Matteuzzo et al. manuscript "Assessing the relationship between the d18O signatures of siliceous sponge spicules and water in a tropical lacustrine environment (Minas Gerais, Brazil)"

Supplementary material : Point by point answers to comments from Anonymous Referee #1 and corrected draft (with modified section in blue).

General comments

Corrected d18Osilica values: This is my first concern for this study. It seems that the applied correction functions are partly reliable for the shown trends (cf. Table 1, column d18Osilica vs. corrected d18Osilica measured). As most of the result and the discussion section is based on thevalues from corrected d18Osilica measured and as this method of corrections has large uncertainties the results are questionable (see comments for the sections "Methods" and "Results"). At least the uncertainties should be mentioned and the theoretic results for the uncorrected data should be discussed and if they provide a similar picture.

This correction was previously discussed in Chapligin et al., 2011 and Alexandre et al., 2012. Although this methodological bias remained unexplained, it is reproducible and could thus be quantified. As pointed out by referee #1, this correction can lead to large uncertainties (Chapligin et al., 2011), although its consistency was verified on independent datasets (Alexandre et al., 2012). In the present case, the simulated uncertainty (calculated using Monte Carlo simulation with the R software) on final corrected $\delta^{18}O_{silica}$ values ranges from 0.5 and 0.8 ‰ (cf Table 1 of the corrected draft).

Moreover corrected $\delta^{18}O_{silica}$ values are linearly correlated with measured $\delta^{18}O_{silica}$ values (corrected $\delta^{18}O_{silica}$ =1.006 * measured $\delta^{18}O_{silica}$ -2.96; r^2=0.96). Consequently, the methodological bias correction is not responsible for the occurrence or absence of relationship found between $\Delta^{18}O_{silica\ -\ water}$ values and water temperature values. When using measured $\delta^{18}O_{silica}$ values instead of corrected $\delta^{18}O_{silica}$ values, as suggested by referee #1, there is still no relationship between $\Delta^{18}O_{silica\ –\ weighted\ water}$ and weighted temperature (r^2=0.03 instead of 0.02 with the corrected $\delta^{18}O_{silica}$ values).

As advised by referee #1, these clarifications were underlined in the methodological section (L 141) and in the results section (L222) of the corrected draft.

The second concern is that the pond is just not ideal for the investigation of temperature isotope relation. For example the authors admit that "significant variations in the pond water temperature [during the day] can be expected" with such a shallow water level and "atmospheric temp variations during the day >10°C". More data from different, more stable ponds would help here to underline the results.

Indeed large daily variations of the environmental parameters may be smoothed by reconstructed averages. This is a drawback which is further underlined in the corrected draft (L242-246). However, as noted in the GBD paper, we checked that although modest, there is still a positive correlation between the reconstructed monthly mean water temperature and water temperature measured at midday (r^2=0.5). Moreover, uncertainties on reconstructed values of water temperature and $\delta^{18}O_{water}$ do not put into question the positive relationships obtained between $\Delta^{18}O_{silica\ -\ water}$ and water temperature when measured data are considered. This excludes that any isotopic equilibrium has been reached and prevents 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 (L246-254 of the corrected draft).

The pond issue leads to the third bigger concern: The authors conclude that due to spicule forming in non-equilibrium conditions this "prevents the use of d18Osilica for reconstructing past d18Owater and temperature changes". This conclusion (mentioned in Conclusion and in a reduced form with a restriction on the locality in the Abstract) is a major aspect of the paper and has to be discussed, and I think partly withdrawn/limited. If there is a clear correlation between d18Osilica (D18Osilica-water) and twater and the latest d18Oprecipitation "imprint" which can be seen in the spicules why should fossil sponge spicules in the sediment not be used as a proxy (not on a monthly base, of course)? I would not use it for this pond, but due to the extremely variable hydrology of this pond I would not recommend to perform a paleoclimate reconstruction on the sediments from this pond from many other proxies anyways. So, in my opinion mainly the hydrology here is responsible for this formulated prevention and the prevention should be formulated as in the abstract or even better with a focus on the limited suitability of this pond.

Cf previous answer. Also the following sentence was added in the conclusions of the corrected draft (L297): "In order to further assess the parameters responsible of the $\delta^{18}O$ imprint in lacustrine sponge spicules, additional calibration experiment are needed, using a single species grown under laboratory controlled conditions of $\delta^{18}O_{water}$, water temperature, dissolved Si and nutrient concentration.”

All $\delta^{18}O_{silica}$ values and $\Delta^{18}O_{silica-water}$ values should be shortened to one digit, for all d18Owater values it is enough to show two digits.
This was modified in the corrected draft (Tables and figures).

The article is voluminous in tables (4) and figures (8) showing partly redundant data (e.g. Table 3 vs. Fig. 3). I recommend shifting Table 2 and 3 to supplementary data .
Tables 3 and 4 were shifted to supplementary data : Tables S1 and S2 of the corrected draft. Table 2 was left as it shows data important for the understanding of the text (e.g. $\Delta^{18}O_{silica-reconstructed\ water}$).

and reduce Fig. 5/6/8 to one Figure with measured data only to further sharpen the focus.
Modified in the corrected draft : fig. 5 and 6 are merged. Fig 8 is left alone (becomes Fig. 7) as it deals with Δ^{18}Osilica-water, not with δ^{18}Osilica (Fig. 5)

Detailed comments for each section/subsection

Title: The title matches the content of the article. However, I recommend a slight adaption as using the delta signature in the title always causes problems when citing etc. I recommend using "oxygen isotope signature/composition" instead.
Modified as suggested in the corrected draft

Abstract
Modified as suggested in the corrected draft

Introduction

The last paragraph is too long and can be shortened.
Modified as suggested in the corrected draft

More detailed thoughts should be given to the different fractionation factors and temperature relations as this is the major topic of the article. Be precise: Most articles agree to the temperature relation of -0.2‰/°C for lacustrine environments. For marine there is still a discussion between -0.2‰/°C and -0.5‰/°C. Differ between the studies on recent and fossil material if mentioning the quartz fractionation as well. Add the recent article by Dodd et al. (2012).
We believe a long discussion on fractionation factors and fractionation coefficients obtained for the different silica/water couples is not in the scope of the present paper. Particularly, clustering thermo-dependant relationships into lacustrine and marine is not straightforward. A complete discussion on that matter was already published by some of us as underlined in the BGD draft (synthesis Alexandre et al., 2012). We added Dodd et al., 2012 to the list of references dealing with this topic.

Delete or shift the last sentence up as it blurs the well formulated aims of the last paragraph.
Modified in the corrected draft, as suggested.

Materials and Methods

Some information can be deleted which is not essential to sharpen the focus of this article, e.g. the introductory sentence about spongillites (leave out the references, too) as well as the footprints of animals around the lake.
Modified as suggested in the corrected draft

Figure 1 : Modified as suggested in the corrected draft

For a better overview in the text: mention all taken samples according to the numbers.
Modified as suggested in the corrected draft

From which depth were the water samples collected? Were these the samples for isotope analysis?Why did you chose to measure water temperature in 20cm depth? The sponges were placed on the floor of the pond which is 1.6 to 3m according to section 2.1. and 0.65 to 2.05m acc. To Table 1. As we look at a big evaporative "pan" the temperatures as well as the isotope values might be quite different on the bottom of the pond (if taken at that depth, too).
Precised in the coreected draft (Fig. 1c and L114-115)

Analytical methods
Cf previous answers of general comments.

Reconstruction of temperature and δ18O water conditions over annual cycles of sponge proliferation
Equation of the water balance was added to the corrected draft.
δD values were added in Table S1, mentioned in the methods section and presented in the results section of the corrected draft : L171 : « In a δ^{18}O-δD diagram, the water samples are aligned on an evaporation line with a slope lower than 8 ($\delta^{18}O = 6.58\ \delta D - 10.94$; $r^2 = 0.96$). »

Sponge growth coefficient: I am not a sponge growth expert, and I might have missed this if any calculation has been performed here, but it might help not to just adapt the numbers/Eq.4 from the Melao and Rocha (1999) for Lagoa Dourada but to add a calculation with own data from the counts (Table 2, Fig. 2, Fig. 3) in Fig. 4 (top cart) and see if it roughly matches the data from Melao and Rocha (1999).
The sponge growth coefficient was calculated from the data obtained by Melão and Rocha, 1999. As we do not have any AFDW measurements we cannot calculate our proper coefficient. However, a similar trend was expected at lagoa verde as both the sponge species and rainfall distribution are similar.

Results

Water level/water temperature/d18Owater: these results are mixed up and should be presented in the order above as suggested by the authors in their introductory sentence.
This paragraph was reorganized as suggested in the corrected draft

On the uncertainty of the reconstructed parameters :
Cf previous answers.

Figures were modified as suggested: Fig 5a, 6a and 8a were gathered as suggested Figures 5b and 5c were shifted in supplementary data S4

On corrected d18Osilica values: Cf previous answers
Equilibrium thoughts: This paragraph might better fit into the discussion section. The gained results are already presented at this point. I would not say "conversely" as the other studies dealt with minerals, quartz and mainly fossil phytoliths and diatoms.
Moved as required

Discussion

The paragraph on biologically controlled mineralisation was left in the discussion section as we believe it is critical for explaining how the latest precipitation gives its d^{18}Oprecipitation “imprint” to the entire spicule assemblage.

Hypothesis that the latest precipitation gives its δ^{18}O imprint to the entire spicule assemblage is presented as an hypothesis.
As previously noted sentence was added in the discussion section of the corrected draft (L297).

Conclusions
Modified in the corrected version as suggested.

References : corrected

I recommend taking not ten but only the most important publications from Volkmer-Ribeiro,C. et al. :
modified

Check references for consistency: leave doi, use official abbreviations for journal names (Geochim. Cosmochim. Acta; Earth Planet Sci. Lett., etc.) : modified

Tables and Figures :

Table 2 : The parameters' indices are partly wrong. Check which equation has been used for each parameter. Corrected

Table 4 (devient S2) : Reduce the number of digits for δ18Osilica−weighted water values to one.
Corrected

Figure 1 : Increase size of brasil (left). Leave out middle magnification. Show lake/pond close-up with (changing) bathymetry and sampling points / transects differing between natural and artificial samples. Modified

Figure 2 : From which samples are these four images from? Same sample, different samples, why different resolution? Corrected

Figure 4
- for clarification in the chart description use a) to f) as in other figures, to be used in the text and in the caption.
- y-axis labels: delete "reconstructed" for the water levels and water temperature as reconstructed as well as measured values are shown.
- x-axis label: delete the first three months as there is no data displayed.
- legend: shift some descriptions to the right (tab) for better reading
- caption: introduce charts from top to bottom
Modified as required.

Figure 5, 6, 7, 8 : Align y-axes of charts, apply same point size for data points, use same scale for x-axes. Modified as required.

Assessing the relationship between the oxygen isotope compositions of siliceous sponge spicules and water in a tropical lacustrine environment (Minas Gerais, Brazil)

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Abstract

Previous attempts to use the oxygen isotopic signature of lacustrine sponge spicules ($\delta^{18}O_{silica}$) as a paleoenvironmental proxy have led to contradictory conclusions. Whether sponges form their silica in oxygen isotopic equilibrium with water, or not, is still to be demonstrated. For this reason, we measured the $\delta^{18}O$ signature of sponge spicules from a single freshwater species (*Metania spinata*) grown on natural and artificial supports over nine months in a small Brazilian pond (Lagoa Verde, northwestern Minas Gerais). The $\delta^{18}O_{silica}$ values were obtained using the infrared (IR) laser-heating fluorination technique following a controlled isotopic exchange (CIE). The $\delta^{18}O$ values ($\delta^{18}O_{water}$) and temperature of the pond water were periodically measured and reconstructed over the course of the sponge growth. A correlation was obtained, with a positive coefficient of 0.3‰°C^{-1} (R^2=0.63), when $\delta^{18}O_{water}$ values and water temperature at the time of sample collection were considered. This result clearly indicates that the freshwater sponge *Metania spinata* does not form its siliceous spicules in oxygen isotopic equilibrium with the pond water and prevents 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. This result also reveals that the latest step of silica formation gives its $\delta^{18}O$ imprint to the entire spicules assemblage, which suggests that one or several biologically controlled kinetic fractionation mechanisms may be in play during the various steps of silica formation.

1. Introduction

Siliceous sponges are filter-feeding animals structured on three-dimensional arrangements of siliceous spicules with lengths of hundreds of micrometres (Demospongiae and Hexactinellida classes) to several meters (Giant Hexactinellida) (Uriz et al., 2003; Wang et al., 2009). These animals have proliferated since the Neoproteozoic at various latitudes in marine and fresh waters (Volkmer-Ribeiro and Pauls, 2000; Müller et al., 2007; Love et al., 2009). In freshwater environments, the spicule assemblages are often used as indicators of changes in water chemistry and budget (Hall and Herrmann, 1980; Turcq et al., 1998; Sifeddine et al., 2001; Volkmer-Ribeiro et al., 2004; Parolin et al., 2008; Machado et al., 2012; Silva et al., 2012). In contrast to diatom frustules, in which silica is deposited from a saturated solution onto organic templates, siliceous spicules in sponges are formed in an enzymatic way (Schroder et al., 2007; Muller et al., 2009; Wang et al., 2012a).

Numerous studies recently indicated the paleoenvironmental usefulness of the oxygen isotope composition ($\delta^{18}O$) of biogenic siliceous particles. These studies demonstrated that the isotope signature of silica ($\delta^{18}O_{silica}$) from diatom frustules and plant phytoliths was dependent on the $\delta^{18}O$ signature of the forming water ($\delta^{18}O_{water}$) and temperature (Juillet-Leclerc and Labeyrie, 1987; Shemesh et al., 1992; Shahack-Gross et al., 1996; Brandriss et al., 1998; Moschen et al., 2005; Crespin et al., 2010; Webb and Longstaffe, 2000;

Alexandre et al., 2012, Dodd et al., 2012). Although the obtained fractionation factors differed significantly from one study to another, the associated temperature coefficients ranged from -0.2 to -0.5‰°C^{-1}, close to the quartz fractionation coefficients measured previously (Clayton et al., 1972; Matsuhisa et al., 1979; Sharp and Kirschner, 1994). Regarding sponge spicules, few investigations led to contradictory interpretations. Matheney and Knauth (1989) found a scatter of the fractionation between water and silica ($\Delta^{18}O_{silica\text{-}water}$) as high as 5‰ for a given seawater temperature in marine assemblages collected from Caribbean and Pacific sites. These authors concluded that the sponges precipitate their spicules in isotopic disequilibrium with seawater oxygen, implying a kinetic fractionation mechanism. However, their data trend (-0.2%°C^{-1}) was in the range of equilibrium fractionation coefficients measured later for various silica-water couples (synthesis in Alexandre et al., 2012). More recently, five modern $\Delta^{18}O_{silica\text{-}water}$ values obtained from an analysis of seawater sponge spicules and one value obtained from the analysis of freshwater sponge spicules were plotted against water temperature (Jochum et al., 2012). The values were scattered and displayed no significant correlation with temperature. However, this result was interpreted as reflecting differences in the species characteristics and/or changes in the isotopic composition of the waters where the organisms lived. The $\delta^{18}O_{silica}$ values obtained from a unique giant deep-sea sponge specimen were finally interpreted as changes in seawater temperature during the growth of the organism. The technique used for exchangeable oxygen removal (melting under an infrared (IR) laser beam with no fluorinating agent and in a vacuum) prior to $\delta^{18}O_{silica}$ analyses was not evaluated by the recent inter-laboratory comparison of oxygen isotope compositions from biogenic silica (Chapligin et al., 2011). This scarcity of $\delta^{18}O_{silica}$ data indicated the need to further investigate whether siliceous sponge spicules form in oxygen isotopic equilibrium with water and can be used as a proxy of past $\delta^{18}O_{water}$ composition.

In this study, we measured the $\delta^{18}O$ signature of *Metania spinata* (Carter, 1881) spicules formed over two annual cycles, on natural and artificial supports, in a small pond in northwestern Minas Gerais (Brazil). The correlations obtained between $\delta^{18}O_{silica}$, $\delta^{18}O_{water}$, and water temperature values were assessed.

2. Materials and methods

2.1. Study area

Large spongillite formations of Pleistocene and Holocene age have been reported in southeastern Brazil (Volkmer-Ribeiro et al. 1998). In northwestern Minas Gerais, more than 80 lens-shaped spongillites that are hundreds of meters in diameters and several meters thick lie beneath ponds where sponges proliferate (Almeida el al., 2009, 2010). One of those ponds, Lagoa Verde (17°42'16"S; 46°23'32"W; 572 m a.s.l.), was investigated for the present calibration.

The pond is located on an 85 km^2 karstic planation surface covered by Cenozoic siliciclastic sediments, including the spongillites (Almeida et al. 2010) (Fig. 1a). The climate is tropical humid with a five-month dry season during the winter (from May to September). The mean annual temperature is 23.2ºC, and the mean annual precipitation is 1,562 mm (INMET, 1961-1990 climate normals, platform Goiânia). The summer rains are convective and related to the southern shift of the Intertropical Convergence Zone (ITCZ) and the influence of the maritime tropical air mass (mT) (Tubelis and Nascimento, 1992). However, in January and February, strengthening of the South Atlantic Subtropical Anticyclone (SASA) typically leads to an Indian summer on the continent (*Veranico*). This weather is characterized by a strong reduction or absence of precipitation, low atmospheric humidity, and high temperature. The regional vegetation is a wooded savanna known as *cerrado* (Veloso et al., 1991), locally subjected to human disturbances (*eucalyptus* plantation, livestock, and mining).

Lagoa Verde has a radius of 0.265 km, a perimeter of 1.8 km, and a surface area of 2.2 km^2. The volume of the pond reached 6.72 10^5 m^3 in March 2011, at the end of the rainy season. In 2011, the water depth in the deepest part of the pond was approximately 3 m but was drastically reduced by at least 1.6 m by the end of the dry season (September). The local vegetation is dominated by the aquatic macrophyte *Eleocharis interstincta* and shoreline grasses, onto which specimens of the *Metania spinata* sponges can attach. Although *Metania spinata* is the unique species observed in Lagoa Verde, it belongs to a group of six sponge species that dominates spongillites and current pond waters in southeastern Brazil and are common to the South American inter-tropical area (Volkmer-Ribeiro et al., 1998; Almeida et al., 2009).

2.2. Sampling and field measurements

Natural sponge samples were collected on grasses at the margin of the pond at the end of the 2010 dry season in August (LV01, 02, 03; Table 1). In September 2010, reproductive structures (called gemmules) of *Metania spinata* sponges were placed in black tulle bags (40×40 cm) and deposited on the floor of the pond along two transects that were approximately 25 m long from the margin to the centre (Fig. 1b and 1c). The bags from each transect were collected at the end of the following wet season. These bags were given designations based on their latest month of growth (March 2011: LV 04, 05, 06; April 2011: LV 09; Table 1). In addition, natural sponge samples were collected on emergent aquatic macrophyte *Eleocharis interstincta* (LV 07, 10, 11 and 12; Table 1) and on submerged sediment (LV08; Table 1). An empty bag was placed on the floor of the pond in May and collected at the beginning of June 2011 (designated Control May 2011: LV13; Table 1).

Five water samples were collected for isotopes and dissolved Si analyses at 12:00 PM (UTC/GMT –3 hours), at 20-30 cm depth, on the days of sponge sampling. Three additional water samples were collected during the period of sponge growth, in September and November 2010 and January 2011 (Table 2). The water level and temperature were measured at 20 cm depth during the sampling campains (Table 1).

Monthly and daily values of atmospheric temperature, precipitation, and evapotranspiration were obtained from the INMET Platform Data Collection #83481 (João Pinheiro), located 21 km east of the site.

2.3. Analytical methods

The spicules were extracted using a three-step chemical protocol commonly used for phytoliths and diatoms (Crespin et al., 2010) that was adapted for sponges as follows: (1) oxidation of organic matter using H_2O_2 (30%), HNO_3, and HNO_3+$HClO_4$ (2:1); (2) clay removal by sedimentation and centrifugation; (3) densimetric separation of the spicules using a 2.3 heavy liquid ($ZnBr_2$). Step 1 was carried out at 50°C (Crespin et al., 2008) and repeated until the organic matter was completely oxidised. The purified spicules were mounted on microscope slides using Entelan® and analysed at 100× magnification. Approximately 300 spicules from each sample were counted and classified as alpha megascleres, beta megascleres, microscleres, and gemmoscleres.

The water samples were stored in amber bottles sealed with paraffin for isotopic analyses. These samples were filtered at 0.45 µm and acidified for dissolved Si analyses. The dissolved Si concentration was measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) at University Federal of Ouro Preto (UFOP).

The $\delta^{18}O$ analyses of the sponge spicules and water samples were processed at CEREGE. The $\delta^{18}O$ values were expressed in the standard δ-notation relative to V-SMOW. Because sponge spicules are made of hydrous amorphous silica, the ground samples were subject to a controlled isotopic exchange (CIE) to set the $\delta^{18}O$ value of exchangeable oxygen (Chapligin et al., 2011). Oxygen extractions were then performed using the IR laser-heating fluorination technique (Alexandre et al., 2006). No ejection occurred. The oxygen gas samples were sent to and analysed using a dual-inlet mass spectrometer ThermoQuest Finnigan Delta Plus®. The measured $\delta^{18}O$ values of each sample were corrected on a daily basis using a quartz lab standard ($\delta^{18}O_{\text{Boulangé 50-100µm}}$=16.3±0.1‰, n=18). Effectiveness of the CIE was checked by verifying that values obtained for one to three aliquots of the phytolith lab standard MSG 60 were always in the standard deviation of the mean value measured during a long term calibration (Crespin et al., 2008). The calculated $\delta^{18}O_{\text{silica}}$ value was corrected for a reproducible methodological bias. The simulated uncertainty on final corrected $\delta^{18}O_{\text{silica}}$ values ranged between 0.5 and 0.8 ‰ (calculated using R software; Table 1). The entire analytical and correction procedure has been previously described in detail (Crespin et al., 2010; Alexandre et al., 2012). During the analysis period, measurement of the international quartz standard (NBS 28) yielded an average of 9.8±0.1‰ (n=8). Replicate analyses of the sponge spicules samples yielded a reproducibility better than ±0.4‰ (1σ).

$\delta^{18}O$ and δD values of the water samples were measured using an automated Thermo-Finnigan equilibrating unit connected to a dual inlet Delta Plus mass spectrometer. Reproducibilities of $\delta^{18}O$ and δD analyses were respectively ±0.05‰ and ±1‰ (1σ).

2.4. Reconstruction of temperature and $\delta^{18}O_{water}$ conditions over annual cycles of sponge proliferation

Sponges proliferate and form their spicules over annual cycles. Spicules categorised as alpha megascleres and microscleres are produced during the growing phase of a cycle. These spicules sustain the sponge’s siliceous reticulate structure and strengthen its pinacoderm. A degenerative phase follows, particularly marked in shallow water environments, when conditions of hydrous stress occur. This phase is characterized by the production of beta megascleres and gemmoscleres (Volkmer-Ribeiro, 1981).

In Lagoa Verde, the proliferation cycles last nine months. A growth phase occurs from November to April and is followed by a degeneration phase from May to July. The sponge samples collected during a given year thus contain sponges growing since November of the previous year. To assess whether sponges precipitate their spicules in isotopic equilibrium with water over their growth period, the water temperature and $\delta^{18}O_{water}$ values were, as a first step, reconstructed from November 2009 to May 2011 (latest sponge sampling).

- The water temperatures (t_{water}) in Lagoa Verde measured at 12:00 PM were correlated with the atmospheric temperature (t_{atm}) measured the same day and time at the INMET station ($t_{water}=1.1098\times t_{atm}+1.0354$; $R^2=0.7$; $p<0.01$; Eq. (1)). Assuming that this relationship is constant over the course of the day, it was used to reconstruct monthly mean water temperatures from the monthly mean atmospheric temperatures from the database of INMET daily values.

- In a $\delta^{18}O$-δD diagram, the water samples were aligned on an evaporation line with a slope lower than 8 ($\delta^{18}O = 6.58\ \delta D-10.94$; $r^2= 0.96$). The measured values of $\delta^{18}O_{water}$ were correlated with the water levels ($\delta^{18}O_{water}=-0.0536\times$water level$+5.9056$; $R^2=0.8$; $p<0.01$; Eq. (2)). This correlation corresponds to the classical behaviour of lake water isotopic composition (Vallet-Coulomb et al., 2006), in which an evaporative isotopic enrichment occurs during the dry season, whereas the rainy season leads to dilution by isotopically depleted precipitation. We used this relationship to reconstruct the $\delta^{18}O_{water}$ values over the entire period of spicule formation. The water levels in the months without water sampling were calculated based on (1) the water volume measured in March 2011 as an initial V_i value (Table 2), (2) a water balance equation taking into account monthly data of precipitation (P) and evaporation (ETP) (water volume$_i$= water volume$_{i-1}$+P_i– ETP_i; Table 2), and (3) the relationship between calculated lake volumes and measured lake levels obtained from our measurements (water level=0.152×water volume–101,919; $R^2=0.95$; $p<0.01$; Eq. (3)). The $\delta^{18}O_{water}$ values in the months without water sampling were thus reconstructed using a combination of water level estimates and Eq. (2).

In a second step, the water temperature and $\delta^{18}O_{water}$ estimates were weighted using a sponge growth coefficient. The nine-month annual cycle of *Metania spinata* was previously monitored in an artificial reservoir, Lagoa Dourada, located 500 km south of Lagoa Verde, in the state of São Paulo (Melão and Rocha, 1999). The annual precipitation patterns at both sites are similar. The sponge dry biomass, expressed as ash-free dry weight (AFDW), exhibited an exponential trend over time at Lagoa Dourada ($AFDW=8.1497^{0.2748\times\text{number of growing months}}$; $R^2=0.85$; Eq. (4)); a similar trend was expected at Lagoa Verde. The AFDW is equal to the dry weight (DW) minus ash, which is essentially composed of silica, and varied proportionally to the DW (Melão and Rocha, 1999). We thus inferred that the sponge growth coefficient, obtained from Eq. (4) (Table 2), could be used as a proxy for silica formation at Lagoa Verde to weight the estimates of temperature and $\delta^{18}O_{water}$ (Supplementary Table S2).

3. Results

Microscopic observation and counting of the chemically treated spicule assemblages indicated no trace of organic remains. The morphological features and abundance of alpha megascleres, microscleres, beta megascleres, and gemmoscleres are presented in Figs. 2 and 3 and Supplementary Table S1. All of the spicules are devoid of dissolution features (Fig. 2). The percentage of spicule categories per samples exhibit the expected pattern (Fig. 3): Alpha megascleres were dominant during the rainy months of March and April (2011); microscleres appeared at the end of the rainy season, in April (2011); and beta megascleres and gemmoscleres were produced during the degeneration phase in the dry season.

The measured and reconstructed values of the water level, dissolved Si concentration, water temperature, $\delta^{18}O_{water}$ and δD are presented with the meteorological data in Table 2 and Fig. 4. The water level estimates are very close to the measured values. The levels were lower in 2010 than 2011as 2010 was drier, warmer, and thus more evaporative than 2011. The dissolved Si concentration ranges from approximately 2.8 to 10.6 mg l^{-1} and decreases as the pond level rises as a result of dilution (Table 2). There is a modest fit between the reconstructed monthly mean water temperature and water temperature measured at midday. Given the important variability in daily and intra-daily atmospheric temperature (e.g. T varies more than 10°C during a day, INMET database 2010-2011, João Pinheiro), significant variations in the pond water temperature can be expected, which explains the difference between the monthly mean values and those measured at 12:00 PM. The $\delta^{18}O_{water}$ estimates are higher in 2010 than in 2011, as they are based on water level variations. Although both the reconstructed and measured $\delta^{18}O_{water}$ values display the same trends, the water sample collected on 8 January 2011, appears particularly ^{18}O depleted (Δ=2.4‰). This discrepancy may be explained by the high rainfalls that occurred just before the sampling. In fact, 70% of the January precipitation occurred during the first eight days of January, thus leading to a heavy dilution of lake water by isotopically depleted precipitation (low $\delta^{18}O_{water}$ and δD values), whereas the monthly water balance does not account for heterogeneity in the rainfall distribution. In addition, the evaporative isotopic enrichment that occurred during the dry period in February is underestimated by our reconstructions. However, despite smoothing the actual variations, our $\delta^{18}O_{water}$ reconstructions reproduce the seasonal trends.

$\delta^{18}O_{silica}$ values corrected for the methodological bias are linearly correlated with measured $\delta^{18}O_{silica}$ values (corrected $\delta^{18}O_{silica}$ =1.006 * measured $\delta^{18}O_{silica}$ -2.96; r^2=0.96) which excludes that any trend discussed below may be an artifact of this correction. For a given month, the $\delta^{18}O_{silica}$ values are significantly scattered (standard deviation ranged from 0.5 to 1.8‰) (Table 1). This scatter is not related to the type of substrate to which the sponges were attached (e.g., natural vs. artificial or submerged sediment vs. *E. interstincta* macrophyte). The average values of $\delta^{18}O_{silica}$ range from 29.6‰ when the sponges were collected during the dry season (August 2010) to 24.0‰ when collected during the rainy season (March 2011). The $\delta^{18}O_{silica}$ values increase with the $\delta^{18}O_{water}$ values of the latest months of growth, either measured (R^2=0.80; Fig. 5a), reconstructed (R^2=0.75, S3a), or reconstructed and weighted (R^2=0.65, S3b). The $\delta^{18}O_{silica}$ values decrease with the water temperature values of the latest months of growth, either measured (R^2=0.77; Fig. 5b), reconstructed (R^2=0.57), or reconstructed and weighted (R^2=0.79). The $\delta^{18}O_{silica}$ values also increase with dissolved Si concentration, although the correlation is moderate (R^2=0.56). When measured and corrected data are considered, the $\Delta^{18}O_{silica\text{ - }water}$ values display a positive correlation with water temperature (R^2=0.63 Fig. 6a). The associated coefficient is 0.3‰°C^{-1} (Fig. 6a). No correlation is observed when reconstructed or reconstructed and weighted values are considered (Fig. 6b and 6c). When measured $\delta^{18}O_{water}$ values are considered, the $\Delta^{18}O_{silica\text{ - }water}$ values display a moderate negative correlation with the dissolved Si concentration (R^2=0.48; Fig. 7). Again, no correlation is observed when reconstructed or reconstructed and weighted $\delta^{18}O_{water}$ values are considered (R^2<0.1).

4. Discussion

As previously underlined, monthly estimates of pond evaporation, $\delta^{18}O_{water}$ and water temperature may smooth their daily and weekly variations. Thus, significant uncertainties must be expected on $\delta^{18}O_{water}$ and temperature estimates put in relation to $\delta^{18}O_{silica}$. This may partly explain the absence of relationship between $\Delta^{18}O_{silica\text{ - }water}$ values and water temperature when reconstructed or reconstructed and weighted values are considered. However the positive relationships between the $\Delta^{18}O_{silica\text{-}water}$ value and water temperature when the data from the time of sample collection are considered clearly indicates the absence of an oxygen isotopic equilibrium between the silica in the sponge spicules and the pond water. Indeed, at equilibrium, mass-dependent oxygen fractionation of a mineral relative to water decreases with increasing temperature (e.g., Faure, 1998). This relationship has been illustrated by negative temperature fractionation coefficients measured for quartz, phytoliths, and diatoms (Clayton et al., 1972; Matsuhisa et al., 1979; Juillet-Leclerc and Labeyrie, 1987; Shemesh et al., 1992; Sharp and Kirschner, 1994; Shahack-Gross et al., 1996; Brandriss et al., 1998; Moschen et al., 2005; Crespin et al., 2010; Dodd and Sharp, 2010; Alexandre et al., 2012; Dodd et al., 2012).

The unique relationships between the $\Delta^{18}O_{silica\text{-}water}$ value and water temperature when the data from the time of sample collection are considered also suggests that successive precipitation/dissolution processes occur over the time of spicule formation, and the latest precipitation gives its $\delta^{18}O$ imprint to the entire spicule assemblage. The $\delta^{18}O_{water}$ value and temperature averaged over the latest month or the entire period of spicule formation may influence the $\delta^{18}O_{silica}$ signature but in opposite ways that cancel each other out.

The biologically controlled mineralization of spicules has been described in detail (Schröder et al., 2003; Schröder et al, 2007; Muller et al., 2007; Wang et al., 2012a). Silica formation is rapid. Schroder et al. (2003) reported that spicules several hundreds of micrometres long grew in several tenths of hours. Dissolved Si is actively taken up by the sponge cells via a Na^{+}/HCO_3^{-} [$Si(OH)_4$] co-transporter (Schröder et al., 2004; Maldonado et al., 2011; Wang et al., 2012b) and stored in specialized cells called sclerocytes. Within the sclerocytes, axial enzymatic filaments termed silicatein are formed, around which silica is deposited. After formation of a first biosilica layer driven by the silicatein enzyme, immature spicules are released into the extracellular space. There, centrifugal and axial growth (respectively "thickening" and "elongation" processes) are driven by extraspicular silicatein. During this step, nanofibrillar bundles condense. A second enzyme, called silicase, localized on similar intra- and extracellular sites as silicatein, is able to dissolve amorphous silica and interact with silicatein during spicule formation (Schroder et al., 2003). The actions of the silicatein and silicase, which respectively polymerize and depolymerize silica, and the reorganization of the silica sheath in the forming spicule may contribute to give the spicule assemblage a late $\delta^{18}O$ imprint.

However, the positive correlations obtained between $\Delta^{18}O_{silica - water}$ values and temperature excludes the possibility that any oxygen isotopic equilibrium between spicules and pond water has been reached, even during this late stage of silica precipitation. Instead, these correlations suggest that one or several biologically controlled kinetic fractionation mechanisms are in play. These mechanisms may occur during the various steps of silica formation, during water and dissolved Si uptake and up to the latest phase of silica polymerization. Removal of water from the area of silica synthesis to the surrounding extraspicular environment (Wang et al., 2012a) may also lead to kinetic fractionation. However, a simple Rayleigh distillation would have increased the $\Delta^{18}O_{silica - water}$ between May, when the spicules were still underwater, and August, when the spicules suffered hydrous stress, which is the opposite of the observed trend.

Other parameters, such as the dissolved Si concentration and nutrient availability, co-varying with temperature may also intervene. Laboratory studies considering marine demosponges previously demonstrated that sponges react to the availability of ambient dissolved Si (Reincke and Barthel, 1997; Maldonado et al., 2011). Although significant variability occurs between individuals, the rate of Si uptake increases rapidly at low Si concentrations and becomes lower at higher concentrations (from 2.3 to 5.6 mg l^{-1} Si in the study by Reincke and Barthel (1997)). This pattern is in accordance with Michaelis-Menten enzyme kinetics (Reincke and Barthel, 1997). In the present case, *Metania spinata* forms its spicules under a range of high Si concentrations (from 2.8 to 6.2 mg l^{-1}, Table 1). The AFDM coefficient calculated by Melão and Rocha (1999) and used as a silica formation coefficient at Lagoa Verde (Table 2) increases with dissolved Si concentration from the rainy to dry season. However, the measurements of dissolved Si are too few to allow for a determination of whether the rate of Si formation decreases at high Si concentrations, as expected in Michaelis-Menten enzyme kinetics. Nevertheless, the dataset obtained in the present study suggests that regardless of the controlling parameters and biological processes leading to kinetic oxygen isotope fractionation, they are less intense during the dry season, when dissolved Si (and possibly nutrients) are more concentrated. In order to further assess the parameters responsible of the $\delta^{18}O$ imprint in lacustrine sponge spicules, additional calibration experiment are needed, using a single species grown under laboratory controlled conditions of $\delta^{18}O_{water}$, water temperature, dissolved Si and nutrient concentration.

5. Conclusion

This study provides clear evidence that the freshwater sponge *Metania spinata* does not form its siliceous spicules in oxygen isotopic equilibrium with the pond water. This fact prevents 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. However, a clear decreasing trend in $\Delta^{18}O_{silica\text{-}water}$ values from the rainy summer season to the dry winter season is observed. Several kinetic fractionations may occur during enzymatically controlled Si uptake, polymerization, depolymerization, and reorganization of the silica sheath inherent to spicule formation. In the present case, the summed amplitude of these fractionations increases with temperature during the latest month of growth at a rate of approximately 0.3‰°C^{-1}. Yet, for a given sponge species, other parameters co-varying with temperature, such as nutrient feeding or dissolved Si concentration, must be considered as potential controlling factors before using any kinetic fractionation coefficient for paleoenvironmental reconstruction purposes.

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7. References

Alexandre A., Basile-Doelsch I., Sonzogni C., Sylvestre F. Parron C., Meunier J.D. and Colin F. Oxygen isotope analyses of fine silica grains using laser-extraction technique: Comparison with oxygen isotope data obtained from ion microprobe analyses and application to quartzite and silcrete cement investigation, Geochim Cosmochim Ac, 70: 2827-2835, 2006.

Alexandre A., Crespin J., Sylvestre F., Sonzogni C. and Hilbert W.: The oxygen isotopic composition of phytolith assemblages from tropical rainforest soils tops (Queensland, Australia): validation of a new paleoenvironmental tool. Clim. Past, 8, 307-324, 2012.

Almeida, A.C.S., Volkmer-Ribeiro, C., Varajão, A.F.D.C., Gomes, N.S. and Varajão, C.A.C.: Espículas de esponjas continentais nos sedimentos cenozóicos do noroeste de Minas Gerais, como indicadores paleoambientais. Revista Brasileira de Paleontologia, 12(2): 123:138, 2009.

Almeida, A.C.S., Varajão, A.F.D.C., Gomes, N.S., Varajão, C.A.C. and Volkmer-Ribeiro, C.: Characterization and origin of spongillite-hosting sediment from João Pinheiro, Minas Gerais, Brazil. J S Am Earth Sci, 29 (2): 439-453, 2010.

Brandriss, M. E., O’Neil, J. R., Edlund, M. B., and Stoermer, E. F.: Oxygen isotope fractionation between diatomaceous silica and water, Geochim Cosmochim Ac, 62, 1119–1125, 1998.

Chapligin, B., Leng, M., Webb, E., Alexandre, A., Dodd, J., Faure, K., Ijiri, A., L¨ucke, A., Shemesh, A., Abelmann, A., Herzschuh, U., Longstaffe, F., Meyer, H., Moschen, R., Okazaki, Y., Rees, N. H., Sharp, Z., Sloane, H. J., Sonzogni, C., Swann, G., Sylvestre, F., Tyler, J., and Yam, R.: Interlaboratory comparison of oxygen isotopes from biogenic silica, Geochim Cosmochim Ac, 75, 7242-7256, 2011.

Clayton, R.N., O'Neil, J.R. and Mayeda, T. K.: Oxygen isotope exchange between quartz and water. Geophys. Res., 77, 3057-3067, 1972.

Crespin J., Alexandre A., Sylvestre F., Sonzogni C., Paille`s C., and Garreta V. IR laser extraction technique applied to oxygen isotope analysis of small biogenic silica samples. Anal Chem, 80, 2372-2378, 2008.

Crespin J., Sylvestre F., Alexandre A., Sonzogni C., Paille`s C., and Perga M.E.: Re-examination of the temperature-dependent relationship between $\delta^{18}O_{diatoms}$ and $\delta^{18}O_{lake\ water}$ and implications for paleoclimate inferences. J Paleolimnol, DOI 10.1007/s10933-010-9436-2, 2010.

Dodd, J.P. and Sharp, Z.D.: A laser fluorination method for oxygen isotope analysis of biogenic silica and a new oxygen isotope calibration of modern diatoms in freshwater environments, Geochim Cosmochim Ac, 74, 1381-1390, 2010.

Dodd, J.P., Sharp, Z.D., Fawcett, P.J., Brearley, A.J., and McCubbin, F.M.: Rapid post-mortem maturation of diatom silica oxygen isotope values. Geochem. Geophys. Geosystems, 13(9), DOI:10.1029/2011GC004019, 2012.

Faure G. (Ed): Principles and applications of geochemistry. A comprehensive textbook for geology students. 2nde ed. Upper Saddle River, New Jersey, Prentive Hall, 600p, 1998.

Hall, B.V. and Hermann, S.J.: Paleolimnology of three species of fresh-water sponges (Porifera:Spongillidae) from a sediment core of a Colorado semidrainage mountain lake, T Am Microsc Soc, 99(1), 93-100, 1980.

Jochum K.P, Wang, X, Vennemann, T.W., Sinha, B. and Müller, W.E.G.: Siliceous deep-sea sponge *Monorhaphis chuni*: A potential paleoclimate archive in ancient animals, Chem Geol, 300-301, 143-151, 2012.

Juillet-Leclerc A. and Labeyrie L.: Temperature dependence of the oxygen isotopic fractionation between diatom silica and water, Earth Planet Sc Lett, 84, 69-74, 1987.

Love, G.D, Grosjean, E., Stalvies, C., Fike, D.A., Grotzinger, J.P., Bradley, A.S., Kelly, A.E., Bhatia, M., Meredith, W., Snape, C.E, Bowring, S.A, Condon, D.J. and Summons, R.E.: Fossil steroids record the appearance of Demospongiae during the Cryogenian period, Nature, 457, 718-722, 2009.

Machado, V.S., Volkmer-Ribeiro, C. and Iannuzzi, R.: Inventary of the Sponge Fauna of the cemitério Paleolake, Catalão, Goiás, Brazil, An Acad Bras Cienc, 84 (1), 17-34, 2012.

Maldonado, M., Navarro, L., Grasa, A., Gonzalez, A. and Vaquerizo, I.: Silicon uptake by sponges: a twist to understanding nutrient cycling on continental margins, Scientific Reports, DOI: 10.1038/srep00030, 2011.

Matheney R.K. and Knauth, L.P.: Oxygen-isotope fractionation between marine biogenic silica and seawater. Geochim Cosmochim Ac, 53: 3207-3214, 1989.

Matsuhisa, Y., Goldsmith, J.R. and Clayton, R.N.: Oxygen isotopic fractionation in the system quartz-albiteanorthite-water, Geochim. Cosmochim. Ac, 43, 1131-1140, 1979.

Melão M.G.G. and Rocha, O.: Biomass and produtivity of the freshwater sponge *Metania spinata* (Carter, 1881) (Demospongiae: Metaniidae) in a Brazilian reservoir, Hydrobiologia, 390, 1-10, 1999.

Moschen R., Lücke A. and Schleser G.H.: Sensitivity of biogenic silica oxygen isotopes to changes in surface water temperature and paleoclimatology, Geophys Res Lett, 32, L07708. doi:10.1029/2004GL022167, 2005.

Müller, W. E. G., Li, J., Schröder, H.C., Qiao, L., and Wang, X.: The unique skeleton of siliceous sponges (Porifera; Hexactinellida and Demospongiae) that evolved first from the Urmetazoa during the Proterozoic: a review. Biogeosciences, 4, 219–232, 2007.

Müller, W.E.G., Wang, X., Cui, F.Z., Jochum, K.P., Tremel, W., Bill, J., Schröder, H.C., Natalio, F., Schoβmarcher, U. and Wiens, M.: Sponge spicules as blueprints for the biofabrication of inorganic-organic composites and biomaterials, Appl. Microbiol. Biotechnol., 83, 397-413, 2009.

Oliveira, A.A.C., Valle, C.R.O. and Féboli, W.L.: Geologia. Nota explicativa do mapa geológico integrado. Folhas SE 23-V-D (partes). Belo Horizonte, Projeto São Francisco, CPRM-COMIG. 2002.

Parolin, M., Volkmer-Ribeiro, C. and Stevaux, J.C.: Use of spongofacies as a proxy for river-lake paleohydrology in Quaternary deposits of central-western Brazil. Revista Brasileira de Paleontologia, 11(3):187-198, 2008.

Reincke, T., and Barthel, D.: Silica uptake kinetics of *Halichondria panacea* in Kiel Bight, Marine Biology, 129, 591-593, 1997.

Schröder, H.C., Efremova, S.M., Itskovich, V.B., Belikov, S., Masuda, Y., Krasko, A., Müller I.M. and Müller, W.E.G.: Molecular phylogeny of the freshwater sponges in Lake Baikal, J Zool Syst Evol Res, 41, 80–86, 2003.

Schröder, H.C., Perovic-Ottstadt, S., Rothenberger, M., Wiens, M., Schwertner, H., Batel, R., Korzhev, M., Müller I.M. and Müller, W.E.G.: Silica transport in the demosponge *Suberites domuncula*: fluorescence emission analysis using the PDMPO probe and cloning of a potential transporter, Biochem J, 381, 665-673, 2004.

Schröder, H.C., Krasko, A., Brandt, D., Wiens, M., Tahir, M.N., Tremel, W. and Müller, W.E.G.: Silicateins, silicase and spicule-associated proteins: synthesis of demosponge silica skeleton and nanobiotechnological applications. In Custódio MR, Lôbo-Hajdu G, Hajdu E, Muricy G: Porifera Research: Biodiversity, Innovation and Sustainability, 2007.

Shahack-Gross R., Shemesh A., Yakir D. and Weiner S.: Oxygen isotopic composition of opaline phytoliths: Potential for terrestrial climatic reconstruction. Geochim Cosmochim Ac, 60 (20): 3949-3953, 1996.

Sharp, Z.D. and Kirschner, D.L.: Quartz-calcite oxygen isotope thermometry: A calibration based on natural isotopic variations, Geochim. Cosmochim. Ac, 58, 4491-4501, 1994.

Shemesh A., Charles C. D. and Fairbanks R. G.: Oxygen isotopes in Biogenic Silica: Global Changes in Ocean Temperature and Isotopic Composition, Science, 256, 1434-1436, 1992.

Sifeddine, A., Martin, L., Turcq, B., Volkmer-Ribeiro, C., Soubies, F., Cordeiro, R. C. and Suguio, K.: Variations of amazonian rain forest environments a sedimentological records covering 30.000 years BP, Palaeogeogr Palaeocl, 168, 221-235, 2001.

Silva, K.C., Parolin, M. and Bissa, W.M.: Espículas de esponjas vs. variações do nível relativo do mar na região de Iguape, sudeste do Brasil, Revista Brasileira de Paleontologia, 15(3), 319-326, 2012.

Tubelis, A. and Nascimento, F. J. L.: Meteorologia descritiva: fundamentos e aplicações brasileiras. São Paulo: Nobel, 374 p, 1992.

Turcq, B., Sifeddine, A., Martín, L., Absy,M.L., Soubiès, F., Suguio, K. and Volkmer-Ribeiro, C.: Amazon Forest fires: a lacustrine reporto f 7,000 years, Ambio 27(2): 139-142, 1998.

Uriz, M. J., Turon, X., Becerro, M., Agell, G.: Siliceous Spicules and Skeleton Frameworks in Sponges: Origin, Diversity, Ultrastructural Patterns, and Biological Functions. Microsc. Res. Tech. 62: 279 –299, 2003.

Vallet-Coulomb C., Gasse F., Robison L and Ferry L.: Simulation of the water and isotopic balance of a closed tropical lake at a daily time step (Lake Ihotry, South-West of Madagascar), Journal of Geochemical exploration, 88, p. 153-156, 2006.

Veloso, H. P., Rangel-Filho, A. L. R. and Lima, J.C.A.: Classificação da vegetação brasileira, adaptada a um sistema universal, IBGE, Rio de Janeiro, 124 p, 1991.

Volkmer-Ribeiro, C.: Porifera, In: S.H. Hurlbert; G. Rodrigues and N.D. Santos (Eds), Aquatic Biota of Tropical South America, Part 2: Anarthropoda, San Diego State University, San Diego, Cal. USA, p. 86-95, 1981.

Volkmer-Ribeiro, C. and Pauls, S.M.: Esponjas de água Dulce (Porifera: Demospongiae) de Venezuela. Caracas. Acta Biológica Venezuelana, 20(1), 1-28, 2000.

Volkmer-Ribeiro, C. Marques, D.M., De Rosa-Barbosa, R. and Machado, V.S.: Sponge spicules in sediments indicate evolution of coastal freshwater bodies. J Coastal Res, 39: 469-472, 2004.

Volkmer-Ribeiro C., Motta J.F.M. and Callegaro V.L.M.: Taxonomy and Distribuition of Brazilian Spongillites.Sponge Sciences, Multidisciplinary Perspectives, 271-278, 1998.

Wang, X. H., Schröder, H.C. and Müller, W.E.G.: Giant siliceous spicules from the deep-sea glass sponge Monorhaphis chuni: morphology, biochemistry and molecular biology. International Review of Cell and Molecular Biology 273, 69-115, 2009.

Wang, X., Schloßmacher, U., Wiens, M., Batel, R., Schröder, H.C. and Müller, W.E.G.: Silicateins, silicatein interactors and cellular interplay in sponge skeletogenesis: formation of glass fiber-like spicules, FEBS J, 279, 1721-1736, 2012a.

Wang, X., Schröder, H.C., Wang K., Kaandorp, J.A. and Müller, W.E.G.: Genetic, biological and structural hierarchies during sponge spicule formation: from soft sol–gels to solid 3D silica composite structures, Soft Matter, 8, 9501-9518, 2012b.

Webb E. A. and Longstaffe F. J.: The oxygen isotopic compositions of silica phytoliths and plant water in grasses: implications for the study of paleoclimate. Geochim Cosmochim Ac, 64(5), 767-780, 2000.

Table 1: Characterization of the samples of pond water and sponge spicules collected at Lagoa Verde: field and geochemical measurements. $\Delta^{18}O_{\text{silica-reconstructed water}}$ values calculated for the latest month of sponge growth.

Pond water				**Sponge samples**			**Isotopic measurements**													
Sponge sampling date	**Measured level**	**Measured t_{water}**	**Measured $\delta^{18}O_{water}$**	**Sample**	**Substrate**		**$\delta^{18}O_{measured\ 1}$**			**$\delta^{18}O_{measured\ 2}$**			**X**	**$\delta^{18}O_{silica}$**	**Corrected[b] $\delta^{18}O_{silica}$**				**$\Delta^{18}O_{\text{silica-measured water}}$**	**$\Delta^{18}O_{\text{silica-reconstructed water}}$**
	(cm)	(°C)	(‰ vs. VSMOW)		Artificial vs. natural	Description	(‰ vs. VSMOW)			(‰ vs. VSMOW)			(‰ vs. VSMOW)		(‰ vs. VSMOW)				(‰ vs. VSMOW)	(‰ vs. VSMOW)
							Average	SD	n[a]	Average	SD	n[a]				Final uncertainty[c]	Average	SD		
Aug 2010	65	19.1	3.25	LV01	Natural	dried grasses	30.4	0.0	2	33.5	0.2	2	0.074	27.6	30.4	0.8	29.6	0.9	27.1	27.6
				LV02	Natural	dried grasses	28.9	0.1	2	31.9	0.2	2	0.071	25.8	28.6	0.7			25.3	25.8
				LV03	Natural	dried grasses	30.0	0.1	2	32.6	0.2	2	0.063	27.0	29.8	0.6			26.6	27.0
Mar 2011	205	30.5	-6.03	LV04	Artificial	bag C8	23.3	0.3	2	25.4	0.4	2	0.059	27.5	22.4	0.5	24.0	1.8	28.4	27.5
				LV05	Artificial	bag A8	25.0	0.2	2	28.6	0.2	2	0.085	28.9	23.8	0.8			29.9	28.9
				LV06	Artificial	bag C13	26.6	0.0	2	29.0	0.1	2	0.057	31.1	26.0	0.5			32.0	31.1
Apr 2011	195	26.8	-3.98	LV07	Natural	*E. interstincta*	26.0	0.1	2	28.3	0.1	2	0.053	29.9	25.4	0.5	25.1	0.5	29.4	29.9
				LV08	Natural	Submerged sediment	26.4	0.1	2	29.2	0.0	1	0.066	30.2	25.7	0.6			29.6	30.2
				LV09	Artificial	bags A7+A13+A16	25.3	0.1	2	27.8	0.0	1	0.06	29.0	24.5	0.5			28.4	29.0
				LV12	Natural	*E. interstincta*	26.0	0.2	2	29.2	0.0	1	0.077	29.6	25.1	0.7			29.0	29.6
May 2011	172	24.0	-2.02	LV10	Natural	*E. interstincta*	26.8	0.1	2	29.4	0.2	2	0.061	30.1	26.2	0.6	27.0	1.0	28.2	30.1
				LV11	Natural	*E. interstincta*	28.1	0.0	2	30.8	0.0	1	0.064	31.6	27.7	0.6			29.7	31.6
Control May 2011	172	24.0	-2.02	LV13	Artificial	1 month control bag	25.8	0.0	2	28.8	0.2	2	0.072	28.8	24.9	0.67	-	-	26.9	28.8

[a] from one CIE; [b] Corrected for methodological bias (Alexandre et al., 2012); [c] Calculated after Alexandre et al.(2012) using a Monte Carlo simulation.

Table 2: Regional meteorological data, pond water parameters, and sponge growth coefficient calculated after Melão & Rocha (1999) for the months of sponge growth. $\Delta^{18}O_{\text{silica-reconstructed water}}$ averages calculated for the latest month of sponge growth.

Measured values are in bold. Reconstructed values are detailed in the text. Monthly mean values of precipitation (P), evapotranspiration (ETP), and atmospheric temperature (T) are from INMET (Station #83481; João Pinheiro).

Samples			Meteorological data					Pond characterization										
Sampling date		**Last month of growth**	**Precipitation**	**ETP**	**P-ETP**	**Monthly mean t_{atm}**	**t_{atm} at the time of collection 12 h**	**Volume**	**Measured level**	**Reconstructed level[a]**	**Measured t_{water}**	**Reconstructed monthly mean t_{water} [b]**	**Dissolved Si**	**Measured $\delta^{18}O_{water}$**	**Measured δD_{water}**	**Reconstructed $\delta^{18}O_{water}$ [c]**	**Sponge growth coefficient[d]**	**$\Delta^{18}O_{\text{silica-reconstructed water}}$**
			(mm)	**(mm)**	**(mm)**	**(ºC)**	**(ºC)**	**(m^3)**	**(cm)**		**(ºC)**		**($mg.l^{-1}$)**	**(‰ vs. VSMOW)**	**(‰ vs. VSMOW)**	**(‰ vs. VSMOW)**		**(‰ vs. VSMOW)**
	1	Nov-09	**85**	**133**	**-48**	**26.2**		670,837		48		30.1				3.32	2	
	2	Dec-09	**413**	**113**	**301**	**24.3**		671,137		94		28.0				0.88	3	
	3	Jan-10	**125**	**116**	**9**	**26.2**		671,146		95		30.1				0.80	6	
	4	Feb-10	**83**	**129**	**-46**	**26.8**		671,101		88		30.8				1.17	10	
	5	Mar-10	**180**	**119**	**61**	**25.4**		671,162		98		29.2				0.68	18	
	6	Apr-10	**77**	**99**	**-22**	**24.2**		671,140		94		27.9				0.86	32	
	7	May-10	**61**	**90**	**-29**	**23.9**		671,111		90		27.6				1.09	57	
	8	Jun-10	**5**	**63**	**-58**	**21.2**		671,053		81		24.6				1.57	101	
	9	Jul-10	**0**	**73**	**-73**	**22.1**		670,980		70		25.6				2.16	179	
16/08/2010		**Aug-10**	**0**	**84**	**-84**	**22.9**	**17.6**	670,896	**65**	57	**19.1**	26.5	**6.2**	**3.25**	**14.9**	2.84	179	26.8
29/09/2010		**Sep-10**	**5**	**64**	**-60**	**25.0**	**21.8**	670,837	**45**	48	**23.7**	28.8	**5.9**	**4.38**	**19.8**	3.33		
		Oct-10	**202**	**134**	**68**	**26.4**		670,905		59		30.3				2.77		
23/11/2010	1	**Nov-10**	**312**	**106**	**206**	**24.3**	**22.4**	671,111	**70**	90	**26.5**	28.0	**10.6**	**0.39**	**-7.3**	1.09	2	
	2	Dec-10	**355**	**131**	**224**	**24.9**		671,335		124		28.7				-0.74	3	
08/01/2011	3	**Jan-11**	**346**	**121**	**226**	**25**	**24.8**	671,561	**150**	158	**29.3**	28.8	**3.9**	**-5.06**	**-39.1**	-2.58	6	
15/02/2011	4	**Feb-11**	**107**	**126**	**-19**	**26.5**	**27.2**	671,541	**160**	155	**29.7**	30.4	**5.8**	**-0.66**	**-19.5**	-2.42	10	
03/04/2011	5	**Mar-11**	**410**	**85**	**325**	**24.4**	**22.4**	**671,866**	**205**	205	**30.5**	28.1	**2.8**	**-6.03**	**-53.1**	-5.06	18	29.1
01/05/2011	6	**Apr-11**	**29**	**100**	**-71**	**24.6**	**24.4**	671,795	**195**	194	**26.8**	28.3	**3.4**	**-3.98**	**-39.7**	-4.48	32	29.7
05/06/2011	7	**May-11**	**11**	**87**	**-76**	**23.2**	**20.8**	671,719	**172**	182	**24.0**	26.8	**6.1**	**-2.02**	**-31.4**	-3.87	57	30.8

[a] According to Eq. (3); [b] According to Eq. (1); [c] According to Eq. (2); [d] According to Eq. (4). (see text for further details).

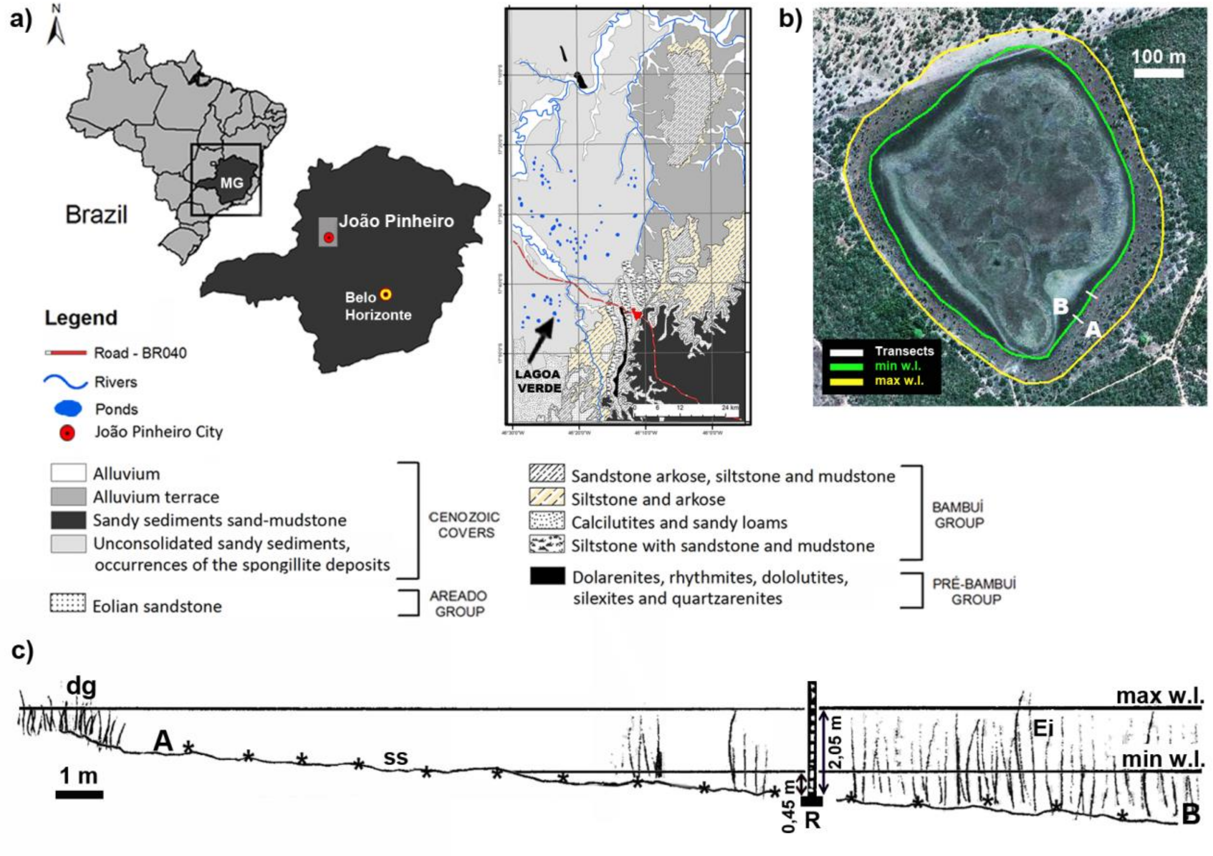


Figure 1: a) Localization area and geological map showing the Lagoa Verde in the João Pinheiro region, Northwestern of Minas Gerais state (MG), Brazil. Modified from Oliveira et al. (2002). **b)** Google Earth image showing minimum and maximum water level in 08/24/2010 and the sampling transects. **c)** Bathymetry of the sampling transects.

AB = transect; max w.l. = maximum water level; min w.l. = minimum water level; R = ruler; * = artificial substrates (bags and control bag); dg = dried grasses; ss = submerged sediment; Ei = aquatic macrophyte *Eleocharis interstincta*.

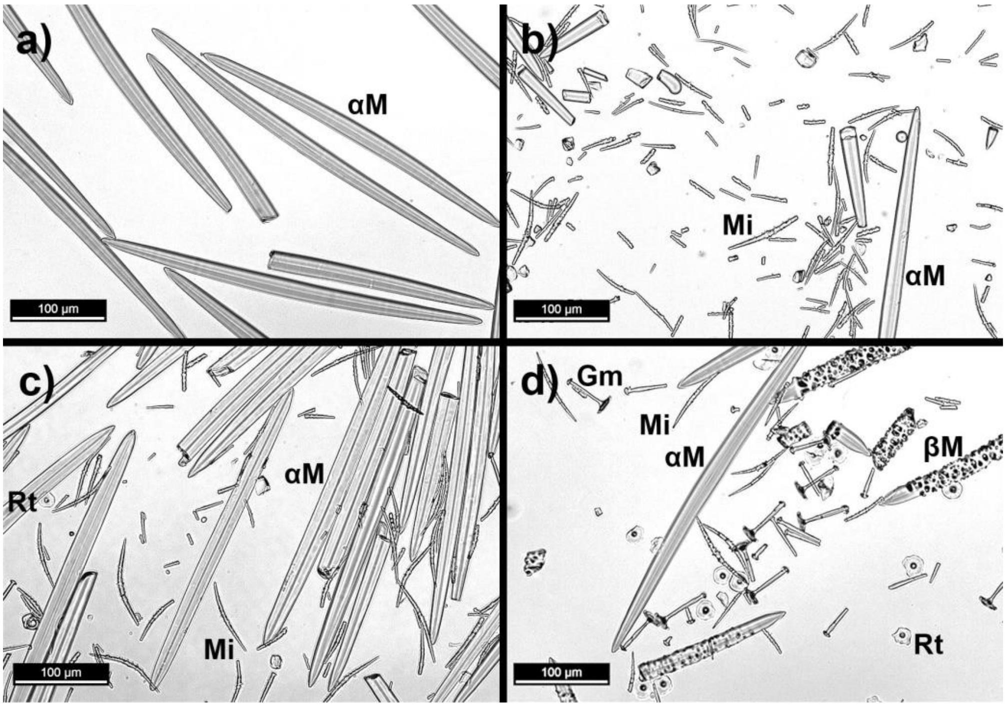


Figure 2: Optical microscopy images of spicules of *Metania spinata* extracted from Lagoa Verde sponge samples: **a**) LV05 , **b**) LV08, **c**) LV10, **d**) LV01. Spicules categories: αM: alpha megasclere; βM: beta megasclere. Mi: microsclere; Gm: gemmosclere; Rt: broken rotule of the gemmosclere.

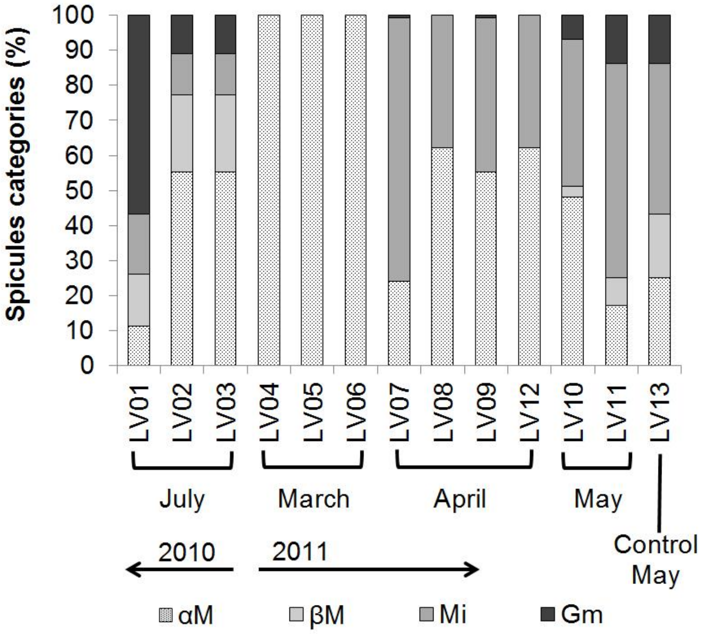


Figure 3: Percentages of spicule categories per sample. αM: alpha megasclere; βM: beta megasclere. Mi: microsclere; Gm: gemmosclere.

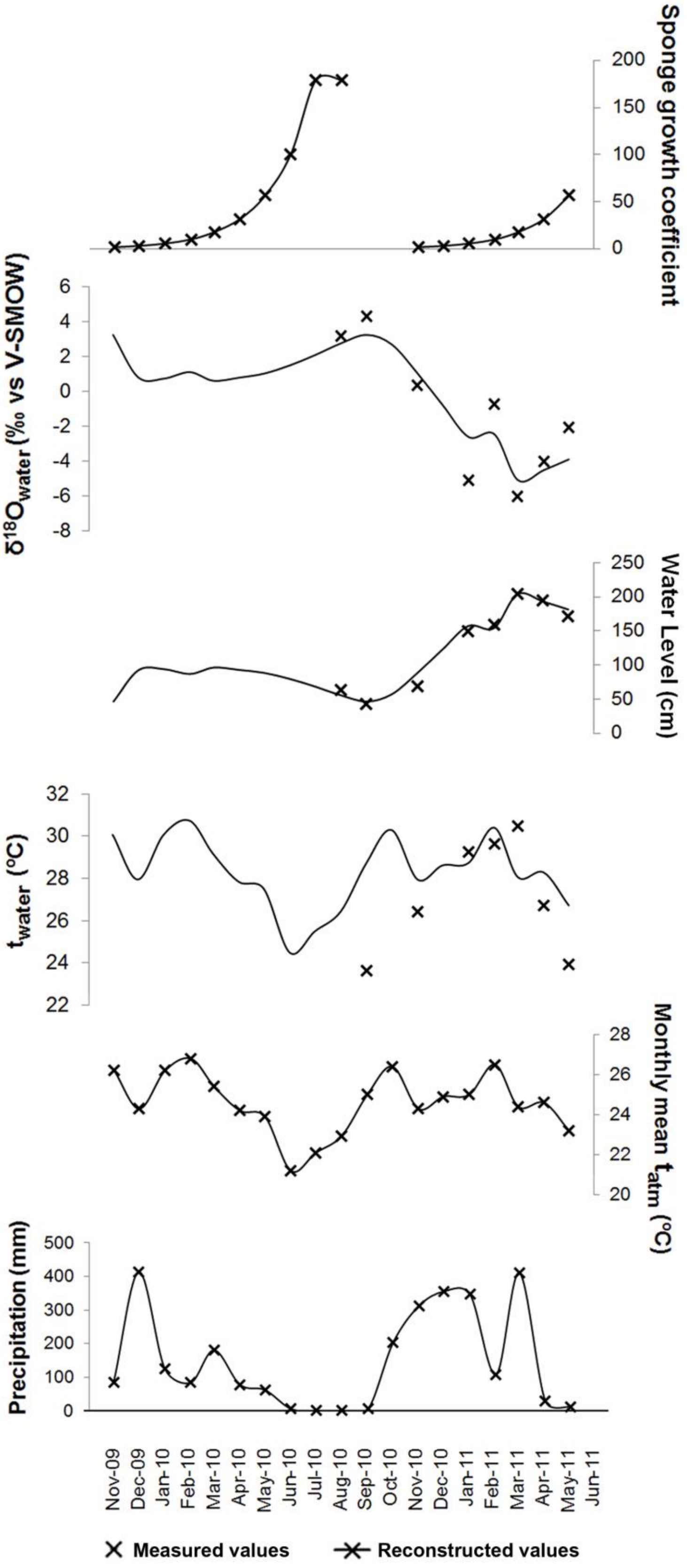

Sponge growth coefficient
200
150
100
50
0
δ18Owater (‰ vs V-SMOW)
6
4
2
0
-2
-4
-6
-8
Water Level (cm)
250
200
150
100
50
0
twater (ºC)
32
30
28
26
24
22
Monthly mean tatm (ºC)
28
26
24
22
20
Precipitation (mm)
500
400
300
200
100
0
Nov-09
Dec-09
Jan-10
Feb-10
Mar-10
Apr-10
May-10
Jun-10
Jul-10
Aug-10
Sep-10
Oct-10
Nov-10
Dec-10
Jan-11
Feb-11
Mar-11
Apr-11
May-11
Jun-11
Measured values
Reconstructed values

Figure 4: Reconstructed and/or measured values of sponge growth coefficient over the investigated sponge growth period, $\delta^{18}O_{water}$, water level of the pond, water temperature (t_{water}), monthly mean atmospheric temperature (t_{atm}), and precipitation.

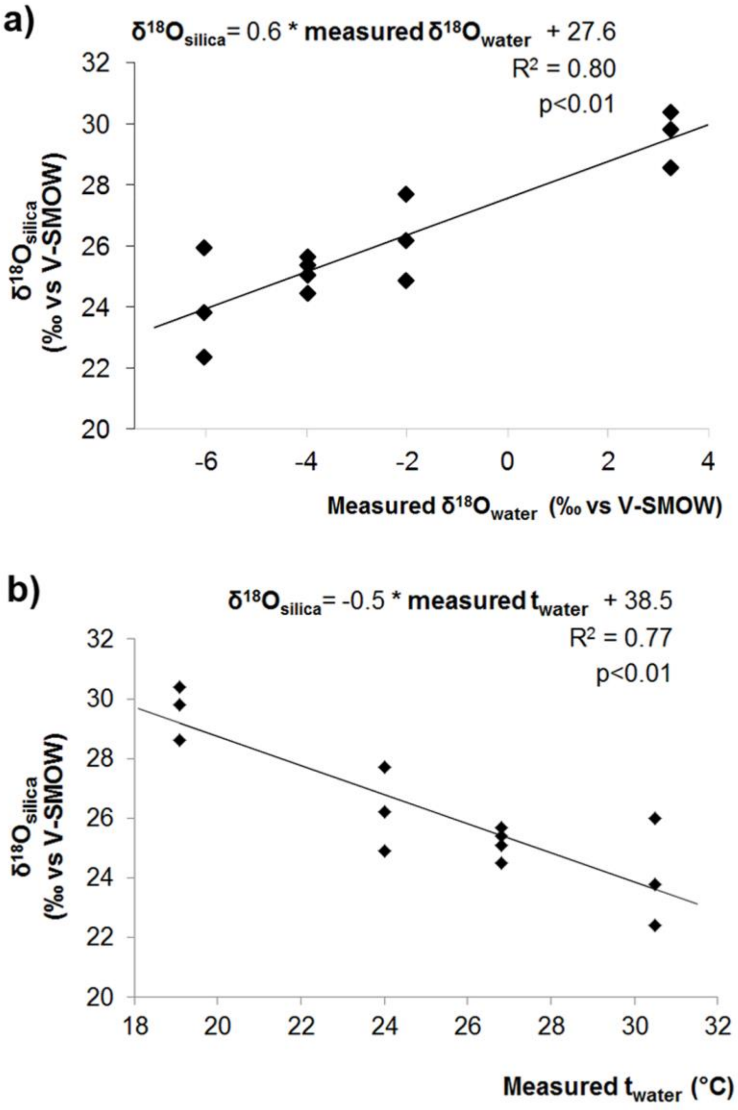
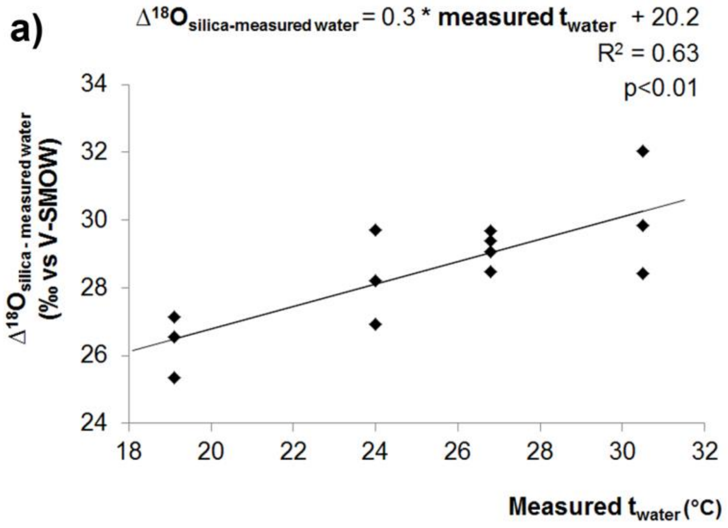
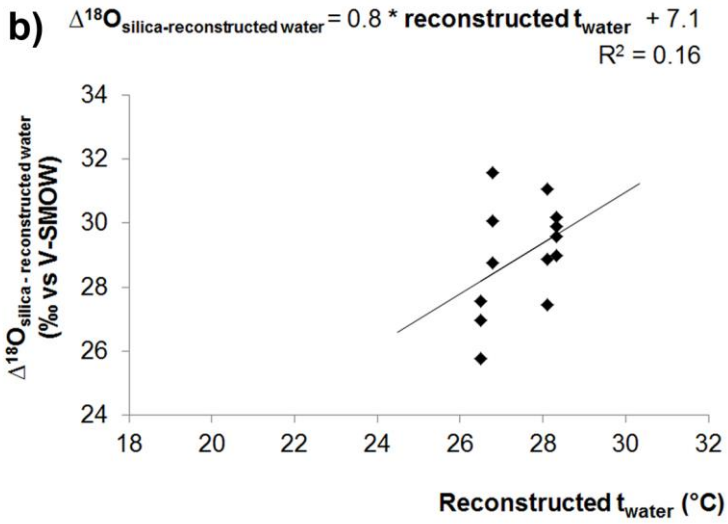


Figure 5: $\delta^{18}O_{silica}$ values plotted *vs.* **(a)** measured $\delta^{18}O_{water}$ values and **(b)** measured temperature, at the time of collection.

a)


$\Delta^{18}O_{silica\text{-}measured\ water}$ = 0.3 * measured t_{water} + 20.2
$R^2 = 0.63$
$p<0.01$
$\Delta^{18}O_{silica\ -\ measured\ water}$ (‰ vs V-SMOW)
34
32
30
28
26
24
18
20
22
24
26
28
30
32
Measured t_{water} (°C)

b)


$\Delta^{18}O_{silica\text{-}reconstructed\ water}$ = 0.8 * reconstructed t_{water} + 7.1
$R^2 = 0.16$
$\Delta^{18}O_{silica\ -\ reconstructed\ water}$ (‰ vs V-SMOW)
34
32
30
28
26
24
18
20
22
24
26
28
30
32
Reconstructed t_{water} (°C)

c)

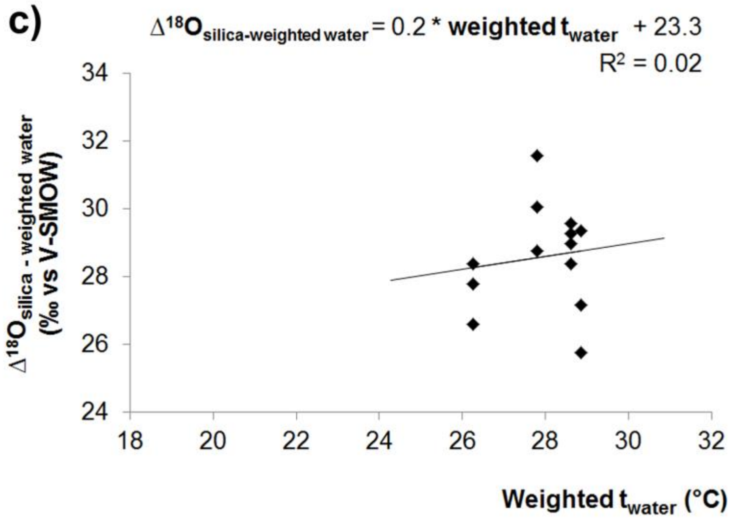

$\Delta^{18}O_{silica\text{-}weighted\ water}$ = 0.2 * weighted t_{water} + 23.3
$R^2 = 0.02$
$\Delta^{18}O_{silica\ -\ weighted\ water}$ (‰ vs V-SMOW)
34
32
30
28
26
24
18
20
22
24
26
28
30
32
Weighted t_{water} (°C)

Figure 6: $\Delta^{18}O_{silica - water}$ values plotted *vs.* **(a)** measured water temperature, **(b)** reconstructed monthly mean water temperature, and **(c)** average of weighted monthly mean water temperature.

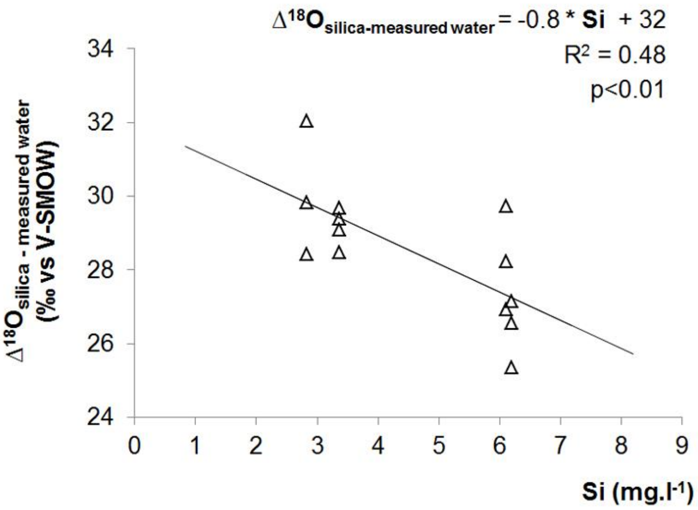


Figure 7: $\Delta^{18}O_{silica – measured\ water}$ values plotted *vs.* dissolved Si concentration.