

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

### **Anonymous Referee #1**

Received and published: 17 December 2020

In this manuscript, Ouyang et al. present an interesting method to measure the  $\delta^{13}\text{C}$ - $\text{CO}_2$  production of various intertidal species using Cavity-Ring Down Spectroscopy (CRDS). The authors aim to link the  $\delta^{13}\text{C}$ - $\text{CO}_2$  production to the food sources of these species and advocate the concept "You breathe what you eat".

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  $\text{CO}_2$  and  $\delta^{13}\text{C}$ - $\text{CO}_2$  production that can be 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

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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.

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  $\delta^{13}\text{C}$ - $\text{CO}_2$  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  $\delta^{13}\text{C}$ - $\text{CO}_2$  isotope values.

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  $\text{CO}_2$  in the container. How did the authors correct for this or did I misunderstand something here?

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,

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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.

4. CH<sub>4</sub> measurements were conducted (line 160) but the data are not presented.

5. Also CO<sub>2</sub> production of differently sized organisms is measured, which gives the rather trivial (yet very useful for doing respiration budget studies) relation of increasing CO<sub>2</sub> 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.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2020-424>, 2020.