General Comments

The authors present a compelling argument for a strong kinetic fractionation effect on the $\delta^{18}O$ values of freshwater sponge spicules from one pond, Lagoa Verde, in Brazil. As the authors indicate, there have been very few studies of $\delta^{18}O$ variations in spicules and essentially no published records of $\delta^{18}O$ variation in freshwater sponges. Therefore, this manuscript is timely and has great potential to advance our understanding of silica-water fractionation by freshwater sponges during spicule formation. To test the relationship between the $\delta^{18}O$ values of freshwater sponge spicules and the water in which the sponges grew, the authors have implemented a monitoring study in which they can assess the growth of the spicules and other parameters (i.e. water $\delta^{18}O$ values and temperature). Although there are a number of assumptions about variations in the water temperature and $\delta^{18}O$ values, the authors have attempted to quantify these variations. The site selection and monitoring seems appropriate for the scope of the study and the field measurements are adequate. Ideally, a more complete sampling campaign would be undertaken to assess the variations in pond water $\delta^{18}O$ values and temperature. Specifically, it not clear when the spicules grew and what exactly they are recording. The authors have also demonstrated that other parameters, particularly the amount of dissolved silica and sponge growth, might have a dramatic effect on the $\delta^{18}O$ values of the spicules.

The authors conclude that the $\delta^{18}O$ variations observed in sponge spicules in this study are significantly affected by kinetic/biologic fractionation and the spicules do not form in equilibrium. Although the data presented in this manuscript support this conclusion, there are a number of problematic assumptions that make it difficult to summarily accept their results as conclusive, including: 1) methodological bias, 2) timing of spicule growth, and 3) water T and $\delta^{18}O$ values that the spicules are recording. I would stress that because of these assumptions, these data do not seem to conclusively support the authors assertion that "this study provides clear evidence that the freshwater sponge *Metania spinata* does not form it siliceous spicules in oxygen isotope equilibrium" with the ambient water.

There are a number of ways in which the manuscript could be strengthened, but I have **major concerns about the $\delta^{18}O$ values used in this study** (see specific comments below). The details of the methodological bias are not addressed in the manuscript (external citations do not clarify the bias, either). Regardless of the observed relationships between the measured $\delta^{18}O$ spicule values and the water in which the spicules grew, the potential for methodological bias undermines all potential results. Unless the authors can identify the source of the methodological bias, there is no way to "quantify" the bias and demonstrate that all samples (within this study and different types for silica) respond equally. **Unless the authors can adequately address the source of the methodological bias in $\delta^{18}O$ measurements, I cannot recommend this manuscript for publication.**

Specific Comments

Methodological Bias:
The authors discus a correction for methodological bias (12894, line 4) and cite a manuscript (Alexandre et al., 2012) that discusses this methodological bias. In the interactive comments, the authors have continued to explain the use of the correction factor for methodological bias so that the $\delta^{18}O$ values internal standards align with the $\delta^{18}O$ values of the interlaboratory comparison study (Chapligin et al., 2011). The effects of the methodological bias are not adequately discussed in the manuscript.

The authors indicate that the cause of the methodological bias is not explained. This is problematic for any $\delta^{18}O$ values reported, but particularly for sample types that have not been analyzed by other analytical techniques (e.g. the freshwater sponge spicules used in this study). There seems to be no indication that the methodological bias is expected to alter all samples in exactly the same way. The authors need to identify the source of the methodological bias before embarking on a calibration study where large analytical uncertainty can significantly affect the calculation of the silica-water fractionation factors. For example, the authors indicate that a standard deviation of 0.5 to 1.8‰ is observed in the spicule $\delta^{18}O$ values for a give month (12897, Line 17-18). In the interlaboratory comparison cited by the authors (Chapligin et al., 2011) the $\delta^{18}O$ values reported by the CEREGE group were all outliers and varied by several pemil. If the empirical relationship used to correct the values has not been independently verified for different amounts of exchangeable oxygen (i.e. different silica types), it is entirely possible that the entire range of standard deviations reported in freshwater spicules measured in this study is compounded error associated with the analytical procedure and methodological correction.

Silica formation
(12889, Line 16: “in an enzymatic way” and discussion on 12899) – This is an interesting discussion on the differences between silica formation in sponges and other biogenic silica like diatoms. I would like to see the authors clarify these differences and the timing of the spicule growth. (12899, line 1). There seems to be a high correlation (R^2=0.80) with the latest water $\delta^{18}O$ values. To me, this would indicate that the spicules are not reflecting average growing conditions and/or temperatures. The authors indicate that spicules grow in “several tenths of hours.” For a real calibration study to occur, there needs to be a more rigorous exploration of when spicule growth occurs and measurements of the water $\delta^{18}O$ values and temperature during spicule growth.

12895, Line 6: “Assuming that this relationship is constant over the course of the day…” This assumption seems like a relatively sophisticated way to reconstruct monthly mean water temperature, but the authors do not adequately demonstrate that it works. When attempting to quantify the fractionation relationship between sponge spicule $\delta^{18}O$ values and pond water $\delta^{18}O$ values at specific temperatures, these assumptions can be very problematic. Was there any temperature heterogeneity in the water body during the day?

12897 Lines 1 – The authors present an excellent assessment of potential variation in the $\delta^{18}O$ values of the water and the water temperature. The authors noted that the pond was in karstic bedrock. What is the contribution to the lake from groundwater? Is this evenly mixed throughout the lake? What is the potential error/variation in temperature and how does that affect the silica-water fractionation relationship?

Measured vs. Reconstructed water values and temperature –Throughout the manuscript the authors discuss how temperature and $\delta^{18}O$ values of the lake were "reconstructed"; however, the $\delta^{18}O$ spicule values demonstrate a higher correlation with the measured water temperature and measured $\delta^{18}O$ values. The reconstructed and weighted water temperature has a slightly higher correlation (R^2=0.79 for reconstructed-weighted T; R^2=0.77 for measured). It is not clear why the authors have gone to such lengths to reconstructed the water temperature and water $\delta^{18}O$ values rather than use the measured values. This becomes a significant problem when constructing the silica-water fractionation relationships, as it seems to indicate that the authors do not know exactly what temperatures and water values to use for this relationship. If the spicules were growing throughout the month, why would it be most strongly correlated with the final $\delta^{18}O$ values? It seems unlikely that the spicules, which are much more dense than other biogenic silica like diatoms, would not record the $\delta^{18}O$ values throughout the entire period of growth.

Conclusion – Generally, I disagree with the authors' conclusion that their results "prevent the use of $\delta^{18}O_{silica}$ values from the spongillites of northwestern Minas Gerais as a direct proxy for past $\delta^{18}O_{water}$ and/or temperature changes." The data appear to support a potential kinetic fractionation, but the $\delta^{18}O_{water}$ values and temperature at the time of spicule growth need further exploration. Lastly, the methodological bias analytical uncertainties need to be clarified to conclusively rule out analytical error as the primary source of variation in the $\delta^{18}O$ values of the silica.

<u>Technical Corrections</u>

12893, Line 26: Chapligin et al., 2011 does not seem to be the most appropriate citation for your $\delta^{18}O$ method.

12894, Line 3: Minor comment, but if the precision is ± 0.1‰, then the $\delta^{18}O$ value should be reported as 16.3‰ not 16.284‰. This is true for the rest of the $\delta^{18}O$ values reported in the remaining lines in this section and throughout the paper. The authors should be consistent with their significant figures. Generally, the precision of $\delta^{18}O$ measurements is not better than ± 0.1‰.