

## ***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.***

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Reviewer 2

**I would like to thank the reviewer for her/his constructive suggestions for our paper. Below, I respond to each of the reviewer’s comments on a point by point basis. Reviewer comments are in plain text and author response is in bold.**

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

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calculated, on the pH of the treatments at the end of the incubation.

**A sentence has been added to express the change in experimental pH as a result of organismal respiration: “Organismal respiration reduced the pH of experimental chambers by ~ 0.1 (n=13) and is consistent with a respiratory quotient between 0.7 and 1.0 when compared with the O<sub>2</sub> consumed during the experiments. This pH end-value was achieved gradually and does not represent the value that the animal was incubated at.”**

**The carbonate chemistry has been explicitly characterized and text has been added to the methods and discussion: “Carbonate chemistry of the region was estimated using WOCE alkalinity values (P-18 1994 and 2008), pH and CTD profiles of salinity and temperature using the CO<sub>2</sub>sys developed by Lewis and Wallace (1998). The system was run using the seawater pH scale, Dickson KHSO<sub>4</sub>, and constants from Dickson and Millero, 1987. WOCE alkalinity data from 200 m was relatively consistent at nearby latitudes between 1994 (2298 ± 9.2) and 2008 (2300.5 ± 4.5), suggesting that these values are reasonable estimates of OMZ alkalinity.”**

**“Assuming an average salinity of 34.7, a temperature of 10° C, a depth of 200 m (CTD data) and incorporating the measured pH with the known alkalinity of the region, aragonite is undersaturated in the OMZ (CO<sub>2</sub>sys: seawater pH scale, Dickson KHSO<sub>4</sub>, and constants from Dickson and Millero, 1987; Ω<sub>Ar</sub> = 0.65).”**

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.

**The chambers remained oxygenated throughout the experiments to prevent the confounding physiological stressor. Text has been added to the methods ex-**

plaining this point: “Most respiration runs never fell below 70% air saturation, and all runs which fell below 35% ( $\sim 80 \mu\text{mol kg}^{-1}$  at  $20^\circ \text{C}$ ) were excluded from analyses. Most animals in the ocean can regulate oxygen independent of environmental availability down to at least 50% saturation (Childress and Seibel, 1998; Seibel, 2011). For species where runs were included which fell below 50% saturation, a statistical tests of species specific metabolic rates show no significant differences between oxygen consumption rate above and below 50% (one-way ANCOVA: *D. quadridentata*  $F(1,9) = 0.81$ ,  $p = 0.39$ ; *C. longirostris*  $F(1,12) = 1.87$ ,  $p = 0.20$ ); *Hyalocylis striata*  $F(1,17) = 0.08$ ,  $p = 0.79$ ). ”

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  $p\text{CO}_2$ , 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 field.

**Although this paper does not seek to address calcification, much of the discussion of ocean acidification emphasizes this metric and I felt that it was necessary to provide some context to readers about the saturation state, particularly because thecosome pteropods are considered sensitive explicitly due to their thin aragonite shells. To address concerns about the strength of carbonate chemistry of the system, text has been added to both the methods and discussion which better elaborate on our estimates of the estimates of the system and the**

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#### **saturation state of aragonite.**

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?

**Text has been added to discuss how differences in pH may have influenced the organismal response during physiology experiments: “Since low O<sub>2</sub> and high CO<sub>2</sub> are inexorably linked in OMZs (Paulmier et al., 2011), we assume that although pH profiles were not taken in 2007 in either the Gulf of California or the ETP, the low O<sub>2</sub> waters found below 200 m at these locations were accompanied by similarly low pH and high CO<sub>2</sub> water.”**

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?

**Text was added to table 1 to indicate the species collected for physiological experiments aboard specific cruises. MOCNESS sampling was not a component of the research in the Gulf of California. Therefore, there are no vertically stratified profiles of pteropod distribution from this region. Text has been added to the methods to clarify this point.**

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)?

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**Fig 5 had lost species specific labels. The figure has been fixed to include this information.**

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.

**Obtaining individuals in good condition and of an appropriate size for respiration experiments was difficult for some of the less common species. Although there is limited replication, the measures of the oxygen consumption and ammonia excretion of *Creseis virgula* reported here are some of the only rates in the literature and may inform later studies. For this purpose, we feel it is important to include the data. Text has been added to explain the unbalanced data sets and to emphasize the low statistical power for *C. virgula* and *C. pyramidata*: “The number of individuals captured in good condition and usable for respiration experiments varied among species, with significantly lower abundances of *C. pyramidata* and *C. virgula*. This low sample size and the large variability in oxygen consumption and ammonia excretion rates suggest that comparison between high and low CO<sub>2</sub> treatments should be treated with caution for *C. virgula*.”**

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.

**Text has been added explaining the choice of temperature and more explicitly stating the implications of high CO<sub>2</sub> at surface temperatures in contrast to high CO<sub>2</sub> in the OMZ: “This temperature was chosen to replicate the average temperature above the thermocline among the three stations. All species of pteropod in this study regularly spend a portion of their day in the mixed layer, and it is here that the effects of ocean acidification will have an impact their physiology.” “Exposure to hypercapnia independent of these other conditions, which will occur as the surface ocean acidifies and as was the case during these experiments, may not be physiologically analogous to their response to hypercapnia at depth in the OMZ.”**

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?

**The sentence has been revised to express the qualitative nature of the data. Fig 5 has been amended to include species specific labels:**

**“Our study of the vertical distribution of thecosomes is the first to describe the presence of four pteropod species in the pronounced OMZ of the ETP.”**

**Although other environmental gradients likely contribute to the distribution of pteropods in the OMZ, the emphasis of this paper is on the response of pteropods to surface acidification. Since this environmental stress will occur**

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at warm temperatures and in well oxygenated waters, I feel it is valuable to address this question separately from the question of what constrains the natural distribution of these animals. The presence of pteropods in the OMZ serves as a description of the natural variability of CO<sub>2</sub> experience by pteropods in this region and as a putative explanation of the unexpected resiliency of these species to short term exposure to hypercapnic conditions.

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?

**Text has been added discussing the O:N ratios: "For all species, the O:N ratio was not significantly different between treatments, indicating that there was no shift in metabolic substrate in response to exposure to CO<sub>2</sub>. This ratio is an indicator of the metabolized substrate. In our study, O:N was highly variable, likely due to uncontrollable differences in the feeding history of captured animals. An O:N ratio below a value of 16 indicates that protein is the primary fuel source for catabolism whereas a ratio of 50-60 is indicative of a diet balanced between lipid and protein catabolism (Mayzaud and Conover 1988). Tropical pteropod species O:N ranged on average between 20-40 suggesting that protein fueled a significant portion of their catabolism."**

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 eutecosomatous 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:

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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 GEOLOGICAL SOCIETY OF JAPAN* 92: 639-652.

**Although there are a number of papers which describe the distribution of pteropod species, there are comparatively only a few publications (Smith and Teal, 1968; Biggs, 1977; Gilmer and Harbison, 1986; Seibel et al, 2007) which address the physiology of the tropical species. This statement was meant to emphasize this point. If we are to understand the effects of changing environmental conditions upon zooplankton we must move beyond simple reports of distributions to a more mechanistic understanding of the effects of hydrographic conditions on their abundances. This sort of analysis is somewhat lacking in the literature.**

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