

## Response to RC2

RC2: Referee #2 comment (in gray)

AC: Author comment (in black)

AC: We thank Referee #2 (A.D. Wanamaker Jr.) for the insightful suggestions to help improve this manuscript. We have addressed each suggestion below and will incorporate them into the manuscript.

RC2: The manuscript is clearly written and the results suggest that the banding pattern (light and dark couplets) in *Mytilus californianus* is largely associated with environmental conditions. A real strength of the study is the abundant environmental data from which the shells were collected. This allowed the authors to investigate which parameters might be most important in controlling the light and dark banding in the shell.

I mostly have some small suggestions that will hopefully make your statements/conclusions a bit stronger and a few editorial suggestions that might improve the flow of the manuscript. Overall, I think this is a strong contribution to the field of sclerochronology.

AC: We greatly appreciate this feedback and value the suggestions to improve the manuscript. We will address each comment, below.

RC2: If you provided additional evidence from x-ray diffraction (XRD) that you have three distinct mineral layers, that would be stronger than the optically derived evidence. Because this is a major finding of this study, this additional line of evidence is warranted. Furthermore, this will be the “go to paper” to cite this mineralogical finding. XRD is quick and relatively inexpensive.

AC: We appreciate the interest in the mineralogical layering of *M. californianus* and we agree that further XRD analysis would be a valuable contribution to the *M. californianus* literature. We are currently planning a study that will feature high-resolution imaging on this species, including XRD, scanning electron microscopy (SEM), and electron backscatter diffraction (EBSD) to examine micro-textures, crystallographic orientation, and crystal sizes. The goal of the present study was to perform optical analysis of *M. californianus* shells to visually characterize the shell structure and determine relationships between environmental conditions and growth band pattern. We were able to identify three distinct layers: (1) in whole valves, where a white chalky calcite layer spanning from umbo to the midpoint of the shell's interior can be observed visually and tactilely, (2) under reflected light microscope, where the microstructural appearance of calcite crystal fabric is distinct from aragonite crystal fabric, and (3) under transmitted light microscope after etching with Mutvei's solution, which accentuates the shape of prismatic calcite crystals. In thin section, the inner and outer calcite layers have a blade-like prismatic microstructure. After immersing in Mutvei's solution, the blade-like calcite prisms laying perpendicular to the shell exterior are even more pronounced. The middle aragonite layer has a brick-like microstructure, with aragonitic 'bricks' laying parallel to the shell's exterior. The aragonite layer also characteristically forms a 'zig-zag' pattern (Dodd, 1964) that we identified in

our samples as well. With the reviewer's comment in mind, we revisited Dodd (1964) (which cites Dodd (1963)) and found that both papers performed XRD analysis to determine mineralogy in *M. californianus*. We will add the Dodd (1963) reference to the Discussion (section 4.1) and make it clear that Dodd (1963; 1964) determined the mineralogy of this species using XRD analysis, and that we were able to confirm this visually using optical microscopy. While some recent studies have mischaracterized the shell structure of *M. californianus*, we suspect that this is because this species is commonly assumed to have only an inner aragonite layer like the rest of its congeners and that its inner calcite layer is therefore frequently overlooked.

RC2: Do you have modern shells from Portuguese Beach? If not, you are "making the argument" that site 3 and site 2 (open coast environments) are similar enough to suggest that changes in shell growth between the modern and archival specimens is related to time dependent growth changes rather than a difference in growth from two different locations (i.e., growth is different because they are at different sites). I think it is warranted to add something to the discussion about this assumption.

AC: We thank the reviewer for the interest in the Portuguese Beach mussels. These were collected by Michael Kennedy in 2002 and 2003 for his dissertation work and donated to us in 2019 by one of his dissertation committee members (Ann Russell). Unfortunately, we had no access to modern shells from Portuguese Beach during the period that we were analyzing shell characteristics for this study; the California Dept. of Fish and Wildlife permitting/licensing centers were closed during this point of the COVID-19 pandemic. We consulted Bodega Marine Laboratory oceanographer Dr. John Largier to ensure that BMR and Portuguese Beach would be similar enough to draw conclusions about time-dependent growth trends. According to J. Largier (pers. comm., 2021), there is little oceanographic difference associated with the 7 km of alongshore separation between site 2 and site 3. Sonoma Coast is well studied oceanographically; it is all part of the same upwelling cell (we cite Largier et al., 1993 to support this), so sites all along this stretch of coast are all well correlated with cold (or warm) periods occurring synchronously at all sites up and down the coast. While we address this in the Methods (section 2.1), we will mention this in the Discussion (section 4.4) as a potential source of variability between modern and archival growth patterns.

RC2: Line – 39 – after ~ 500 years add Butler et al., 2013; Butler, P. G., A. D. Wanamaker, J. D. Scourse, C. A. Richardson, and D. J. Reynolds (2013), Variability of marine climate on the North Icelandic Shelf in a 1357-year proxy archive based on growth increments in the bivalve *Arctica islandica*, *Palaeogeography Palaeoclimatology Palaeoecology*, 373, 141-151, doi: 10.1016/j.palaeo.2012.01.016

AC: We agree with the suggestion to cite the Butler et al. (2013) paper. We will incorporate this reference here.

RC2: not everyone would support this statement about obvious/clear daily growth increments in *A. islandica*. Better to say Schone et al concluded ...

AC: We agree and will rephrase this sentence by adding in the word “concluded” to reflect that we are reporting Schone et al. (2005a) and Schone (2013)’s interpretations regarding daily growth increments.

RC2: Table 1 – consider adding Wanamaker et al 2008 for Mg/Ca in *Mytilus edulis*  
Wanamaker, A. D., K. J. Kreutz, T. Wilson, H. W. Borns, D. S. Introne, and S. Feindel (2008), Experimentally determined Mg/Ca and Sr/Ca ratios in juvenile bivalve calcite for *Mytilus edulis*: implications for paleotemperature reconstructions, *Geo-Marine Letters*, 28(5-6), 359-368.  
We found differing Mg/Ca ratio relationships based on ambient seawater salinity. Thus, there is likely a physiological response/control over elemental incorporation.

AC: We value the reference suggestion and we will add the Wanamaker et al. (2008) paper to the Mg/Ca row of Table 1.

RC2: I think the last paragraph in the Introduction should be the aims of the study. Thus, I suggest making the paragraph (line 75) about banding the first paragraph of the Introduction. I think the Introduction lost clarity after reading about the aims which was followed by a very broad discussion of banding.

AC: We thank the reviewer for this suggestion. We agree that this paragraph about banding feels somewhat misplaced; it was moved around multiple times during the initial writing of this manuscript. We will move it back up to the first paragraph so that the Introduction (section 1.1) ends with the enumerated study objectives.

RC2: Line 116 – add standard deviation to salinity range and report if it is 1 or 2 standard deviations.

AC: In 2018, mean daily BOON salinity was 33.4 PSU with 1 standard deviation (SD) of 0.34. We will report this here. We used the year 2018 to calculate mean daily salinity since the BOON instrument malfunctioned in 2019 and 2020; 2018 was the most complete record for salinity.

RC2: I found figure 6 a bit confusing/hard to follow. Perhaps adding “range” to Seasonal SST for the x-axis on panel B would eliminate the possibility of thinking panels A and b are nearly identical.

AC: We thank the reviewer for this suggestion, which we agree would greatly improve the figure. We will re-work Figure 6b to feature seasonal range on the x-axis and change the point shape to denote the season of collection to highlight the relationship between season, seasonal temperature range, and terminal band color. See the last page of this PDF document for the revised figure and new caption.

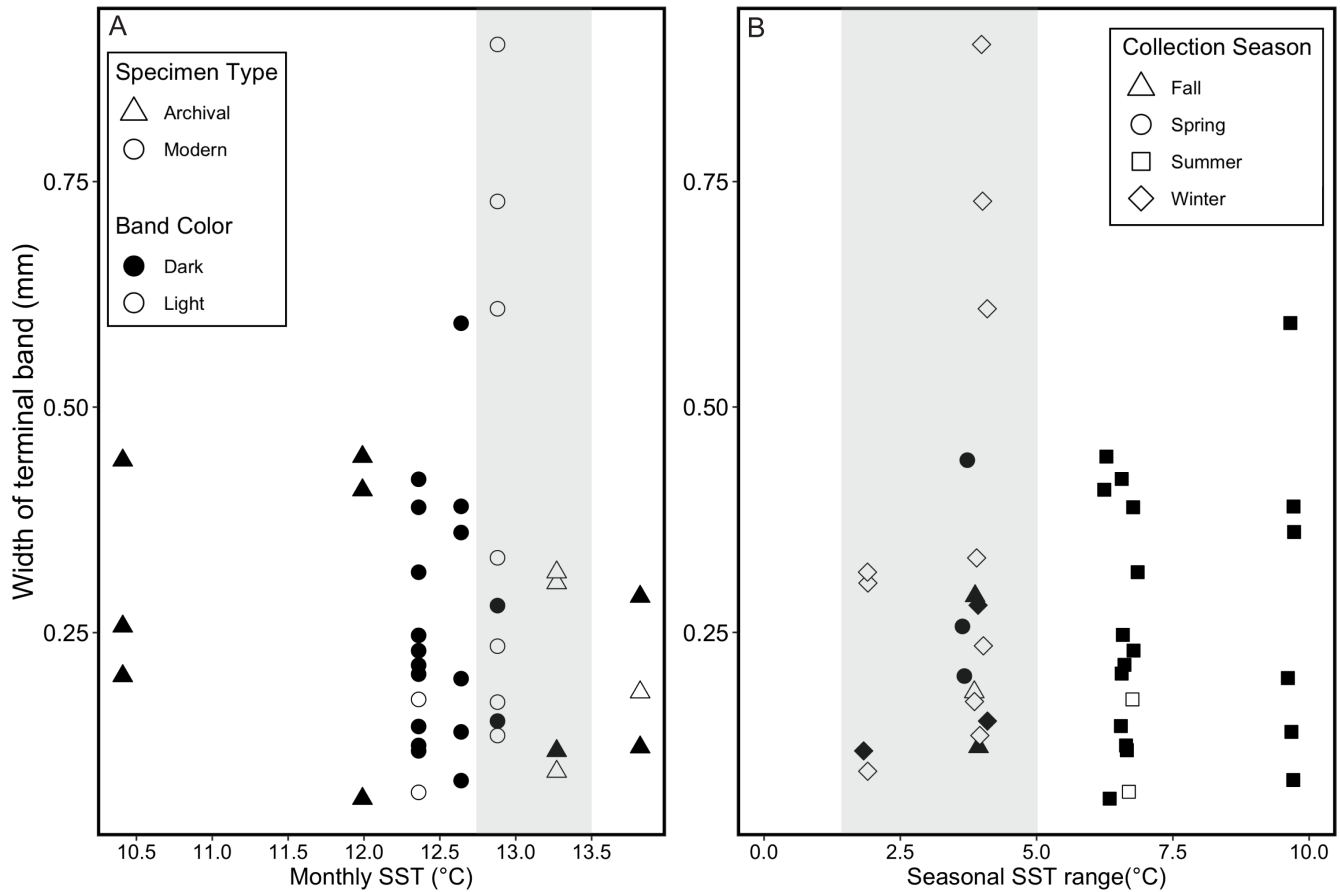
RC2: Line 390- onward – high resolution sampling (representing weekly or so) of oxygen isotopes in the outer calcite layer would help solve this issue right? Is this planned? Some discussion of this possibility is warranted in the Discussion (or future work?).

AC: We agree that stable isotope analysis would be useful for estimating growth rate and reconstructing annual cycles in *M. californianus* shells. Multiple studies cited in our paper (e.g., Jones and Kennett (1999), Jazwa and Kennett (2016), Ford et al. (2010)) performed high-resolution (weekly to monthly) subsampling of the outer calcite layer of *M. californianus* shells and documented seasonal oscillations of oxygen isotopes recorded by this species. While we could perform the same analyses (high-resolution oxygen isotope sampling of the outer calcite layer) in our samples, it would be difficult to compare this outer calcite oxygen isotope profile to dark-light banding in the inner calcite layer in any accurate or useful way. We know that the outer calcite layer grows extensionally and the inner calcite layer grows inward adding to shell thickness, but we do not know if these layers calcify simultaneously or at proportional rates. The banding in each layer is certainly a different resolution (i.e., tidal banding in the outer calcite layer and ~ seasonal to multi-annual banding in the inner calcite layer based on environmental conditions). Ideally, one could sample both the inner and outer calcite layer at a very high resolution and then match up the two sinusoidal oxygen isotope profiles to (1) estimate and compare growth rates of each layer and (2) estimate the amount of time represented by a pair of dark-light bands. Unfortunately, this would require *in situ* oxygen isotope analysis since drilling/milling for powdered samples would not be possible in the inner calcite layer, which is very thin (~ 2 mm on average) and could not accommodate a drill bit. Further, dark-light bands are extremely thin (on the order of microns), which would be challenging to sample even with costly instrumentation such as secondary-ion mass spectrometry (SIMS) or sensitive high-resolution ion microprobe (SHRIMP). We will address this challenge briefly in the Discussion (section 4.3).

RC2: Also, when considering future work, Goodwin et al found that *Mercenaria mercenaria* clams grew during the warmest part of the day throughout the year whereas oysters in the same setting had no preference. If we were to sample these clams and oysters for oxygen isotopes, we might then conclude that they grew in different environments, but they did not. Thus, I wonder if monitoring daily high and low temperatures might provide some additional insight on your work. This is just a thought- no action needed.

Goodwin, D.H., Gillikin, D.P., Jorn, E.N., Fratian, M.C., and Wanamaker, A.D., (2021) Comparing contemporary biological archives from *Mercenaria mercenaria* and *Crassostrea virginica*: Insights on paleoenvironmental reconstructions, *Palaeogeography, Palaeoclimatology, Palaeoecology*, 562, doi.org/10.1016/j.palaeo.2020.110110.

AC: We thank Referee #2 for this thoughtful comment. We will read the Goodwin et al. (2021) paper recommended here and consider incorporating tidal SST monitoring for future work.



**Figure 6.** Relationships between SST, width of terminal band, color of terminal band, specimen type, and collection season for all 40 specimens. Filled (black) points represent dark terminal bands and open (white) points represent light terminal bands in both plots. (a) Relationships between mean monthly SST during month of collection, terminal band color, and terminal band width. Shaded bar represents approximate monthly SST range over which most specimens are associated with light terminal bands (12.75-13.5°C). Specimen type specified with point shapes (see legend A). (b) Relationships between the seasonal SST range (mean daily SST maximum – mean daily SST minimum for each season of collection), terminal band color, and terminal band width. Shaded bar highlights that most terminal light bands are associated with a seasonal SST range < 5°C. Collection season specified with point shapes (see legend B).