

## ***Interactive comment on “Ocean acidification dampens warming and contamination effects on the physiological stress response of a commercially important fish” by Eduardo Sampaio et al.***

**Anonymous Referee #1**

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General comments:

This manuscript aims to address a topic of significant importance, namely the interaction between climate change stressors and contamination in coastal regions, and particularly its impact on species of commercial importance. This is certainly a topic of great interest and an area that has been identified as a significant knowledge gap in the field at present. Despite this potential and the undoubted requirement for such a study within the field, regrettably the manuscript presented here does not adequately address this question. As it stands there is insufficient detail presented throughout the

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methods to adequately appraise what has been done, there appear to be a number of methodological oversights that hamper the interpretation of the results and this has, to a large extent, led to many of the conclusions drawn not being supported by the data. Based on these factors I believe the manuscript at least requires major revisions to include this required detail, as well as restructure the conclusions to match what has actually been undertaken. It would then require re-review to appraise the manuscript in its new form. If it is not possible to include this required detail in full, in its current state the manuscript is not of sufficient quality to be published.

Specific comments:

Abstract, discussion and conclusions – Throughout the manuscript the authors suggest the reduced accumulation of mercury in tissues under combined exposure is due to metabolic depression, and a subsequent reduced appetite/ingestion of food, initiated by elevated CO<sub>2</sub>. However, the authors do not measure any parameters in the current study that could confirm or counter this suggestion. There is no indication that these fish ingested less food so the conclusions, certainly as they are presented, are unfounded. Reduced accumulation could in fact be caused by a number of different mechanisms in the organism by which elevated CO<sub>2</sub> augmented Hg accumulation, either by metabolic depression, reduced appetite (could be caused by alternative mechanism), reduced digestive efficiency, reduced uptake across the gut epithelium, greater egestion of Hg or impacts of Hg transport and complexation in plasma to reduce delivery to measured tissues. All are potentially feasible and at present insufficient information is known about this to surmise it is metabolic depression. It is vital to indicate that whilst altered accumulation is noted, which differs between specific tissues, the mechanism is not known. Following this point, the authors have not cited two key references on ocean acidification and mercury contamination recently published (Li et al Scientific Reports 7;324 2017; and Wang et al ES&T 51:5820 2017). It is possible these were published after initial submission of the current manuscript, but in light of altered mercury accumulation under elevated CO<sub>2</sub> these two manuscripts are key as

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they support the current finding.

â€” Abstract, discussion and conclusions – Similarly to the point above, the authors repeatedly suggest elevated H<sup>+</sup> impacts mercury accumulation/toxicity at a molecular level, but no acid base measures were made. Also it is a common misunderstanding that elevated CO<sub>2</sub> results in chronic acidosis in fish plasma, this is not the case. Elevated CO<sub>2</sub> results in acute acidosis which is rapidly compensated for by an elevation in bicarbonate, returning the plasma H<sup>+</sup> to normal levels. Therefore the suggestion that elevated H<sup>+</sup> impacts on mercury toxicity/accumulation is not supported, especially as acid-base parameters are not presented that counter this common response noted in acid-base compensating species such as fish. The authors need to again re-interpret data and re-write conclusions to better reflect the demonstrated results and not make broad unsupported conclusions, pinned loosely on previously published literature that has been misinterpreted/misunderstood.

â€” Discussion – The authors suggest the Fulton condition may diminish under mercury contamination. Whilst AIC indicates the best fit model as slightly negative the statistic (p-Value) clearly indicates no significant effect and therefore suggesting this is not the case may mislead readers to interpret a result that is not supported statistically, even if it may support a previous publication.

â€” Intro and methods - The justification of mercury, and methylmercury, in fish from coastal regions is insufficient. There is no quantification of levels within the environment from different regions globally, how coastal compares to open ocean and how this then translates into a burden for fish populations. As it stands this is not adequate for a contaminant manuscript, and belies the statement that an environmentally relevant concentration was used, as stated in the methods. What is an environmentally relevant concentration, where does the level chosen fit with measured environmental levels from different regions globally, and even just within the region the study was undertaken in. Finally, if the route of uptake is solely dietary for fish then how do environmental levels correspond to burdens in prey species and thus exposure in the experimental organ-

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ism? Is the level chosen a typical contaminant level in prey species in an impacted environment or the level in water/sediment? This needs clarifying, and fully justifying in relation to existing literature and levels previously used.

â€” Methods - Following this, the total amount of mercury (mg per kg of food), is higher than the content of methylmercury added as an additive (8.02 MeHg, 8.28 HgT). This is not possible. Also how were these levels measured (or is it nominal)?

â€” Methods (page 4 lines 1-7) - The description of the conditions, and particularly their maintenance is not sufficient. It states ammonia, nitrate and nitrite were regularly monitored and kept within recommended levels. How was this tested, what were the accepted levels and what were the levels measured within the experiment? Also how were high levels mitigated against and how often? Furthermore, it mentions salinity was kept at  $35.0 \pm 1.0$  g/l NaCl? The probe listed is a conductivity probe so does not measure in g/l of NaCl but gives a conductivity measure or salinity as a psu. Also how was salinity maintained? i.e. is this addition of deionised water to compensate for evaporation? Or addition of additional NaCl? The description is confusing, and could be interpreted as additional NaCl addition. Any further addition of NaCl would significantly alter osmolality thus this needs clarifying to explain if input water fluctuated in salinity. A better description of this process is therefore required.

â€” Methods - There is no measure (or data presented) of methylmercury or total mercury in experimental water. This is a major omission, and gives no indication as to what proportion of the contaminant leaches from food into water, particularly if any food remains uneaten and in the tank for any time. It also prevents the discussion of amounts of methylmercury that egested immediately into the water by this fish, not being taken up or bioaccumulated.

â€” Throughout - Given the commercial importance of the species, one surprising oversight is the fact that no discussion on different tissue burdens were made with respect to human consumption and climate change impacts. The only place this is

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alluded to is in the title! This is particularly relevant given the possibility that elevated CO<sub>2</sub> reduces Hg accumulation possibly reducing transfer of Hg into humans directly via consumption of muscle tissue, which could be an important result. This would provide some wider context in which to place the importance of this study generally, as well as contaminant/climate changes studies more generally.

Technical corrections:

Page 1, Line 18-19 – Sentence beginning “Despite the more than likely co-occurrence. . .” is weak and doesn’t read well. Needs stronger justification (see above) to enable stronger conviction in abstract, as well as explicitly highlight that contaminant/climate change stressor interactions are largely overlooked, rather than just “these stressors”.

Page 1, Line 29 – should read mechanisms not mechanism

Page 2, Line 2 (and throughout) – should be CO<sub>2</sub> sub-scripted, this error occurs in a number of positions throughout manuscript, also sometimes is sub-scripted so inconsistent.

Page 2, Line 4-5 – I would argue greenhouse gas effect is increasing global temperatures, and this is resulting in projected further increase (already increased by 0.76°C from pre-industrial) in surface ocean temperature of . . . by end of the century.

Page 2, Line 22 – Should read “. . . Sampaio et al., 2016) and ultimately mortality (Cocchini et al., 2000).”

Page 3, Line 7 – protein not proteins

Page 3, line 11 – responses not response

Page 3, Line 16 – remove the before estuaries

Page 4, Line 10 – should be pH controllers not controller

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Page 5, Line 5 – Length<sup>3</sup> should be super-scripted

Page 5, Line 19 – remove with before nitric acid

Page 5, Line 23 – should be gill not gills

Page 5, Line 25 – remove posteriorly

Page 5, Line 26 – rewrite as “. . . response concentrations, quantified” removing were

Page 6, Line 18 – assume is potassium periodate not potassium per iodate

Page 6, Line 23 (and page 7, line 16) – mg<sup>-2</sup> needs super-scripting

Page 6, Line 25 – insert space before Superoxide

Page 7, Line 5 – is the % inhibition of SOD activity calculated as maximum inhibition, average inhibition at each 5 minute time point or from initial and final, just measured every 5 minutes over 25 minutes so potentially have different rates of inhibition and total overall inhibition over this time course

Page 7, Line 23 – insert space before and

Page 8, Line 2 – insert space in mg<sup>-1</sup>total

Page 9, Line 19 (and other places) – *A. regius* needs italicising

Page 10, Line 2 – notoriously is an odd choice of words, suggest just removing as reads fine without replacing

Page 10, Line 23 (and page 11, line 15) – H<sub>2</sub>O needs subscripting

Page 18 – Why is the x-axis reversed on figure 1, d, compared to b and c. This confuses comparisons.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-147>, 2017.

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