

bg-2016-230

The metabolic response of thecosome pteropods from the North Atlantic and North Pacific Oceans to high CO₂ and low O₂

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We would like to thank the referee for their appreciation of our approach and their helpful comments for improving the clarity of the text. We have responded to each suggestion on a topically grouped point by point basis with referee comments in plain text and author response in bold.

- Section 2.4: How many organisms were incubated per respiration chamber

Each chamber contained one individual pteropod. The text has been modified to read:

“Post-gut-clearance, healthy organisms were put into separate glass syringe respiration chambers, one individual per chamber, with a known volume of 0.2 µm filtered seawater and 25 mg L⁻¹ each of streptomycin and ampicillin.”

how many replicates were possible to set up? Is N in table 4 the number of replicates? Please clarify.

- Is N consistent with number of replicates?

The number of replicates was different for each species, treatment and region. The N in table 4 (previously labeled as the number of individuals) is indeed the number of replicates. In the case of this experimental design individuals and replicates are synonymous. The word ‘individuals’ has been replaced with ‘replicates’ for clarity.

The discussion is in general ok, but I miss some consideration relating to the specific life stage(s) these experiments were done on. Was it all the same life stages? If not, could that affect results and conclusions?

Of what stage were the pteropods (all adults?)? If different stages, how could that affect results and conclusions drawn?

Were all individuals of the same developmental stage?

To address this point, text is now included in the methods, results, discussion and conclusion specifically stating that the work was done only with adult life stages.

“Species were targeted specifically for their abundance and the likelihood of their presence in both ocean basins and only adult individuals were used.”

“Only relatively large adult specimens were used in respiration trials, in part to avoid any confounding effects of ontogeny and in part to ensure a measurable change in oxygen levels.”

We agree with the reviewer that juveniles of all the species we studied could potentially be more sensitive to the tested conditions. There is experimental evidence from our group that the veliger stage of *L. retroversa* is more susceptible to CO₂ (Thabet et al. 2015) and clearly as projections are made about population-level responses to changing environmental conditions the sensitivities of all life stages must be considered. The vertical distribution of pteropod juveniles has not been well documented (in part because good morphological keys to that stage do not exist), but it would be interesting to determine whether they are absent from depths where high CO₂ / low O₂ is present naturally. Interestingly many diel vertically migratory species exhibit different vertical distribution throughout ontogeny in relation to midwater regions of high CO₂ / low O₂ (i.e. Wishner et al. 2013; Maas et al. 2014). It seems logical that juvenile pteropods would avoid regions of high CO₂ during initial shell formation as this is a period of sensitivity as has been shown in some pteropods (Thabet et al. 2015) and in other species (Kurihara 2008; Kroeker et al. 2013; Waldbusser et al. 2015).

Since the text only focuses on adults we have chosen not to discuss this thoroughly; we did, however, reiterate our focus on the adult life stage at the start of the discussion:

“This study reveals that short term exposure to low O₂ and high CO₂, similar to what would be experienced by individuals in the Pacific during diel vertical migration, does not influence the oxygen consumption of adult individuals of most of the thecosome pteropod species examined from either the Atlantic or Pacific.”

And made mention the importance of the consideration of the various sensitivities of different life stages as:

“Furthermore, although adult individuals may show no change in metabolic rate, there is evidence that juvenile stages of many calcifying species are typically more sensitive to CO₂ exposure (i.e. Connell et al. 2013; Waldbusser et al. 2015) and emerging evidence supports the idea that eggs, veligers and juveniles of *L. retroversa* and *L. helicina* are more vulnerable to acidification than adults (Lischka et al. 2011; Thabet et al. 2015; Manno et al. 2016). Thus, although adults may be capable of surviving short term exposure, as acidity in surface waters increase there may be population level stress due to ontogenetic sensitivity.”

Please give also size ranges of different species incubated.

- L319: Size ranges of all species would be really helpful to see!

- Section 3.1: Please include information on the size ranges of the different pteropod species.

Table 4: - Please include a size range for all different species

The size range (average mass and standard error) for the pteropods is already reported in Table 4, and it seems unnecessary to report the range (smallest to largest). If the reviewer instead means size in length, the information is not available for some of the samples which have been used for other purposes. Furthermore, the species documented here vary substantially in shape and have different growth patterns, making it impossible to report a standard size (length/diameter, width). It seems that if length is what the reviewer refers to, this is tied into the previous concern about life stage and we hope that the changes made to the text are sufficient to allay the reviewers' concerns.

- Section 2.6: Assumptions proved in case of significant results found for *L. retroversa*?

We assume that the reviewer refers to the assumptions of normality and heterogeneity of variance – please correct if otherwise. These tests were performed and *L. retroversa* met all assumptions. Text has been added on line 370 as follows:

“The datasets were tested for normality and homoscedasticity.” Information about the statistical assumptions has been added to Table 5.

- Section 3.2: This section needs some clarification with respects mentioning of geographical positions, temperatures. . . in the text and the respective table.

- L409/410: Can not find geographical position in table 2?

- L415: geographical position not found in Table 2?

- L432/433: 400 or 385 m? geographical position can not be found in Table 1.

Text has been added to clarify the geographical positions of each station in the text (latitude, longitude and station number are now specifically described). The range reported in the text describes collectively all of the stations found in the portion of the study region, and hence is a summary of numbers found in Table 2 rather than re-stating exact values from that Table. In addition to the above changes, text has been added to the legend in table 2 to help point out the geographical locations of the hydrographic regimes. Line 1024:

“Each basin was characterized by multiple hydrographic regimes (see text and Fig 2); transitions between regimes are denoted by dashed horizontal lines.”

- L411: 250 or 209 m?

The sentence was intended to indicate that at all stations in this hydrographic regime, the oxygen fell below 130 within the range of the organisms. To better clarify this point the text has been changed to (Line 420-421):

“At these stations O₂ fell below 10% (~130 $\mu\text{mol kg}^{-1}$) at depths less than ~250 m”

- L412: 110 or 130 m?

It was between 108.3-131 m. To clarify the sentence was changed to (Line 421-422):

“At these stations in the northern part of the transect, pH fell below 7.7 at depths less than 130 m,...”

- L417: 10–17_C?

The change has been made.

- L421–425: How can I see that *Clio pyramidata* experienced these conditions (in Table1)?

Table 1 reports that *Clio pyramidata* is a known vertical migrator with a typical range of 0-500 m, and can be found as deep as 1500 m. Based on this information, we would expect that its range similarly extends 500 m in the Pacific, which would put it into conditions of 10% O₂, 800 μatm pCO₂ and aragonite undersaturation in the Pacific as per the values documented in Table 2. To clarify we have referenced Table 2 in the sentence (which as a note has been moved to the discussion as per the request of reviewer 2).

- L427: 200 $\mu\text{mol kg}^{-1}$ corresponds to what % air saturation O₂?

The information has been added to Line 436-438 as:

“In contrast to the Pacific, along the entire Atlantic transect O₂ concentration was above ~200 $\mu\text{mol kg}^{-1}$ (~15%) in the top 500 m, while pCO₂ never reached 800 μatm and aragonite undersaturation never occurred throughout the top 1000 m.”

- L428/429: Would it be possible to indicate the dominant hydrographic regimes in

Fig. 1, would be helpful in connection with the sampling location of different pteropod species.

An attempt was made to modify the figure, but there was no way to show the regimes clearly on what is already a busy figure. We hope that reviewer agrees that with the new additions to table 2 and the text there is now enough information to make this clear.

- L437: below 5_C? I calculated 5.2_C?

The sentence was intended to indicate that at all stations in this hydrographic regime, temperatures within the 25-100 m all reached below 5 C. The sentence has been modified as:

“Stations conducted in this water were typified by a temperature and salinity anomaly with temperatures falling below 5°C from 25-100 m and a salinity signature < 33, contrasting significantly with the surface salinities of the northern portion (~34) and southern portion (~36) of the Atlantic transect.

- Could salinity be included in Table 2?

Salinity has been added to Table 2 and the table caption has been modified to reflect the change.

- L478/480: This sentence is unclear. According to Table 3, ar was never below 1? And 1.2 is not under-saturated? Please clarify!

The point of the sentence was that omega reached a minimum of 1.2 and hence *approached*, but didn't reach, under-saturation. To clarify the sentence has been changed as follows:

"The experimental conditions of the high CO₂ treatments reached their lowest value in the middle part of the transect ($\Omega_{Ar} = 1.2$ at mid-latitudes; Table 3), where cold northern waters of low salinity were encountered. Experimental Ω_{Ar} had a range of 1.5-2.0 for the rest of the transect in the Atlantic."

- L482: In situ values are meant here, right? Maybe indicate in the text, easier for the reader.

We meant the experimental and have now indicated this in line 492-494 as:

"The values of experimental Ω_{Ar} were lower overall in the Pacific, although the high CO₂ treatments also never reached under-saturation (Ω_{Ar} 1.3-1.8)."

- Section 3.4: As indicated earlier, please clarify how many replicates were measured and how many individuals were incubated per chamber and experiment and species.

The word 'individuals' has been replaced with 'replicates' in table 4 for clarity. We do not think it is appropriate to re-state that the method was to place single individuals in a chamber in the results, and hope that the reviewer agrees that the clarification in the methods and table are sufficient to clarify the point.

- L526: Fig 4 not 4A

The correction has been made

- L552/553: What stage of *L. helicina* was it? Could the high mortality also be associated with life cycle issues and less so with temperature, i.e. die off after reproduction?

It is possible, but based on our previous experiences with *Limacina spp.* these organisms are capable of laying eggs throughout their later adult life and typically don't die due to spawning (Maas pers. obs.; Thabet et al. 2015). It is true that they do eventually reach their largest size class and then die. An analysis of the available size class data of all the individuals does not support the idea that the older or younger (larger or smaller respectively – pteropods grow continuously throughout their lives) were differentially susceptible. Live individuals ranged in mass from 0.5-10.4 mg, while dead individuals were 0.9-5 mg. We did not measure individuals from the high mortality events, however, so size may have played a role? In any case, a sentence about alternative hypotheses has been added (Line 569-571):

"Alternative hypotheses are that these were population reaching senescence, or that they were collected in a hydrographic regime with low food availability."

- L617: How likely is it that O₂ saturation below 10% resulted in a substantial difference compared to the results obtained at 10% O₂ saturation? In other words, any idea where a critical threshold level could lie?

The lower than 10% O₂ saturation that was documented in the wild was never explicitly tested in our experiments. Our lowest experiment only ever reached ~8% oxygen over the course of the respiration. Since these were end point measurements there is no way to determine the P_{crit}. Based on previous observations we would hypothesize that the Pacific populations have a lower P_{crit} than the Atlantic. Importantly some of these species in the Eastern Tropical Pacific have been shown to survive and respire at 1% O₂ (Maas et al. 2012). An explicit study of differences in P_{crit} between the ocean basins using the more modern optical spot sensors (such as in (Kiko et al. 2016; Maas et al. 2016), would be informative and productive.

In general, please next time indicate numbers of figures and tables directly on the page where figures and tables are shown. The way they are presented here led to a continuous turning around of printed pages and searching for a particular one.

We apologize that the reviewer found the lack of labels on the figures and tables confusing and will be sure to take this into consideration during our next submission.