibly aligning with the twice daily rise and fall of the tide, but as seen in the data, slightly out of phase with the modeled daily cycle, and not perfectly symmetrical, as the tides on a particular day aren’t exactly equal.

We kindly note that all LAICPMS data required to produce the plots in this manuscript is provided in the online Zenodo repository (see Data Availability statement in manuscript for the link). We realize that this data was somewhat “hidden” in the large supplement and created a separate “raw data” folder at the top level of the supplement’s folder structure to make it easier to find for the reader.

Killam, D., Al-Najjar, T., and Clapham, M.: Giant clam growth in the Gulf of Aqaba is accelerated compared to fossil populations, *Proceedings of the Royal Society B: Biological Sciences*, 288, 20210991, <https://doi.org/10.1098/rspb.2021.0991>, 2021.



Zoomed-in plot of (semi-)diurnal variability in *P. maximus* specimen 2. The lines represent 51-point Savitzky-Golay filters showing the hour to daily scale variability while filtering out the higher-order measurement noise.



Zoomed-in plot of (semi-)diurnal variability in *T. maxima* specimen 29. The lines represent 21-point Savitzky-Golay filters showing the hour to daily scale variability while filtering out the higher-order measurement noise.

Once this is achieved, the data can be reassessed with respect to the implications of their spectral analysis. To be honest, I doubt that their existing dataset will reveal such sub-daily cycles due to the insufficient combination of laser-spot size and laser scan speed, but maybe I'm missing something and thus this should

be added. If this is not possible, then the samples may need to be re-analyzed with much smaller laser spot sizes and slower scan speed.

2) LA-ICP-MS data: While there is overall good documentation, it is necessary to get data documenting accuracy from MACS-3 and BAS752 and JCp1. Please provide some general details for BAS752 as this is less well-known standard material. I doubt that the sweep time (run cycle time) is 100 ms, given that the sum of all 6 m/z is exactly 100 ms, and some time is spent between the masses. Why was B not analysed, given that B/Ca can show very well-resolved daily cyclicity in giant clams?

We will add details on the accuracy of LAICPMS values on the check standards in the appendix (**S12**). In summary, the accuracy on the matrix-matched MACS-3 carbonate standard is better than 5% for all elements in all LA-ICP-MS sessions from which data is used in this study.

The reference material called “BAS ECRM 752” is also known as “BAS-CRM 393” and is a well-known limestone standard (see https://rrr.bam.de/RRR/Content/EN/Downloads/RM-Certificates/RM-cert-iron-steel/RM-cert-ceramic-materials/b752_1e.pdf?_blob=publicationFile). We will rename it to “BAS CRM-393” in the text to avoid confusion.

Our reported ICP-MS sweep time indeed does not include the time needed for the ICP-MS to move between masses. The total cycle time including down time on the ICP-MS is 109 ms, and we add this to the supplement describing the LA-ICP-MS settings (**S11**).

The element B was not measured to limit the number of elements and keep the total cycle time as short as possible (maximizing the spatial resolution of the measurements).

3) Definition of terminology: What exactly is meant by ‘semi-diurnal’? Does it mean half daily (12 h=tidal?) or *approximately* daily? Semi may mean half or approx. Please define.

Fair point, we define semi-diurnal as half-daily (=12h) and will make this explicit in the revised version of the manuscript.

4) Shell growth – a few issues: How useful is it to utilize maximum shell height (L_{inf}) from the literature since the authors did growth band counting and interpolation in between? How does one unequivocally identify growth breaks visible on the outer margin? Doesn’t the statement (L257) “ [...] distinction between diurnal (24h) and tidal (~12h) pacing of growth increments [...] imply some form of circular reasoning?

In Tab. 2, e.g. TS85, how does a diurnal width of 40.3 μm correspond to an annual width of 20.2 mm? On my reckoning, it is 14.7 mm.

These are fair points, and we acknowledge that these aspects could have been explained more clearly in the manuscript. Initially, we aimed at expressing the full length of the tridacnid shells in counted lamina (as was done for the pectinids). The tridacnid shells were stained with Mutvei solution as described in Killam et al. 2021, to reveal internal growth lines. However, the stain did not make clear all growth lines visible through the growth record, which is a function of the organic material content of the shell. This varies through the ontogeny of the animal with relation to season and other factors. The identification of daily lines is still a field of active development in *Tridacna*, with some shells staining to reveal clear daily lines throughout the record (Komagoe et al., 2018), while others require more labor-intensive methods to reveal any daily lines at all (Liu et al. 2022).

Therefore, we opted for a hybrid method in which we measured the width of increments in a sample of the shells where they were well developed and used these measurements in combination with the annual growth breaks (which are easily recognized on the shell) to create age models for all specimens. This is also the reason why we needed to use literature values for L_{inf} to anchor our growth model. Note that we

used well established L_{inf} values from the same species in the same area to avoid biasing our growth model estimates (see section 2.5).

We do not believe that this method implies circular reasoning, since we inferred the (semi-)diurnal timing of the increments by comparing them with the independently measured annual growth breaks and use values for L_{inf} from separate studies. In the revised version, we will attempt to better clarify our description of this methodology to take away the reviewer's concerns.

The apparent mismatch between (semi-)diurnal increment widths and mean annual growth (Table 2) stems from the fact that the former is the mean of the measured increments and the latter is the annual growth rate based on growth break distance. Since growth rates vary throughout the lifetime of tridacnids (both inter- and intra-annually) and these measurements were not necessarily made in the same part of the shell, the annual growth is not a simple multiple of the increment width.

Killam, D., Al-Najjar, T., and Clapham, M.: Giant clam growth in the Gulf of Aqaba is accelerated compared to fossil populations, *Proceedings of the Royal Society B: Biological Sciences*, 288, 20210991, <https://doi.org/10.1098/rspb.2021.0991>, 2021.

Komagoe, T., Watanabe, T., Shirai, K., Yamazaki, A., and Uematu, M.: Geochemical and Microstructural Signals in Giant Clam *Tridacna maxima* Recorded Typhoon Events at Okinotori Island, Japan, *Journal of Geophysical Research: Biogeosciences*, 123, 1460–1474, <https://doi.org/10.1029/2017JG004082>, 2018.

Liu, C., Zhao, L., Zhao, N., Yang, W., Hao, J., Qu, X., Liu, S., Dodson, J., and Yan, H.: Novel methods of resolving daily growth patterns in giant clam (*Tridacna* spp.) shells, *Ecological Indicators*, 134, 108480, <https://doi.org/10.1016/j.ecolind.2021.108480>, 2022.

5) Results overall: Keep the results description to a minimum overall, refer to figures and tables upfront, and move sections such as the comparison between P & T (~L327-L347) to the discussion. These are calcitic vs aragonitic shells, so differences are to be expected simply based on K_d 's. What is a 'typical' seasonal pattern (L350) – this is again mixing results with interpretation, which has to be avoided. L346 – don't mix ratio with concentration presentation and use quantitative rather than qualitative comparative statements (L376, 377).

These are valid comments, and they echo the concern the reviewer voiced above about the length of our manuscript. During our revision, we will try to shorten the results section and integrate the indicated segments into the Discussion. We also double-checked our reference to element concentrations and ratios throughout this section.

L319: Instead of a fixed value of 0.05 mmol/mol, such differences should be given as %deviations.

We will calculate the relative deviations and provide these in the revised manuscript.

6) Results of spectral analysis: Fig. 3 (Pecten) – even for these fast growing clams, the respective peaks at daily and half-daily (12 h) timing are not very clearly resolved. Instead, what is the meaning of the peaks between 1 d and 7 d? And in Fig. 4 I find no convincing peaks for the *Tridacnids* that indicate <~7 day periodicities, so I do NOT understand where the assertion in L436 is derived from. Hence my worry about temporal resolution of the LA-ICP-MS input data raised upfront!

We tried to plot the spectral analysis results of these long, high-resolution records in many ways before settling on the current figure. The problem with plotting these is that the scale of the "power" value (y-axis) differs by more than an order of magnitude over the period domain (i.e. longer cycles have much higher power than shorter ones). This characteristic is inherent to spectral analysis, but causes the periodicities associated with shorter cycles (higher frequencies) to show much lower spectral power than the longer periods. We tried plotting these powerspectra on a log-log scale, but this compromised

recognition of peaks for the longer periods as well as on the shorter end of the spectrum. Therefore, we decided to show the confidence levels (percentage values) associated with peaks in the period domains highlighted in the figures. We fully agree that this is not the most intuitive way to plot these results, and we would have preferred to highlight the important peaks differently.

One thing we can try is to break the horizontal axis and highlight specific intervals of periodicity associated with the cycles of interest. In principle, breaking axes like this is not good plotting practice, but we are willing to try it to see if it is a better way to highlight the peaks in the powerspectra. If the reviewer has any alternative suggestions on how these results could be plotted in a way that ameliorates the large differences in power over the period domain, we would be very happy to hear them.

7) Further issues:

a) Fig. 1: While it is a good figure in general, two issues should be changed. A-K is mixed between the two groups of organisms, and more importantly, I'd prefer to see much larger images that showcase the LA-ICP-MS profiles. B, C, K are too small and don't give sufficient detail.

We appreciate this feedback and will make panels B, C and K bigger in the revised version. We assume that with the comment "A-K is mixed between the two groups of organisms", the reviewer means that the figure caption would be clearer if the letters were ordered by species (e.g. A-D for pectinids and E-I for tridacnids). We will implement this in the revised manuscript.

b) Fig. 5: The content of this figure could be better assessed if we saw truly daily-resolved data, see above. Same for Fig. 6. If there is crucial information in some SOM-Figs, then move them into the main text please.

In reply to these comments, we add excerpts of the records through tridacnids and pectinids to the manuscript showing examples of the (semi-)diurnal variability in the trace element ratios.

c) In L574-576 there is a certain amount of contradiction to previous statements about Mn incorporation.

We did not find contradiction in this section, but rephrased part of the discussion here to clarify our line of reasoning.

d) L588 The authors did not resolve daily periodicity in Sr/Ca in tridacnids in my view, so they can't make statements like this.

We respectfully disagree and hope to have demonstrated in reply to the major comment above that our methodology does resolve daily periodicity even in the slowest-growing tridacnids.

8) Referencing: The references are in part incomplete with journal titles missing and others in Arabic font. Please proof read before submission. Killam et al 2022 missing.

We thank the reviewer for pointing this out and will update and complete the reference section before submitting our revised manuscript version.

9) Minor issues: This list is not comprehensive.

L120: 7.2 m – space between, here and elsewhere

We will add the space throughout the manuscript

L158 parallel

Rephrased

L327 'contain' is wrong wording, better 'are characterized'

Agreed, this will be rephrased

L389 (Tab. 1): increment width – specify daily increment width; and L399 (Tab. 2): what is semi-diurnal?

We added “daily” and define “semi-diurnal” on first mention in the revised manuscript (see reply to comment above)

L502 Fig. 7 – good idea as a summary

Thanks!

L541 this appears misplaced here

Correct, this should read “Short-term changes in shell composition in pectinids” and will be rephrased.

Taken together this is an important study on a timely subject. The ideas conveyed in the abstract are broadly fine but can in detail not be assessed due to issues with the initial data raised above. I hope that this can be re-addressed. In its current form, the manuscript is not suitable for publication.