

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

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In this manuscript, Ouyang et al. present an interesting method to measure the $\delta^{13}\text{C}$ - CO_2 production of various intertidal species using Cavity-Ring Down Spectroscopy (CRDS). The authors aim to link the $\delta^{13}\text{C}$ - CO_2 production to the food sources of these species and advocate the concept "You breathe what you eat".

Response: We thank the reviewer for the compliments on our manuscript.

Identifying food sources from field-collected organisms is an important and timely topic in food web research, so I think any attempt to add an useful technique to the toolbox of food web researchers is welcome. The authors show that there is a lot of potential in obtaining precise measurements of CO_2 and $\delta^{13}\text{C}$ - CO_2 production that can be

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achieved with the CRDS for intertidal fauna. Unfortunately, I also think that this study does not live up to this potential. The manuscript describes a series of four, only loosely connected, experiments that are poorly described and have only marginal scientific significance. Most importantly however, the advocated concept "You breathe what you eat" is not confirmed by the experiments nor it is clear why the labor-intensive and expensive CRDS is superior to the traditional $\delta^{13}\text{C}$ -tissue analysis. I detail my major concerns below.

Response: We shall rearrange the structure of the manuscript to make the linkage of the four experiments be more apparent. Specifically, in the Results section, we will arrange the result of each experiment clearly corresponding to that described in the Materials and Methods section. We do not agree that CRDS is labor-intensive and expensive. For measurement of gas samples, CRDS offers a quick and inexpensive alternative to traditional IRMS, as there is less need for calibration. The standalone G2201i used in our study is less expensive compared with most IRMS.

1. The concept "You are what you eat" is simple: It assumes that the isotope composition of an organism is a simple mixture of its food sources (in case of $\delta^{13}\text{C}$) or a fixed fractionation factor heavier (in case of $\delta^{15}\text{N}$). This means that any researcher can 'simply' collect a large number of organisms from the field, analyse them for their isotope signature and reconstruct its diet, making it a very powerful technique. Of course, there are several potential caveats and problems. The concept "You breathe what you eat" in contrast is methodologically significantly more complex and expensive. If it would resolve some of the caveats associated with the "you are what you eat" concept, then it would be a very welcome addition. Table 1 shows however that organisms do not breathe what they eat. Instead, there is a clear, species- and diet-specific fractionation factor between diet consumed and $\delta^{13}\text{C}$ - CO_2 produced. So why bother going through all the hassle of this more complex method? In addition, the authors do not show or discuss how the classical tissue isotope analyses (samples are measured though, see line 155-156) compares to the produced $\delta^{13}\text{C}$ - CO_2 isotope values.

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Response: We acknowledge that there is fractionation between diet consumed and $\delta^{13}\text{C}$ of benthos respired CO_2 which also exists between diet consumed and $\delta^{13}\text{C}$ of an organism. The advantages of our method lie in the following aspects: (1) it can provide information about both the most recently consumed diet and the integrated diet over longer periods while the classic tissue isotope analysis only tracks the integrated diet over time. (2) breath $\delta^{13}\text{C}$ can be repeatedly measured non-destructively for the same animals and thus can track the changes in its food sources while animals must be sacrificed for the classic tissue isotope analysis which cannot track the change in food sources for the same animal. Our method is useful since some marine crabs remain dormant most of the time with a short active period (e.g. 90 days, Katz 1980). Our experiment has monitored the changes in $\delta^{13}\text{C}$ of benthos respired CO_2 and CO_2 production when they are fasted or fed on leaf litter/microphytobenthos to reflect their active and dormant status. Some species of aquatic migratory species occupy intertidal habitats during specific seasons of the year. Our experiment has shown the changes in $\delta^{13}\text{C}$ of benthos respired CO_2 and CO_2 production under different feeding regime to reflect their changes in food during migration. CRDS can be replaced by alternatives which can fulfil the same objective. For example, gas samples can be collected in sealed vials and analysed via a gasbench/gas chromatography coupled with Isotope Ratio Mass Spectrometer. We have shown the comparison of $\delta^{13}\text{C}$ of CO_2 respired by crabs with classic tissue isotope analysis but found no significant difference between them (Line 235-7). We shall revise the text to strengthen the description on the advantage and repeatability of our methods.

2. I am not sure how to interpret the experimental design from lines 132-139, but it seems that fauna were kept in a 800-mL container that was covered with punctured aluminium foil. Air samples (30-mL) from the container were taken at several sequential time points. I may be wrong, but from this I understand that each sample extraction will 'suck in' ambient air into the container, which will dilute the produced CO_2 in the container. How did the authors correct for this or did I misunderstand something here?

C3

Response: The small hole (diameter: 2mm) on the lid is designed to keep the pressure balance between the inside and outside of the containers but will not result in abrupt air exchange. The small hole used for ventilation has been demonstrated in the previous studies (e.g. Carleton et al. 2004).

3. Overall, the experimental procedures are not well described. I found it difficult to reconstruct exactly how the different experiments were conducted, why some organisms were starved, whether starved was considered similar to dormant (not the same in my opinion, but see line 128-129), how many replicates were done etc. In addition, the experiments are 'numbered' in the Materials and Methods section for clarity, but this numbering is not followed in the Results section, so linking results to methods is cumbersome.

Response: We thank the reviewer for reminding us of describing the experiments and results more clearly for repeatability. Some animals were starved to simulate the dormant status under which they would not feed. There are 15 replicates for each group in experiment 1, 10 replicates for each group in experiment (2), 15 replicates for each group in experiment (3). We'll supplement this information in the revised version and rearrange the results, corresponding to the numbering in the Materials and Methods section. See our response to the reviewer's overall comments.

4. CH_4 measurements were conducted (line 160) but the data are not presented.

Response: We have responded to this query in response to the specific comment 5) of reviewer #2.

5. Also CO_2 production of differently sized organisms is measured, which gives the rather trivial (yet very useful for doing respiration budget studies) relation of increasing CO_2 production with body size. Many authors have used and use the cheaper and easier method of continuous measurements of oxygen concentration of submerged organisms in an incubation chamber. I would really like to read what the complex CRDS measurements offer in addition to providing straightforward respiration data.

C4

Response: The measurement of CO₂ production of different organisms is useful if combined with population structure and/or size distribution of the benthos to determine how much marine fauna contribute to CO₂ effluxes from mangrove forests. Our method measures CO₂ production from organisms in mangroves, which are emersed during low tides. There are alternatives for our instrument as indicated in our response to the reviewer's major concern 1. If the purpose is just to measure CO₂ production of organisms, cheaper options (e.g. infrared gas analysers) can be applied. We shall state these points in the revised version.

References Carleton, S. A., Wolf, B. O., & Del Rio, C. M. (2004). Keeling plots for hummingbirds: a method to estimate carbon isotope ratios of respired CO₂ in small vertebrates. *Oecologia*, 141(1), 1-6. Katz, L. C. (1980). Effects of burrowing by the fiddler crab, *Uca pugnax* (Smith). *Estuarine and Coastal Marine Science*, 11(2), 233-237.

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