

Response to RC1

RC1: Referee #1 comment (in gray)

AC: Author comment (in black)

AC: We thank Referee #1 (Daniel Killam) for the supportive feedback and helpful comments to improve this manuscript. We have addressed each comment below and will incorporate the suggestions in the manuscript.

RC1: The study from Vriesman et al is an investigation into biomineralization patterns of *Mytilus californianus*, reporting on the potential biological and environmental causes of the semi-periodic growth lines present in the shells of this species. *M. californianus* is an iconic and well-studied species in the fields of intertidal ecology and marine invertebrate physiology, but is comparatively under-studied in terms of its biomineralization and sclerochronology. This is partially because as the authors note, mytilids are enigmatic in their shell growth patterns, often lacking the clear, consistent annual increments of shells in other bivalve taxa. Thus, the work of Vriesman et al represents a long-overdue sclerochronological revisit of *M. californianus*. The study provides a characterization of the tripartite shell layer structure of *M. californianus*, which is unusual among the mytilids and a point of error in some recent studies of the species (who shall remain unnamed), and then investigates environmental determinants of growth bands within each respective shell layer. Prior sclerochronological work on this species has been stymied by the lack of true periodic growth bands, preventing the creation of an age model, so the authors take the alternative approach of characterizing the terminal growth band (dark or light) and the environmental conditions concurrent with those bands. They propose that the formation of light bands is often concurrent with "goldilocks" (my term) conditions associated with stable, moderate temperatures and a lack of upwelling. The study also looks at whether variations in the contrast of the dark-light bands might have environmental significance related to microenvironment and other factors. As such, the study represents a worthwhile addition to the limited literature on mytilid sclerochronology and I recommend its publication. Below I provide line-by-line questions/comments/suggestions that came to mind while reading.

AC: We greatly appreciate the feedback regarding our manuscript's contribution to sclerochronology and we value the line-by-line questions/comments/suggestions (addressed below). We will incorporate them into the manuscript as well.

RC1: 105: If permission or permit from the reserve was required for collection, mention that here.

AC: We obtained a permit to access and collect *M. californianus* shells directly from the Bodega Marine Reserve (BMR) since it is part of the UC Natural Reserve System. We will specify this in the Methods.

RC1: 138: Were you able to identify the terminal band as dark or light easily across all shells? Or were there edge cases where identification was difficult, such as for the shells with low contrast? Your Fig. S3 was helpful as an example.

AC: In some cases, it was difficult to definitely identify the terminal band as either light or dark with visual inspection alone. We will state this explicitly in section 2.3, and we will update our methodology to be transparent about how we identified band coloration in these cases. Lines ~160-164 will be edited to the following: “In some cases, it was difficult to visually determine the terminal band color; to supplement visual inspection, gray values were obtained from the 8-bit image through a transect of dark-light banding at the region of interest. To determine the proportion of light banding in each individual specimen and confirm the coloration of the terminal band, gray values greater than the mean were considered light bands, and gray values less than the mean were considered dark bands.”

RC1: 143: Reflected, transmitted light or both?

AC: We used a microscope equipped with both transmitted and reflected light sources. We used both for our analysis but the photographs shown in the paper were taken under reflected light. We will specify that in the Methods section. We will also add this to the Figure 2 caption.

RC1: 153: This gray-value variance approach seems to me rather novel and merits greater elaboration in the methods. Have any other references used a similar approach? I couldn't find too many prior uses of this technique; one for fish otoliths (<https://doi.org/10.1016/j.seares.2006.09.006>) but not a whole lot else. Did you have any prior expectation of what these results would mean? I.e. did you expect greater contrast to correspond to greater growth disruption? Also, for reproducibility, provide more info on how you collected and standardized the gray values. Was this via the transect tool in Fiji/ImageJ?

AC: We thank the reviewer for the interest in our gray-value variance technique. We developed this technique for this paper in particular and we are not aware of any other references that utilize gray-value variance as an estimate of dark-light band expression. We were aware of the Katayama and Isshiki (2007) paper, which uses image opacity and gray values to examine otolith structure. While we did not base our methodology off of this, this is a valuable reference to explain the use of imaging software to examine growth structures, so will cite this paper in our methods (section 2.3).

We developed the gray-value variance method after we had made first-order observations of all 40 thin sections; we noticed that many samples had strongly expressed, visually clear growth bands (i.e., ideal for a sclerochronologist). Other samples had weakly expressed, cloudy bands that made it more difficult to distinguish dark from light (i.e., very poor for sclerochronological analysis). We were curious if the variation in growth band expression/clarity was due to micro-environment and/or a temporal shift. In this case, we expected greater contrast (higher gray-value variance) to correspond to more “normal” growth patterns (i.e., alternating deposition of distinguishable dark and light layers) and lower contrast (lower gray-value variance) to correspond to more disturbances or intervals of halted growth (i.e., more dark banding or little difference between dark and light bands).

We will specify that this technique was carried out using the transect tool in Fiji. We will also elaborate further on how we obtained gray values, how we calculated and standardized gray-value variance, and how we interpreted gray-value variance.

RC1: 163: I assume the percent of light bands was calculated as (light band number)/(total dark + light band number)*100%? Might want to note that explicitly.

AC: Yes, this is correct. We will add this immediately after our explanation of how gray values were used to determine band color.

RC1: 202: Can you provide more background on your identifications of polymorphs for each respective layer? Is this based on the prior observations of mineralogy of this species, or were you also identifying based on their microstructural appearance, response to plane polarized light, etc?

AC: We appreciate the interest in the calcium carbonate polymorphs in the shell of *M. californianus*. Referee #2 asked a similar question and recommended using X-ray diffraction (XRD) to determine the mineralogy of this species. We value both questions and we have future high-resolution imaging analysis planned on this species to observe crystallographic orientation, micro-fabrics, and crystal sizes on a much finer scale. For the present study, we were able to visually distinguish calcite from aragonite based on their appearances under reflected light microscope. The inner and outer calcite layers have a blade-like prismatic microstructure. Mutvei's solution accentuated the appearance of these blade-like calcite prisms, which lay perpendicular to the shell exterior. The middle aragonite layer has a brick-like microstructure, with aragonitic 'bricks' laying parallel to the shell's exterior. Extensive visual observation of many *M. californianus* thin sections gave us confidence in our identification of three mineralogical layers. We will also cite Dodd (1963) in addition to Dodd (1964), which both utilized XRD to determine the mineralogy of *M. californianus*.

RC1: 228: Do you have any data on the average thickness of these different types of bands? A quick mention of those descriptive stats would assist in placing these bands in context relative to the animal's shell height.

AC: This is a very good question. While we were collecting shell characteristic data, we intended to measure the thickness of each dark and light band in every specimen. We discovered that this would be difficult since dark-light bands often taper, appear at an angle, and/or are inconsistent throughout the shell. For this reason, we chose to measure/describe characteristics that we could more definitively quantify or distinguish (e.g., terminal band color, thickness of the inner calcite layer at the region of interest, growth band expression, etc.). Figure 6 contains information about the widths of the terminal bands, which are ~ 0.15 to ~ 0.8 mm thick and Table S1 contains cross-sectional thickness of the inner calcite layer (0.3 mm to 3.6 mm thick). The thicknesses of dark and light bands are highly variable, but all are on the order of a few hundred micrometers or less. This makes fine-resolution sub-sampling (such as drilling for oxygen isotope analysis) extremely difficult in this inner calcite region. This point relates to a question posed by Referee #2, so we will address the narrowness of this region and the fine scale of the banding in the Discussion.

RC1: 380: You could note here that the anaerobiosis-dissolution idea had been originally proposed as a mechanism for growth line formation across bivalves including subtidal taxa like *Mercenaria*, but has been since been dismissed by some workers (see Schone and Surge,

sclerochronology treatise chapter). However, it is still a theory of interest in intertidal taxa like mytilids, which some work (including the McCoy study you cite) has determined have much greater swings in intra-shell redox conditions during tidal emersion. Basically, bringing up the anaerobiosis theory could be controversial in some quarters of the sclerochronological community (I would not be surprised if the other reviewer has reservations), but it seems your results in this paper merit greater investigation of whether dissolution is at play in the creation of dark growth bands in *M. californianus*. So you could add greater mention of the fact that the theory is still controversial but merits further investigation, and might help explain why mytilids have such unusual growth patterns compared to other bivalves.

AC: We thank the reviewer for the insights about the anaerobiosis-dissolution theory. We find this extremely interesting as well. We will add the Schöne and Surge (2012) citation to be transparent about the controversy regarding dark-light band formation in bivalves. We will also mention their alternative hypothesis (dark bands = visual expression of slower growth and smaller crystals) in the Discussion (section 4.1). We will make it clear that there are multiple theories regarding growth band formation and that further research is required to determine the mechanisms responsible for such complex growth features in intertidal bivalves like *M. californianus*. We will also add the Gordon and Carriker (1978) reference to this paragraph since this is the study cited (and rejected) by Schöne and Surge (2012).

RC1: 430: Do you have any data on emersion time at the different intertidal positions at the study sites? If so, does mean emersion time have an influence as an ordinal predictor on band contrast across sites? I just notice your MIP population has a higher variance than the other two and wonder if it's hiding a couple of subgroups. Even if you don't have data on tidal emersion time, might be useful to have the point shape in Figure 5B correspond to site of origin, to see if there's any separation.

AC: We appreciate this question; information on emersion time at each intertidal position would be extremely useful. We will find a way to achieve this for the next intertidal field experiment. To address the point about highlighting site of origin, Figure S5 features the same x- and y-axes (tidal position and standardized gray-value variance, respectively) and omits all Portuguese Beach specimens to emphasize only BMR shells. Per the reviewer's suggestion, we attempted to map point shape to site of origin in the existing Figure 5B, but this substantially changes the figure by grouping each intertidal position into separate, individual box plots (i.e. LIP would feature two boxes, MIP would feature three boxes, HIP would feature two boxes). We were concerned that this would distract from the categories of interest (intertidal position and habitat), so we concluded that leaving the figure as is and pointing the reader to Figure S5 is the best way to address the reviewer's question.

RC1: 510: You could cite the Bullard study again here.

AC: We will cite the Bullard et al. (2021) paper again here and mention that it documented a recent decline in aragonite relative to calcite in *M. californianus* shells from southern California. It fits in very well here and we thank the reviewer for this suggestion.