Interactive comment on "The silicon isotopic composition of choanoflagellates: implications for a mechanistic understanding of isotopic fractionation during biosilicification" *by* Alan Marron et al.

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The manuscript "The silicon isotopic composition of choanoflagellates: implications for a mechanistic understanding of isotopic fractionation during biosilicification" authored by Marron et al. investigates the silicate [DSi] uptake fractionation factor for two choanoflagellate species. As this is important work to better understand the marine Si cycle and a first step in using choanoflagellates as a paleoproxy for past [DSi] utilization, I recommend its publication in Biogeosciences. However, I am not convinced about the way, the authors calculated the fractionation factor (ε), which deviates

from previous culture studies in diatoms and therefore is not comparable. Further-more, clarifications in terms of the experimental set-up are necessary. A re-calculation of the fractionation factor might have great importance on the discussion, I consider this manuscript as major revisions. Please see detailed comments below

Major comments:

1) Calculation of the fractionation factor (ε):

The equation (3) is adapted from Varela et al., (2004), which is a field study estimating ε via a Raleigh system approach. Even though this is not necessarily incorrect, the authors should use the same equation, that was used in previous diatom culture studies (e.g. de LaRocha et al., 1997; Sutton et al., 2013) in order to make it comparable to the diatom fractionation factor. This will shift the fractionation factor to significantly lower values, which has a strong influence on the discussion and comparison with diatoms and sponges.

In several parts, the authors use ε for the fractionation factor in sponges. Isn't that only defined as Δ (apparent fractionation factor)? ε only equals Δ in steady-state systems or in case $\delta30BSi$ represents the instantaneous BSi in the Rayleigh model approach. This has to be considered comparing ε in diatoms with Δ in sponges.

This part is very important, as this is the first study on fractionation factors in choanoflagellates and it is necessary to calculate the fractionation factor correct and comparable to other systems!

2) Experiment set-up.

How long were the Choanoflagellates adapted to their new growth "medium" or were they grown in the same medium before the start of the experiment (with the same d30Dsi composition?)

How are growth rates? According to your study, the choanoflagellates only took up 4% of the DSi, which is more or less within the error of your DSi measurements. As this

is a fairly low amount, I was wondering, if they might use up previously-stored DSi (not sure, if they do that) for biosilification!

In line 17 (P5) you mention, that d30DSi was measured before and after, but I do not find these values!

Minor comments:

P1, L10: it should say -0.5 to -2.1 ‰ as the fractionation factor of Chaetoceros is -2.1 (Sutton et al., 2013)

P1, L16: the sentence is imprecise: The Si cycle does not only describe the transport of Si into the biosphere, also vice versa (otherwise it wouldn't be a cycle). Furthermore, it also describes the geochemical cycling of DSi, e.g weathering, vs. inverse weathering. Please rephrase.

P1, L20: Not all diatoms have "heavily" silicified shells.

P2, L 11: The study by Meyerink et al., 2019 actually shows that there might be an influence of iron on the fractionation factor.

P2, L 12: By now, there are many more culture studies about diatoms besides de LaRocha et al., 1997 and Sutton et al., 2013, eg. Milligan et al., 2004 Sun et al., 2014 and a mesocosm study by Meyerink et al., 2019

P2, L 13: Isn't it a linear relationship, otherwise it would not work as proxy?

P3, L3: delete the word "vital", this is at term geologist use, if they do not know what is happening and they are confused by biology.

General: The authors do not discuss the different fractionation factor between both species, possibly growth rate?

It would be nice to have some more information (maybe two sentences) about habitat of choanoflagellates (marine, brackish, freshwater (which species did you use); water

depth, pelagic, benthic, importance in paleo records (Where, when?)

Best, Patricia