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***Interactive comment on “The metabolic response of pteropods to ocean acidification reflects natural CO<sub>2</sub>-exposure in oxygen minimum zones” by A. E. Maas et al.***

**Anonymous Referee #2**

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This interesting manuscript represents an important attempt to interrogate the subtle, but significant, differential response of closely related marine species to changing environments. The authors have identified significant reductions in oxygen consumption and nitrogenous excretion in one species of pteropod (*Diacria quadridentata*) when incubated at 0.10% CO<sub>2</sub>.

This work is important as it contributes valuable data to the growing body of published work which identifies the potential resilience of some calcifying marine species to predicted changes in the world's 'future ocean'. The manuscript is strengthened by the attempt to correlate the findings of experimental manipulations with field data on the

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distribution of pteropods within a Pacific OMZ.

Nonetheless, in a number of areas a more detailed or considered discussion is required and the limitations of the current study need to be fully identified.

In the introduction the authors describe both facets of the problem of increasing seawater pCO<sub>2</sub> to marine species, namely: 1) the dissolution of carbonate structures (more readily seen in aragonitic minerals) as a function of changes to the seawater carbonate system and 2) the direct effects of changing pH on intracellular acid base balance and the downstream effects to metabolism. However, the manuscript fails to constrain the carbonate system or end pH of the experiments. The authors have exposed the pteropods to 1000ppm CO<sub>2</sub> but have not quantified the changes imposed on the carbonate system – they present no data on the aragonite saturation state in response to this exposure and present no data, either measured or calculated, on the pH of the treatments at the end of the incubation. They also do not identify what the oxygen concentration of the experimental treatment was – did the syringe respiration chambers (10-50 ml) remain sufficiently oxygenated during the experiment so as not to directly impact pteropod respiration rate. As such it is difficult to interpret the results of, for example, Figs 2-4 in the context of the pH and O<sub>2</sub> profiles shown in Figs 1 and 5. Furthermore, in the water column profiles taken in 2007 and 2008 there are no clear measurements of the carbonate system (e.g. total alkalinity, DIC, carbonate saturation states etc); the reader has to accept statements such as those that appear at the top of page 10302 on the approximate alkalinity in the region from WOCE data (is this alkalinity at the surface or at depth, at what temperature?) and also the assumption that aragonite is: “thought to be undersaturated”. These weaknesses reduce the impact of this manuscript. It could be argued that consideration of calcification and aragonite saturation is a distraction to this manuscript. The authors present data on metabolism in response to high pCO<sub>2</sub>, perhaps the manuscript should be restricted to consideration of the literature on metabolic suppression vs metabolic stimulation in response to acidosis; undoubtedly this manuscript would still make a useful contribution to that

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field.

The manuscript also presents a compilation of pteropod respiration and excretion data from different locations and different years. Nowhere in this manuscript is there any discussion of the potential for differences in pH profile between locations and years and how this might have influenced the organism response data that they present? Which species were collected and experimented on during the Gulf of California cruise in 2007 on board RV New Horizon? Also, the data presented in Fig 5 seem to be a compilation of profiles only from the Costa Rica Dome and the Tehuantepec Bowl in 2007 and 2008; are there no data in this figure from sampling in the Gulf of California in 2007? Fig 5 also does not provide much insight on the distribution of particular species with depth, just 'all pteropods' and could presumably be changed easily to identify the profile of individual species? For example, what is the exact profile established from MOCNESS hauls for *Diacria quadridentata* (of relevance to the statement on lines 8-10 on page 10302)? There seems to be no adequate explanation for the unbalanced data sets used in the experiment. In Fig 2 this is highlighted with apparently only 3 individuals contributing to the 0.10% CO<sub>2</sub> exposure for *Creseis virgula* and an n=1 for the excretion data. How does the limited data set affect the validity of the ANCOVA of respiration rate with wet mass at the two pCO<sub>2</sub> levels. Additionally, how does this affect the power of the experiment to identify statistical difference with such low n and such high variability (see Figs 3 and 4)? Perhaps the data for this species should be excluded.

The experiments were conducted at 20 oC; how does this relate to the in situ temperature the pteropods experience in the water column? The implication of Fig 1 is that by approximately 100m the water temperature dropped to at least 15 oC, reaching approximately 10 oC by 400m - the maximum depth of the MOCNESS data in Fig 5. There is no real indication as to why a temperature of 20 oC was used in this experiment. Do all of the pteropod species regularly occur in surface waters at temperatures of 20 oC. As the authors know, temperature has an overriding effect on metabolic rate and they

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suggest as much in the discussion on page 10302 (around line 20) but the implications of this to their experiment and interpretation should perhaps be expanded.

The opening statement of the Discussion is not supported –this manuscript only reports qualitative data on the diel vertical migration of ‘all pteropods’. The data presented is also only presence/absence. Surely this manuscript and Maas et al., when published, must be viewed together to support this statement; Maas et al. presumably presenting quantitative data by species? Indeed, the suggested title of Maas et al. in prep (lines 20-23, page 10305) implies that additional environmental gradients contribute to the observed distribution and physiology of pteropods in this system – how definitive is the current manuscript?

Minor:

1) There is no real discussion of the relative significance of the O:N ratios in the current manuscript, if they are not considered then why are they presented?

2) The statement that: ‘little is known of the physiology of tropical pteropod species’ does not seem to be supported by the literature. The authors should consider:

Cummings FA; Seapy RR (2003) Seasonal abundances of euthecosomatous pteropods and heteropods from waters overlying San Pedro Basin, California *VELIGER* 46: 305-313, which discusses vertical migration, also:

Bhattacharjee D; Mallik TK (2000) Pteropod occurrence in relation to aragonite compensation depth - An example from Carlsberg Ridge (Indian Ocean) *INDIAN JOURNAL OF MARINE SCIENCES* 29: 305-309, and:

Davenport J; Trueman ER (1985) Oxygen uptake and buoyancy in zooplankton organisms from the tropical eastern Atlantic *COMPARATIVE BIOCHEMISTRY AND PHYSIOLOGY A-PHYSIOLOGY* 81: 857-863, and possibly:

Ujihara A (1986) Pelagic gastropod assemblages from the Kazusa group of the Boso Peninsula Japan and Pliocene-Pleistocene Climatic Changes *JOURNAL OF THE GE-*

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