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Biogeosciences Discussions

Interactive comment on "Architecture, Growth Dynamics and Biomineralization of Pulsed Sr-Labelled *Katelysia rhytiphora* (Mollusca, Bivalvia)" *by* Laura M. Otter et al.

Anonymous Referee #1

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Otter and colleagues exposed specimens of a veneroid bivalve from Australia to episodically strongly elevated Sr levels (18 times above normal marine levels) in order to make the shell growth visible. They studied the effect of high Sr levels in the water on shell ultrastructure, crystallographic orientation, shell chemistry and growth rate. Except for the shell chemistry, all above mentioned shell properties remained unchanged. Sr/Ca values in the shell increased proportionately to that in the water, i.e., ca. 18 times, which still is way below expected thermodynamic equilibrium, a result supporting previous studies. Findings were interpreted to indicate an "intracellular, diffusion driven, selective transport" of ions across the mantle epithelium and subsequent shell formation processes via amorphous calcium carbonate.

The experiment and analyses were superbly executed and I really enjoyed reading the results. A broad variety of different machines (EBSD, nanoSIMS, μRaman spectroscopy, EPMA, TGA, optical microscopy and FEG-SEM) were employed to study physical and chemical properties of the shells. Yet, the study contains a number of flaws that need to be addressed in a significantly revised version of the ms.

(1) Authors need to specify the overarching goals of their study more clearly and formulate specific hypotheses. For example, I do not think that the main goal was just "to visualize growth" with Sr labeling as stated in the first (= most important) sentence of the Abstract. The title lists at least two other topics. In contrast to the great data presented in this manuscript, the Abstract and Introduction are very weak, poorly structured and organized, and the overarching (and far-reaching) purpose of the study remains elusive. The text is full of juxtapositions, i.e., sentences and paragraphs need better transition. In the Abstract, actual numbers of key data must be given, i.e., the 18 times enrichment in the shell (at least in the outer portion thereof; see below) following exposure to 144 μg/g Sr instead of 8μg/g (translate these data into molar Sr/Ca ratios, please). In the Introduction, authors should first place their study into broader context and identify the motivation for this investigation (which is not that existing in-situ staining methods affect the physiology of bivalves! See below). They need to describe open research questions and how they were addressed here. At the end of the Introduction and later in the Conclusions section, authors need to describe the implications of their finding, e.g., that bivalves likely serve as faithful recorders of the ocean chemistry etc. (which essentially emerges from the observation that Sr/Cashell changes proportionately to Sr/Cashell if the Srwater level is increased, or, as the authors expressed it – an interesting point of view by the way – irrespective of the Sr level of the water, Cashell/Cawater and Srshell/Srwater remained the same).

(2) Authors erroneously speak of outer and inner shell layer, but, in fact, they have only studied the outer shell layer, which in almost all bivalves is divided into two ultrastructurally different portions, i.e., the outer and inner portion of the outer shell layer (in the

following, oOSL and iOSL). The inner shell layer (ISL) is located way back (below what is depicted in Fig. 4C) and (in a cross-sectioned shell) starts where the myostracum intersects with the inner shell surface (= aka pallial line) and ends somewhere at the hinge portion. In Figure 1B, the inner shell layer is formed approx. inside the brown areas, whereas the brown section and portions outside thereof largely belong to the iOSL; the oOSL is likely not seen in this image. The pallial line delimits the ISL from the iOSL. I recommend to look at Fig. 2A in Schöne (2013).

(3) Surprisingly, a number of relevant recent papers dealing with very similar issues remain uncited. For example:

(3a) In-situ labeling: Mouchi et al (2013) labeled oysters with manganese to study growth rates, and Mouchi et al. (2016) used immunogold to obtain insights into biomineralization processes of Crassostrea gigas. Riascos et al (2007) tested three different stains in abalone and the surf clam, i.e., calcein, alizarin and strontium chloride.

(3b) Zhao et al (2017a) recently demonstrated that Sr/Ca in the outer shell layer of Corbicula fluminea increases proportionately to Sr/Ca in the ambient water and is not affected by growth rate effects. A very similar finding as reported here.

(3c) An alternative mechanism of how the bivalve controls the trace and minor element levels in the shell – brought forward by Shirai et al. (2014) and based on Stephenson et al. (2008) – was also ignored: Organic macromolecules near the shell formation front exert control on which and how many ions are incorporated into the carbonate phase of the shells. If the overall production of biomass and thus growth rate decreases (e.g., during times of low food availability), less of such organic substances are produced and the level of trace impurities in the shell carbonate automatically increases. This in turn, affect the morphology of biominerals and likely explains the more primitive ultrastructure at growth annual and even daily growth lines (biochecks) (Füllenbach et al. 2017), i.e., irregular simple/spherulitic prismatic ultrastructure (Schöne 2013). Data in Table 1 also indicate that different microstructures in your study contain different Sr

levels, likely for the very reason described above. However, you did not discuss this or the fact that the relative change in the iOSL is only ca. 14 times, not 18.

(4) The alternative mechanism of element incorporation mentioned in 2c does not require any control on uptake of elements. Although the chemical composition of the extrapallial fluids or gels (outer EPF forming the OSL, inner EPF the ISL) of marine bivalves have rarely been measured, the few available studies (e.g., Wada & Fujinuki 1976) unequivocally show that they have nearly the same ionic strength and chemical composition as the ambient seawater (Crenshaw 1972, Lorens & Bender 1980). This is no surprise, because bivalves are osmoconformers, like all other marine organisms. Imagine which energetic efforts were otherwise required if the bivalves had to constantly pump these ions out of the body fluids. Some elements such as strontium, magnesium and sodium reach the body fluids as ions from the ambient water through the gills and the gut (Wilbur & Saleuddin 1983) and across the mantle epithelium (passive diffusion). I have prepared a table for you summarizing data from Wada & Fujinuki (1976) (Table 1).

Table 1 see extra file

Despite this, shells are strongly depleted in many trace and minor elements. For example, if measured with a spatial resolution of ca. 50μm Sr/Ca in aragonitic OSL of Arctica islandica ranges between ca. 1-3 mmol/mol and Mg/Ca remains below 0.8 mmol/mol (e.g., Schöne et al. 2011). Even when measured by much higher spatial resolution (nanoSIMS) which might be advantageous given the strong chemical heterogeneity of the shell at the μm-scale, Sr/Ca in aragonite of Cerastoderma edule does reach values expected for equilibrium fractionation (Füllenbach et al., 2017). In calcitic shells of various species, Mg/Ca ranges between ca. 4-28 mmol/mol (see summary in Vihtakari et al. 2016). These findings lend support to the hypothesis that unwanted elements are actively excluded from the shell by specialized organic macromolecules directly at the site of shell formation (Schöne 2013; Shirai et al. 2014). How this mechanism fits to the ACC-mediated shell formation processes needs to be discussed.

Since the chemistry of body fluids of bivalves resembles that of seawater, there is no need for any active transmembrane element transport. Zhao et al. (2017b) recently demonstrated very clearly that Sr, Mg and Ba levels in shells of Corbicula fluminea were not transported by active transport mechanisms and did not use the same pathways as Ca. These authors have poisened Ca2+ATPase and blocked Ca2+ channels. According to the finding by Zhao and colleagues, a passive diffusion pathway across the mantle epithelium is much more likely and would perfectly fit to the incorporation control by organic macromolecules at the shell formation front. I strongly feel that these alternative explanations must be presented and discussed.

(5) Another argument against ATP-mediated uptake mechanism is unchanged growth rate of the bivalve. If the hypothesis by Otter and colleagues holds true according to which an “intracellular, diffusion driven, selective transport” of ions is responsible for the observed low Sr shell concentrations, then it is surprising that shell growth rate remained unchanged. A selective transport consumes energy = ATP), and the energy demand for such a transport process increases if the Sr level in the water rises. If more energy is devoted to the control of Sr incorporation into the shell, less energy is available for shell formation resulting in lower growth rate.

(6) There is a confusing usage of the term “uptake” (e.g., P2L8). ‘Uptake’ refers to way elements take from the environment to body fluids. This can either occur through mantle epithelia (in ionic form, potentially by one of the pathways listed in your paper) or during digestion of food. Is this really what you mean here on page 2 or rather the ’incorporation’ of elements into the shell at the site of shell formation? From the context, I assume you meant the latter: “Recent studies showed that the uptake of some trace elements, such as strontium, are strongly influenced by crystal growth rates, shell curvature and ontogeny in addition to physiological effects”.

(7) A number of observation were only presented, but not discussed and combined with other aspects of the study, e.g., different amounts of organics in different ultrastructures.

(8) Interpretation of the timing of shell growth, meaning of microgrowth increments (= daily), major biochecks (= annual) and greyscale changes (= fortnights) is purely speculative and not supported by the data presented. This would require mark-and-recovery experiments. Though not unlikely that the regular change in greyscale results from fortnightly changes, you need to cite at least relevant papers dealing in detail with such tide-controlled growth patterns (Evans 1972, Ohno 1989, Schöne 2008, Hallmann et al. 2009). B the way, you did not say where the bivalves lived: in the intertidal zone?

You also noticed that you observed 6 lines in portions formed in tanks during 6 (solar) days suggesting that at least these growth patterns are circadian. However, you have no evidence that the same applies to shell portions formed in nature. Given that the specimens lived in the intertidal zone (please provide details on tidal regime: diurnal or semidiurnal, tidal range etc.), it is reasonable to assume that they have formed circalunidian growth patterns (lunar days). Perhaps, acclimatization to circadian lab conditions were sufficient to reset biological clock resulting tin switch from lunar to solar daily. However, all this needs some discussion (in the Discussion section, not results as currently presented).

(9) Since you are aiming to publish your paper in a journal that is often read by people of the proxy and paleoclimate communities, you need to translate oxide values into element concentrations (as well as molar element/Ca data), and all element/Ca data into molar ratios (required for easier, direct comparison with published data). Likewise, instead of reporting Ca/Sr ratios, please turn this around and give Sr/Ca data.

(10) I do not think your results allow any conclusions on whether higher Sr levels in water have or have not affected shell growth rate. If growth conditions remained invariant (aside from changing Sr levels), shells should have grown much more homogeneously. But in fact, there is a significant slowdown from LE1 over NE1, LE2 to NE2 suggesting that growth conditions deteriorated through time (Table 2).

Other issues:

- Please check orthography in entire ms. I am not familiar with the Australian English, and whether this represents a mix of American English (e.g., analyze, labeling, meter) and British English (analyse, labelling, metre).

- Consistent use of hyphenation is required in entire ms: crossed-lamellar, cross-acicular, 3 mm-thick, high-resolution, crossed-lamellar, crossed-acicular, organic-rich etc. need a hyphen

- Headings: Consistently capitalize heading or use sentence case.

- No colon at the end of headings! E.g., P8L21: “The inner crossed[-]acicular [shell] layer:”, P9L1, etc.

- P1L16, “aragonite crystals”: As you noticed in the following sentence, "the smallest mineral units are nanogranules" which are enveloped by proteinaceous materials. I suggest to employ the term "mesocrystals", because the definition of an abiogenic aragonite crystal does not include nanocomposites consisting of aragonite and organic material.

- P1L19, replace “shells” by ‘shell portions’ or ‘ultrastructures’. There are no bivalves consisting entirely of nacre. I assume you intended to say that different ultrastructures contain different amounts of organics.

- P1L19/20: I do not understand this sentence. Growth rates = growth patterns? Outer structure = outer shell layer. Prisms can be correlated to growth rates? Do you mean that each 3rd order prism forms in one day? Moreover, you did not mention anywhere in the text sub-daily growth patterns.

- P1L20, “outer structure”: You used the term “structure” in two different ways: as a synonym for “ultrastructure” and “shell layer” (e.g., P6L32). Be consistent. Do not use “structure”, but one of the other terms above. Check and change throughout ms.

- P1L23, “physiological processes during calcification have no lag”: Rephrase, this is hard to understand. Shells do not just consist of CaCO3, but also organics which need

to be fabricated, and the building blocks for these substances derive from ingested food. Digestion of food and fabrication of organic molecules that end up in the shell need time. There is hence a lag between ingestion of food and shell production. Or what do you mean with “physiological processes . . . have no lag”.

- P1L23, “calcification” is the wrong term here (and used improperly in many other studies). Calcification rate includes density and is not synonymous to growth rate! Calcification rate = amount CaCO3 precipitated per time interval per area. Replace all instances with ‘shell growth rate’

- P1L25, “Sr-conditions”: no hyphen; ‘Sr level’ or ‘Sr concentration’ sounds better

- P1L26, “Sr-enrichment”: no hyphen

- P1L26, ”Sr-enrichment factors for labelled and ambient conditions”: This remains insufficiently explained and is oddly phrased. Do you mean artificially elevated Sr levels vs. normal marine Sr levels? Give actual numbers! What do you mean with "identical enrichment factors": Sr levels in shell increase proportionately to that in the water (i.e., 18 times)? As far as I can tell from Table 1, this does not apply to both shell layers (and ultrastructures).

- P1L31, “aragonite or calcite”: replace “or” by ‘and/or’. Note there are species with different CaCO3 polymorphs in the outer and inner shell layers. Further note that some species also come with vaterite, ref

- Introduction: better transition between paragraphs needed

- P2L3: delete “recent and fossil”, superfluous

- P2L4+5: None of these papers used trace elements of shells as environmental proxies. Replace by suitable citations:

(a) temperature: Klein et al. (1996a), Wanamaker et al (2008), Schöne et al. (2011), Zhao et al. (2017a)

(b) salinity: Klein et al. (1996b)

(c) pH: Zhao et al. (2017c)

- P2L5: "uptake" refers to element uptake from the environment either through mantle epithelia (in ionic form, potentially by one of the pathways listed in your paper) or during digestion of food. Is this really what you mean here or rather the 'incorporation' of elements into the shell at the site of shell formation? (see main comments above)

- P2L10: "Urey et al., 1951" is neither a "recent" study nor a study that looked at trace elements. One reference that must be added here is Shirai et al. (2014) which discussed another potential mechanism that controls Sr incorporation into the shell (see main comments above).

- P2L12: substitute "shell" with 'trace and minor elements in shells'

- P2L14: substitute "but" with 'and'

- P2L14: Firstly, always say 'ultrastructure', not "structure", because at other places you use "structure" as a synonym for shell layer. Secondly, this statement needs a reference.

- P2L15-16: Delete sentence starting with "Apart...". Then start next sentence with "Apart from those,"

- P2L17: replace "which are found" by 'which occur'

- P2L21: The homogeneous ultrastructure forms an own category and is not a subgroup of the crossed-acicular category (compare Marin et al. 2012)

- P2L21: "venerid" must not be italicized

- P2L22: "Shimamoto, 1986" is outdated (?), check most recent revision of ultrastructures by Carter JG et al. (2012)

- P2L24: "Pulsed strontium labelling ... understanding of other marine calcifiers": You

have used exposure to higher Sr levels not only to study the effects of this trace element on the ultrastructure and as a time gauge, but primarily to identify potential mechanisms of element incorporation into shells. And the latter has been already done with bivalves by Zhao et al (2017a).

- P2L28: delete hyphen after "micro"

- P2L33: replace "between umbo and ventral margin" by 'parallel to the main growth axis' or 'parallel to the umbo-ventral margin axis'

- P3L5-6: "Growth lines..." show/refer to figure

- P3L7: crossed-acicular ultrastructure is not a subcategory of the homogeneous ultrastructure. The latter forms an own category. Refer to more recent studies (Marin et al. 2012, Carter et al. 2012).

- P3L16: Two main clauses combined by conjunction require comma; check and correct throughout ms: ', and'

- P3L17: Specimens: Much more information needed here: sediment type, tidal height, intertidal zone(?), how many specimens collected/prepared/used for which analytical technique, when collected. Table would be best. Part of this information is relevant for the temporal alignment of the shell growth patterns.

- P3L17: replace "live-collected" by 'collected alive'

- P3L20: use '$\times$' as mathematical operator (consistently throughout ms)

- P3L23: "which is a reliable sign for the absence of handling stress"... says who? This claim is unsupported. And looking at the decline in daily shell growth (Table 2) during the experimental phase, the specimens do not seem to have liked the new environment. So, handling stress cannot be precluded.

- P3L24-26: Has the element composition of the food been measured as well? How do you know that all Sr and Ca comes from the water? Has always the same amount

of food being offered? When were they fed, during simulated day or nighttime?

- P3L29-30: An "event" is a very short-term incident. This sentence should be rephrased, e.g., "exposure to background conditions, i.e., normal marine Sr levels".

- P4L7: P400-P2000

- P4L12: thickness of gold-coating?

- P4L21: 20,000$\times$

- P5L9: replace “was used” by “were used”

- P5L21: μm2 (superscript)

- P5L27 “The inner and outer layer of a K. rhytiphora shell were separated with a DREMEL tool and mechanically cleaned.” Be more specific here: Have you obtained powdered material or fractions of the two portions of the outer shell layer? How have you managed exactly to separate them?

- P6L3: Actually wrong. You have only studied the outer shell layer, which consists of two portions with different ultrastructure, an outer and inner portion, respectively (oOSL, iOSL)!

- P6L8: Rephrase (and italicize genus and species names): ‘The outer shell layer of studied K. rhytiphora specimens is ... near the ventral margin’

- P6L10: “in agreement with previous studies. . .” This phrasing means that the other species studied by Carré and Soldati and colleagues lived in Australian waters. Rephrase.

- P6L11: “growth periods”: delete “periods”

- P6L13: “troughs” Odd phrasing. Something like this is better: ‘Cyclic changes in greyscale near the ventral margin correlate strongly with tidal cycles, i.e., light grey and dark grey portion fall together with full and new moon cycles, respectively.’ The main

problem is that you do not provide any evidence for the timing of shell growth! Where is the evidence that the dark and light portions really have formed during new and full moon? This is an interpretation at most, and as such belong to the Discussion section (where you need to refer to previous studies of intertidal bivalves which found narrower increments and thicker growth lines formed during spring tides, and these portions then appear darker than shell portions formed during neap tides when viewed at lower magnification and under reflected light. More suitable Refs: Evans 1972, Schöne 2008, Hallmann et al. 2009)

- P6L16-25 also needs to be moved to Discussion. Only keep descriptive part here. You have no evidence that these grey bands formed on a circalunidian basis, but you can certainly interpret them as such based on previous work.

- Timing of shell growth: You later noticed that you observed 6 lines in portions formed in tanks during 6 days suggesting that at least these growth patterns are circadian. However, you have no evidence that the same applies to shell portions formed in nature. Given that the specimens lived in the intertidal (please provide details on tidal regime: diurnal or semidiurnal, tidal range etc.), it is reasonable to assume that they have formed circalunidian growth patterns. Here, please stick to descriptions, not interpretation.

- P6L25: ‘in two other specimens’, not “on two other specimens”

- Section 3.2: Title is more suitable for Discussion. – This section should be expanded as it is an essential component of the ms and forms the basis for your hypothesis on element incorporation. Describe Table 1 in much more detail. Report molar ratios as well. Compute and tell reader by how much the Sr levels increased in the shell when exposed to 18 times higher Sr levels in water. This will then show that the Sr levels in oOSL increased by 18 to 20 times, whereas the iOSL only by ca. 14 times. This needs to be discussed later.

- P6L27: “Sr incorporation”: no hyphen - P6L28: “Sr concentration”: no hyphen

- P6L32: replace “structure” by “shell layer

- P7L2: “. . .were identical within uncertainty”: i.e., they have remained invariant, stayed the same? I suggest you rephrase this to avoid confusion.

- Title Section 3.3: Section heading should inform about content of section, not which method has been used.

- P7L31: “This species develops annual growth checks” On what evidence is this statement based? How did you analyze when the shell portions formed? Likely correct, but pure speculation... or is there previous work on this species?

- Section 3.4.3: Interesting information, but what is the purpose of having this measured and reported?

- P9L10: crystallographically

- P9 “Calcification Rates” includes density, not synonymous to growth rate! Calcification rate = amount CaCO3 precipitated per time interval per area; this is not what you mean.

- P9L28-29: “Due to the geometry of first order prisms without- and inward bending in cross-sections,. . .” No sentence

- P10L2: Provide image showing where you determined increment widths, or even better trace two growth lines to show that growth in oOSL is faster than in iOSL due to shell geometry.

- P10L4-5: quite complicated phrasing: absolute growth rates vary among specimens

- P10L5: grew, on average, 5.6... same for the other "on average": separate by comma and place before number

- P10L12: “Also, rates tend to decrease effectively with increasing distance to the ventral margins (Fig. 4A).”: Unclear what you mean and purpose of mentioning this. You need to trace fortnights in Figure 4A to support your statement.

- “bivalve species”: you listed genera not species rephrase: ... structure of other bivalves, e.g., Pinna..., the aragonitic...

- P10L23, P11L5, P11L23: “In K. rhytiphora the first order prisms” comma after species name

- P11L10: Equally-sized (adverbial usage)

- P11L30: Since you did not capitalize "aragonite", you should also use lower case here (except for the acronym/abbreviated form). Besides that, you used lower case in the Abstract.

- P12L16: replace “shell” by ‘shell portions’

- P12L30: replace “the outside of their shells” by ‘outer shell surface (Fig. 1), and’

- P13L1: “growth time”: Firstly, you have no evidence that these growth checks formed annually. Secondly, no bivalve grows 365 days. Note also that such ornamentation patterns do not agree with growth patterns in other species, and likely this is a coincident and only true for shell portions near the ventral margin in the studied specimens. Rephrase.

- P13L12-13: Perfect! This is your time gauge. It verifies the circa daily nature of these growth features and could further be used to support your hypothesis of fortnightly growth bundles appearing as greyscale changes.

- P13L14-15: replace “higher” with ‘faster’, “short” with ‘narrow’, “longer” with ‘broader’

- P13L15: “day”: An interesting question that you need to discuss is that these are probably circadian (24h) periods entrained by the 12/12 light/dark cycle experimental conditions. The adjustment interval was probably long enough that the natural, tide-entrained shell formation cyclicality (resulting in circalunidian, 24.8h, periods) vanished. Under natural conditions though, you would need to have circatidal (12.4h) and circalunidian increments, because otherwise your interpretation of the other 48 or 50

dark cycles representing fortnight periods would not hold true.

- P13L22: “We suggest a diel physiologically controlled variation of calcification” Not sure exactly what you mean. Circadian clock controlling growth/calcification rate? This has been reported previously elsewhere.

- P13P29“physiological processes involving Sr incorporation”, rephrase: ‘physiological processes controlling Sr incorporation’

- P13L29 “have no lag”? Well, this depends on the temporal scale you are looking at. Where is the evidence that there was no gradual increase in shell Sr levels during the course of minutes or so? Diffusion of Sr through the mantle epithelium takes at least some time.

- P13L30-31: I do not think that the implications provided are supporting an ACC-mediated growth of shell in bivalves.

- P13L33-34: “A fundamental observation of this study is that the calcification front runs evenly across all structural units and architectural orders of the shell independently of the current growth rate. This” But this is known and no a new finding of this study!

- P14L1: “show the labels to cut across the different architectural building blocks”: could also occur if extrapallial space is gel-filled or epithelial cells are in direct contact with shell

- P14L2 “where the label would rather follow a zig-zag trend between fully labelled and unlabelled units” Impossible to understand what you intend to say here. Rephrase please. Do you mean that the growth front is uncoupled from the ultrastructures? This is known as well: In freshwater bivalves the large prisms continue to grow over many years and daily growth lines cross them perpendicularly (studies by Dunca, Mutvei etc.).

- P14L2-4: “This is clearly visible from the sharply defined change between labelled and unlabelled shell areas (Fig. 4B and D), as well as from the cyclic variations in

short-term growth rates (discussed above). Our” Likewise hard to understand

- P14L10 “active selective transport consuming Ca2+-ATPase enzymes”: transport consumes energy which is provided by ATP, and the enzyme that accomplishes the transportation is the Ca ATPase. Rephrase.

- P14L14-15 “We observed virtually identical enrichment factors for Ca and Sr (CaShell/CaSeawater and SrShell/SrSeawater) in labelled and ambient conditions (Table 3).”: Interesting point of view! But this does not mean anything else than Sr/Ca shell increases proportionately to that of Sr/Ca seawater, and this has already been shown by Zhao et al. (2017), which you did not cite.

- P14L15: “Sr-ion transport is independent from. . . ” if so, the energy demand of the bivalve increases in order to keep the Sr out of the shell. Do you see a decrease in growth rate during Sr enrichment as opposed to ’normal’ Sr levels in water?

- P14L16-17: “Sr ion would be at the expense of a Ca ion”: Not really clear what you mean; Since this is the essence of your paper, you need to describe this more clearly and convincingly. Why exactly can transport mechanism 1 not be true?

- P14L17: Replace “Sr-enrichment” by ‘shell Sr concentrations’

- P14L18: Ca/Sr: please also or only report Sr/Ca

- P14L19-20: Replace “Hence, the strong enrichment of Ca from seawater to shell” by ‘strong enrichment of Ca in shell’

- P14L25: “Ca to be transported as ACC-nanogranules to the calcification front (Loste et al., 2004; Addadi et al., 2006; Jacob et al., 2011; Zhang and Xu, 2013).” Check if all cited studied were using bivalves (not gastropods or other taxa), and which ultrastructures were analzed, report this here.

- Section 4.5: Here you discuss more (and different stuff) than what the heading implies.

- P15L12: italicize genus and species names

- P15L14-15: “a systematic change in growth increments during Sr-enriched periods”: Do you mean ’growth increment widths’? You need to highlight here again that food levels and other extrinsic factors that could potentially have affected growth rate remained unchanged during the experiment, and you would have expected invariant increment widths if Sr had no effect on growth rate... see comment further above on relationship between growth rate and Sr exclusion from shell

- P15L18: Replace “calcification” by ‘growth rate’

- P15L23-24: “Reduced growth rates in aquaculture conditions cannot be explained by ontogenetic trends alone but result from missing tidal cycles.” Sorry, but this is pure speculation and likely wrong. Much more likely is that you did not provide proper food and the animals did not really ’like’ the tank conditions.

- P15L30: ‘nanometer’?

- More comments in pdf with annotated figures and tables.

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Table 1. Average element-to-Ca ratios in the inner extrapallial fluid of marine bivalves in comparison to seawater. Calculated from chemical data reported in Wada & Fujinuki (1976).

	Seawater	EPS during growth	EPS during resting
Na/Ca (mol/mol)	44.3	44.1	42.4
Li/Ca (mmol/mol)	2.1	2.6	2.7
Mg/Ca (mol/mol)	5.0	5.1	4.9
Sr/Ca (mmol/mol)	8.3	9.4	8.0
Mn/Ca (mmol/mol)	30.2	291.1	223.9

Fig. 1.