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Interactive comment on "Do marine benthos breathe what they eat?" *by* Xiaoguang Ouyang et al.

Anonymous Referee #2

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This paper aims to determine if "you breathe what you eat", specifically, whether the d13C value of carbon dioxide respired by benthic fauna (crabs and gastropods) from a mangrove forest reflects the d13C of the dietary source. The authors used four separate experiments to determine how d13C of respired carbon is affected by 1) taxon (crabs vs gastropods), 2) feeding mode (detritivores vs deposit-feeders), and 3) food source (MPB vs senescent mangrove leaves, and 4) how the quantity of respired CO2 is affected by animal size. The flux and d13C of respired CO2 was determined through incubation of fauna within sealed chambers, with CO2 sampled periodically via syringe, and analysed using a Picarro CRDS.

Although there are some very clear differences and trends in the data collected, I unfortunately remain unconvinced that this study represents a useful advance in isotope

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techniques and I remain uncertain of the conclusions. The manuscript should be revised to improve readability through correction of grammatical errors. However, it is most important that the manuscript be revised to more clearly and thoroughly outline the basis for the study and the hypothesis of the various experiments, and to clearly explain how the outcomes of the study advance the field and can be practically applied elsewhere. My main concerns are as follows:

1) As per the comment above, the justifications, aims, and hypotheses of each of the experiments should be clearly outlined.

2) Given that d13C values for the leaf and MPB are similar, and 13C fractionation between diet and respired CO2 is apparently ∼3-10 per mil (Table 1) and presumably somewhat variable, it seems to me that it would be impossible to distinguish leaves and MPB as potential dietary sources on the basis of breath analysis. Even for sources with more distinct d13C values (e.g., C3 and C4 plants) it would be difficult to determine diet, given the large variability in fractionation of d13C-CO2resp vs d13C-diet based on taxa, diet, and feeding mode (based on data in Table 1). It appears that very specific data would need to be collected through targeted experiments before applying the proposed method, which seems to make application of the proposed technique too complex to be practical.

3) Assuming it 'works', the advantage of using the proposed technique is unclear. The d13C of respired CO2 (d13C-CO2resp) has been used to determine diets of higher order consumers where it is not feasible to collect tissue samples for 'traditional' isotope analysis using an EA-IRMS. However, this is typically not an issue for intertidal invertebrates, and CRDS is still typically less accessible than EA-IRMS analysis. Where might the proposed method be of use? Given the rapid shift in d13C-CO2resp it is likely that the CO2 represents very recent diet, and could be used in combination with tissue sampling to determine diet shifts. Could the authors indicate where this might be useful? Furthermore, how might it be possible to distinguish between a shift in recent diet vs a shift to use of stored carbon (e.g. lipids)? How is this distinct from simply

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analysing tissues with different turnover rates to look at recent changes in diet? It could also be noted that the method does not rely on use of a CRDS, but CO2 samples could also be collected into sealed vials and submitted for analysis via a gasbench/GC-IRMS where a CRDS is unavailable.

4) The title of the paper “Do marine benthos breathe what they eat?” does not reflect the content of the manuscript in its entirety. There is considerable focus on whether marine benthos breathe more when they are larger (CO2 flux vs size relationships). With regard to this focus, it is not clear how an understanding of CO2 production vs size is of practical use. The authors mention the potential incorporation of this relationship when determining the contribution of fauna to CO2 effluxes from mangrove forests, but this would presumably also rely on some understanding of population structure and/or size distribution of benthic taxa. This should be outlined to make clear why this should be of interest.

5) The implications of the large differences in fractionation with taxa, diet, and feeding mode are not fully discussed. Fauna may breathe what they eat, but how can we determine what they eat based on what they breathe?

6) What is the potential impact of confinement and the conditions of the chambers (no sediment, no burrow) on respiration (both in terms of quantity of C respired, and its source (e.g. lipids vs carbohydrates vs proteins))

My more specific comments are as follows:

The methods appear quite straightforward, but some additional information should be provided. Specifically:

1) What was the potential for dilution of gas within each chamber with air entering through the hole in the foil and/or around the edge of the foil (was this sealed in place)?

2) How many animals of each taxa were used in each experiment? How many animals were in each chamber?

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3) Ln 143: Presumably the MPB fed to the fauna were obtained through sieve and spin, otherwise what was the source of MPB? If MPB were from sieve and spin, was the MPB confirmed to still be living? How was the MPB provided, given that the final step was concentration on a filter?

4) Was experiment 4 run separately to the other experiments? Were animal sizes standardised in the other experiments?

5) CH4 analysis is mentioned, but no data is presented.

6) How many leaf and MPB samples were analysed? What area were these collected from? Where were these collected vs where were animals collected?

7) Crab tissues were apparently analysed after incubation, but this data is not presented. It would be interesting to see how d13C values for muscle and other tissues compared to d13C-CO2resp. Were gastropods analysed for tissue d13C?

8) Confirm sampling times: 20 minutes after incubation began then every 10 minutes over 50 additional minutes?

Throughout the ms, replace lower/higher with 13C-depleted/13C-enriched (or similar)

It would make reading easier if the authors replaced “d13C of deposit feeders respired CO2”, which is grammatically awkward, with “d13C-CO2resp for deposition feeders”. This abbreviation (or similar) could be used throughout the ms to improve readability.

Ln 14-16 and elsewhere: It is not immediately clear what is meant by “feeding regime”

Ln 26: “on field collection” – it is unclear what this means without having read the remainder of the paper

Ln 39: Remove ‘past’

Ln 47 onwards: Rework. The focus here on predators seems at odds with the focus of this manuscript on detritivores and deposit feeders.

Ln 48: Provide references for previous use of d13C-CO2 techniques

Ln 75-76: Unclear.

Ln 125-130: Some of this information would be better placed in the introduction

Ln 126: Apparently crabs and gastropods were compared, but it seems likely there could be just as much different between the crab groups (ocypodids vs sesarmids) as between crabs and gastropods.

Ln 140: remove one mention of “collected”

Ln 143: Unclear what is meant by “gas samples were collected separately”.

Ln 153-154: Presumably the samples were homogenised. Were the crabs dissected to remove tissues first?

Ln 197&Ln 200: Specify what the groups were (e.g. crabs vs gastropods, or different crab groups vs gastropods?)

Ln 218-219 seems repeated in ln 219-220.

Ln 227-230: Somewhat misleading. The pattern is consistent, but the magnitude is different.

Ln 245 (and elsewhere): reduce repetition, “CO2 production significantly increased with carapace length” (remove “there was a significant relationship. . .”_

Ln 249-250: Sentence is unclear.

Ln 259: It is not clear what is meant by ‘categories’.

Section 4.1: The point of this section is unclear. It seems to mainly repeat the results, with no new inferences apparent. Can the authors specifically explain how d13C-CO2resp can indicate dietary sources in some useful way?

Ln 294: The relevance of the similarity of d13C for fasting crabs and those fed on

leaves is unclear

Ln 297: Interesting that fractionation of d13C-CO2resp vs d13C-diet for crabs is similar to the range here, but gastropods seem to have far greater fractionation. Why? Where the diet of crabs was switched, is it possible that they were still using stored C (e.g. lipids), and this would have diluted the d13C value of the respired CO2 and affected results seen here?

Ln 327: Specify that C. perspicillata is a bat, and check the spelling of the species name.

Ln 334: Check spelling of detritivore

Figure 1: There is one purple point in among the blue points – is this an error?

Figure 4: The order of the bars (left to right) appears non-intuitive and does not match Figure 3. Also, the colours in this and other figures is unnecessary.

Table 1: Is it possible to provide an error estimate for the fractionation values? E.g. d13C of individual animals – d13C of diet?

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