

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.

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General comments

The interactive effects of acidification, warming and the presence of the metal Hg was assessed in the Fish *Argyrosomus regius*. Bioaccumulation of Hg was measured in different organs of the fish and sublethal toxic responses were also analyzed by the use of biomarkers. The topic is highly relevant since research regarding global change issues should preferably focus on a multi-stressors approach. Furthermore, mercury is an

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important persistent contaminant found in coastal environments around the world and information regarding its interactive toxicological effects with other parameters such as acidification and warming are of great value. In general, the writing is clear and the data obtained is interesting. However, some issues regarding the methodological approach used are not well explained and there are some information at the results and discussion section that should be included. Therefore I recommend that the authors perform the suggested corrections before the article is published.

Specific comments

Introduction and discussion

The focus of the study is the evaluation of toxic responses of the metal Hg in a global change scenario. It is mentioned that concentration of Hg was chosen according to environmental measurements, however data on the range of toxic concentrations of this metal to this species or other fish species is not included. Considering that the article uses an ecotoxicological approach and therefore it is based on dose-response concentration it is crucial that more details on this subject is included, such as values of toxicity for fishes and environmental values within contaminated and non contaminated areas, especially in the area where the study was conducted.

In the discussion section, comparative results of mercury accumulation and biomarker response are missing. The study of Biomarkers is quite complex as responses can be influenced by many parameters. In this sense, there are several studies on biomarker response to mercury in the literature. Such studies should also be mentioned to provide information on the sensitivity of this species comparing to others, as well as to know the relevance of the used Hg concentration.

In the abstract, (page 1 line 20), introduction (page 3 line 20) and methodology (page 4 line 23) pCO₂ concentration is given as 1100 μ atm, while the actual value used was 1500 μ atm. Please correct.

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Methodology

The fishes were taken from an aquaculture station. Were the physico-chemical parameters measured at the station? This is relevant to know the levels of pH and temperature that organisms were acclimated at the long-term.

Page 4 Line 5- Ammonia levels is an important issue at toxicity tests, especially with fishes, as it can interfere on the toxic responses. Authors mention that ammonia (along with nitrate and nitrite) levels were kept within recommended levels. How was this performed? What are the recommended levels? Please give more details.

Salinity should be given as psu or without unit.

Page 4 line 13- Please give more details on alkalinity measurements, such as the equipment used, storage of samples, the use of certified materials. . .

Page 4 Line 20- The method for mercury contamination is confusing. MeHg exposure was performed by food intake and fishes were fed two to three times a day. How was the difference between food intakes measured? Authors states that ingestion decreased due to changes in metabolism, but how was this measured? Where is this result? How much mercury was given as total in the experiment? How much of this metal remain dissolved in the water column?

In the experimental set-up, the setup "IV" is the same as the setup "II", 19 °C, 400 pCO₂ μatm and contaminated feed (MeHg: 8.02 mg kg⁻¹; HgT: 8.28 mg kg⁻¹). Setup IV should be 19 °C , 1500 μatm and contaminated feed.

Results

In the methodology section, it is mentioned that Reference material was also used to validate measurements of metal content. However, results of recovery percentage in not given. Please include this data as it validates the measurements.

Page 8 line 20-25 concentration of Hg was lower in muscle but concentration in liver and

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gills was actually the same considering error between replicates.

Figure 1d the 400 and 1500 μatm are inverted.

Page 8 line 27- As expected, catalase activity was affected by mercury contamination, but was this biomarker affected by pCO₂ also? What about warming? This is briefly mentioned in the discussion section, but the results are not given.

While the values for Hsp70 are given in each organ analyzed, the results for the other biomarkers are not specified. Were they measured only in the liver or other parts? Please include this information in the results and also in the methodology.

Discussion

Page 9 lines 15-20 the information "However, our AIC-chosen best model indicated that mercury may diminish organism Fulton condition" is contradictory to what is mentioned on the results : "Fulton condition (K) did not show any significant differences between treatments (MeHg, $p > 0.05$, GLM analysis in Table 1)."

Technical corrections

Page 2 Lines 1-2: CO₂ should be subscript Page 4 Line 2: m³ should be superscript Page 4 Line 10: CO₂ should be subscript Page 5 Line 5: lenght³ check type error Page 6 Line 12: mg⁻¹ should be superscript Page 6 Line 23: mg⁻² should be superscript, Page 10 Line 20: H⁺ should be superscript Page 9 line 17: the word non-lethal could be replaced by sublethal, which is more often used in toxicity studies Page 9 line 19: A. regius should be written in italic

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