Review of

De Winter et al. **Ultradian rhythms in shell composition of photosymbiotic and non-photosymbiotic mollusks**

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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.

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.

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 ~250 µm/day and thus 20 µm indeed nominally represent ~2 h. However, their subtropical counterparts, Tridacna, only grow between 22-40 µm/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 µm/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 µm/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.

I thus invite the authors to show comparative plots, at very high spatial resolution, namely ~400 µm for Tridacnas 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.

Once this is achieved, the data can be reassessed with respect to the implications of their spectral analyis. 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?

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.

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.

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).

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

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!

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.

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.

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

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.

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.

9) Minor issues: This list is not comprehensive.

L120: 7.2 m – space between, here and elsewhere

L158 parallel

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

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

L502 Fig. 7 – good idea as a summary

L541 this appears misplaced here

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.