Comment on bg-2021-219
Daniel Killam (Referee)

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 revisitation 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.

105: If permission or permit from the reserve was required for collection, mention that here.
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.
143: Reflected, transmitted light or both?
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.
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?

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.

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?

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.

380: You could note here that the anaerobosis-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 anaerobosis 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.

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.

510: You could cite the Bullard study again here.

Regards,

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