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Interactive comment on “Effects of increased $p\text{CO}_2$ and temperature on trace element (Ag, Cd and Zn) bioaccumulation in the eggs of the common cuttlefish, *Sepia officinalis*” by T. Lacoue-Labarthe et al.

Anonymous Referee #1

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General comments: This is an interesting and timely study addressing the effects of warming and reduced pH (mimicking the CO_2 -driven ocean acidification) on the uptake of trace metals into the cuttlefish *Sepia officinalis*. Indeed, understanding the interactive effects of temperature and low pH (which affect both the rates of biological uptake mechanism and solubility of metals thus potentially mobilizing sediment-bound metals and altering the amount of bioavailable metals in the water column) will be very important for understanding and forecasting the potential effects of ocean acidification and global climate change on metal toxicity to marine organisms.

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Interactive Discussion

Discussion Paper



Specific comments: Materials and Methods: 1. From the M&M description it appears that there was only one replicate bottle per each temperature-pH combination; is this correct? If yes, this is a typical pseudoreplication design for temperature and pH exposures. This issue may be less serious in the case of pH where three pH gradations were used, so that low pH is “replicated” twice; however, in the case of temperature no such replicates are available. The authors should address this issue and explain which implications it has for their experimental results and interpretations. 2. “Seawater was spiked with 110mAg (1 kBq L⁻¹), 109Cd (1.5 kBq L⁻¹) and 65Zn (1 kBq L⁻¹). These activities corresponded to an addition of 86, 16, 64 pg L⁻¹ stable Ag, Cd and Zn, respectively.” – Are these the total metal concentrations in the incubation media? Were any non-radioactive metals added? Please explain. 3. “. . . total activity/concentration ratio (LCR; g; ratio between radiotracer content in the vitellus or the embryo – Bq – and time-integrated activity in seawater – Bq g⁻¹) over time” – this indeed is a non-standard measure of the metal uptake and a more detailed description of this measure including integration procedures would be useful. 4. I would like to see how the concentration factor was calculated; it might explain why there was a decrease in the concentration factor during prolonged metal exposures on Fig. 3. My guess is that it is probably a result of using cumulative dose (exposure time x concentration) for the denominator of the CF but without an explanation it may also look like depuration. 5. The first paragraph of discussion is too long and too speculative for a simple finding that low pH prevented the normal increase in the volume of perivitelline fluid. In fact, most of this paragraph starting from the sentence: “It is worth noting that low temperature, i.e. 16C, reduced the egg swelling compared to 19C” and ending with “. . . embryonic form of the haemocyanin (Decleir et al., 1971), which differs from the juvenile or adult forms” can be omitted without the loss to the interpretation or understanding of the data, and replaced by the statement that the authors do not have data to choose between the two explanations proposed in par. 5 on p. 4877.

Technical comments: Abstract: Spell out CF when first mentioning it.

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P. 4867. Change “are considered as complex organisms” to “are complex organisms”.

P. 4867. What is “low low oxygen-carrying blood protein”?

P. 4867. Change “reported their low oxygen-carrying blood protein as a key of their expected vulnerability to the ocean acidification” to “reported their oxygen-carrying blood protein as a target of their expected vulnerability to the ocean acidification”.

P. 4868 Change “The subsequently incorporation” to “the subsequent incorporation”.

P. 4872. Please spell out LCR (lad concentration ratio) when the abbreviation is first mentioned.

P. 4873. Change “no combined effect of both pH and temperature was observed” to “no interactive effect. . .”

P. 4873. “The lower pH of incubation seawater of eggs, the more the hatchlings accumulated 110Ag in their tissues”. – Do you mean that the the accumulated concentrations were higher? Or do you truly mean that there were more individual hatchlings that accumulated Ag (as opposed to those that did not)? Please clarify.

Abstract and results are in contradiction to each other; the abstract states that 109Cd CF decreased with increasing pH, whereas in the results (p. 4874) the statement is opposite (“109Cd CF decreased with increasing pCO₂” and thus decreasing pH) – please correct.

P. 4874. Change “tracer was no longer accumulated in the eggshell, but only being depurated from it” to “tracer no longer accumulated in the eggshell, but was only depurated from it”.

Table 1. Asterisks and “ns” signs are redundant and should be removed from the Table – all the necessary information is given by P values.

P. 4876. This statement is unclear: “110mAg CF in the perivitelline fluid did not vary with the pH for either temperature, except at normal pH and at 19C”; please clarify. Do

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you mean “except a significantly lower CF found at the normal pH compared to pH 7.85 and 7.60 in the 19C-incubated group”?

P. 4878. Change “Then, 110mAg, 109Cd and 65Zn uptake kinetics decreased while the eggs were under exposure conditions” to “During prolonged of the exposure to metals (>XX days), uptake rates of 110mAg, 109Cd and 65Zn decreased”.

P. 4879. Par. 5. Change “two specificities” to “two characteristics”. Replace 1) with “Firstly,” and delete 2) in this paragraph.

P. 4879. “. . .whereas 110mAg and 20 65Zn penetrate earlier in the pooled vitellus and embryo” – change to “with 110mAg and 20 65Zn penetrating earlier in the pooled vitellus and embryo” and specify, earlier than what?

P. 4879. “Regarding the 110mAg, 109Cd and 65Zn activities in the hatchlings, it appeared that 1) 110mAg and 109Cd uptake showed a linear relationship with the increasing pH, whereas 2) 65Zn was best accumulated in the embryo at the intermediate pH. This observed dichotomy was consistent with the non-essential (Ag and Cd) and essential (Zn) character of the studied elements”. It is not clear why essential and non-essential elements should have different pH-dependence of their uptake? Please explain. And please remove 1) and 2) from the 1st sentence of this paragraph.

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