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Assessing the relationship between the δ^{18}O signatures of siliceous sponge spicules and water in a tropical lacustrine environment (Minas Gerais, Brazil)

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Abstract

Siliceous sponge spicules constitute an important siliceous component of lacustrine sediments, together with widespread diatom frustules. In contrast to diatom frustules, siliceous spicules are formed in sponges in an enzymatic way. Previous attempts to use their oxygen isotopic signature ($\delta^{18}O_{silica}$) as a paleoenvironmental proxy have led to contradictory conclusions. These attempts demonstrated the need to further assess whether sponges form their silica in oxygen isotopic equilibrium with water. 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. Assuming that silica may form continuously in the spicules, temperature and $\delta^{18}O_{water}$ values over the months of growth were weighted using a sponge growth coefficient previously established for *Metania spinata*. The $\delta^{18}O_{silica}$ values of sponges grown simultaneously and on similar substrates were scattered. No relationships were observed between the $\Delta^{18}O_{silica-water}$ and water temperature when the reconstructed values were considered. Conversely, a positive correlation was obtained, with a 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. Such a positive temperature coefficient clearly indicates that the freshwater sponge *Metania spinata* does not form its siliceous spicules in oxygen isotopic equilibrium with the pond water. Instead, one or several biologically controlled kinetic fractionation mechanisms may be in play during the various steps of silica formation. Our results suggest that the latest precipitation gives its $\delta^{18}O$ imprint to the entire spicules assemblage. The amplitude of the apparent fractionations increases with temperature, but other controlling parameters, such as dissolved Si concentration and nutrient availability, co-varying with temperature may

intervene. These 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.

1 Introduction

Siliceous sponges are filter-feeding animals structured on three-dimensional arrangements of siliceous spicules with lengths of hundreds of micrometres (Hexactinellida and Demospongiae classes) to several metres (Hexactinellida class). These animals have proliferated since the Neoproteozoic at various latitudes in marine and fresh waters (Volkmer-Ribeiro and De Rosa-Barbosa, 1979; 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, 2010; Volkmer-Ribeiro and Machado, 2007; 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, such as diatom frustules and plant phytoliths. These studies demonstrated that the isotope signature of silica ($\delta^{18}O_{silica}$) 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; Dodd and Sharp, 2010; Crespin et al., 2010; Webb and Longstaffe, 2000; Alexandre 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 ($\Delta^{18}\mathrm{O}_{\mathrm{silica-water}}$) 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 ($-0.2\,\%\,^{\circ}\mathrm{C}^{-1}$) 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 $\Delta^{18}\mathrm{O}_{\mathrm{silica-water}}$ 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 $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ 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 $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ analyses was not evaluated by the recent inter-laboratory comparison of oxygen isotope compositions from biogenic silica (Chapligin et al., 2011). This scarcity of $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ 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 $\delta^{18}\mathrm{O}_{\mathrm{water}}$ composition.

In this study, we measured the $\delta^{18}\mathrm{O}$ 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 $\delta^{18}\mathrm{O}_{\mathrm{silica}}$, $\delta^{18}\mathrm{O}_{\mathrm{water}}$, and water temperature values were assessed. To our knowledge, the $\delta^{18}\mathrm{O}$ signatures of freshwater sponge spicules have never been investigated except for the unique sample from Lake Baikal analysed by Jochum et al. (2012).

2 Materials and methods

2.1 Study area

When sponge spicules dominate a sedimentary deposit with secondary amounts of clay, sand, organic matter, and diatom residues, the sediment is classified as a spongillite (Traxler, 1895; Volkmer-Ribeiro, 1992; Volkmer-Ribeiro and Motta, 1995). 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 metres in diameters and several metres 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. 1). The climate is tropical humid with a five-month dry season during the winter (from May to September). The mean annual temperature is 23.2 °C, and the mean annual precipitation is 1562 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 characterised 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). Footprints of wild animals, such as antas (*Tapirus terrestris*), porcos-do-mato (*Tayassu pecari*), onças-parda (*Puma concolor*), and tatus (*Euphractus sexcintus*), were also recognised near the pond.

Lagoa Verde has a radius of 265 m, a perimeter of 1810 m, and a surface area of 22.06 10^5 m^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; Volkmer-Ribeiro and Costa, 1992; Volkmer-Ribeiro and Motta, 1995; Almeida et al., 2009).

2.2 Sampling and field measurements

In September 2010, at the end of the dry season, reproductive structures (called gemmules) of *Metania spinata* sponges were placed in black tulle bags (40 cm × 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. 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). 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). In addition, natural sponge samples were collected on emergent aquatic macrophyte *Eleocharis interstincta* (April and May 2011: LV 07, 10, 11 and 12; Table 1), on submerged sediment (April 2011: LV08; Table 1), and on grasses at the margin of the pond (August 2010: LV01, 02, 03; Table 1).

Five water samples were collected at 12.00 p.m. (UTC/GMT −3 h) 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 water temperature at a depth of 20 cm were measured during water sample collection (Table 2).

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 UFOP. The pond water temperature and pH values had been measured at midday at depths of 20–30 cm during the sampling campaigns.

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. No ejection occurred. The oxygen gas samples were directly sent to and analysed using a dual-inlet mass spectrometer ThermoQuest Finnigan Delta Plus®. The measured δ^{18}O values of each sample were corrected on a daily basis using a quartz lab standard ($\delta^{18}\mathrm{O}_{\text{Boulangé } 50\text{–}100\,\mu\text{m}} = 16.284 \pm 0.1$ ‰, $n = 18$). The calculated $\delta^{18}\mathrm{O}_{\text{silica}}$ value was corrected for a reproducible methodological bias. The entire procedure commonly applied to diatoms and phytoliths has been previously described in detail (Alexandre et al., 2006; Crespin et al., 2010; Alexandre et al., 2012). During the analysis period, measurement of the international quartz standard (NBS 28) yielded an average of 9.78 ± 0.14 ‰ ($n = 8$). Replicate analyses of the sponge spicules samples yielded a reproducibility better than ±0.39 ‰ (1σ). The δ^{18}O values of the water samples were measured using an automated Thermo–Finnigan equilibrating unit connected to a dual inlet Delta Plus mass spectrometer. The reproducibility of the δ^{18}O analyses was ±0.05 ‰ (1σ).

2.4 Reconstruction of temperature and δ^{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 (Volkmer-Ribeiro, 1981). 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 characterised 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 p.m. were strongly 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.

- The measured values of $\delta^{18}O_{water}$ were strongly 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) a water balance equation based on monthly data of precipitation (P) and evaporation (ETP) (Table 2) and (2) the relationship between calculated lake volumes and measured lake levels obtained for our measurements (water level = 0.152 × water volume − 101 919; $R^2 = 0.95$; $p < 0.01$; Eq. 3).

  The changes in the water volume were estimated using the equation $V_{i+1} = V_i + (\text{P-ETP})_{i+1}$, using the water volume measured in March 2011 as an initial V_i value (Table 2). 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 ($\text{AFDW} = 8.1497^{0.2748\times\text{ number of growing months}}$; $R^2 = 0.85$; 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 $\delta^{18}\text{O}_{\text{water}}$.

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 Table 3, respectively. 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 gemoscleres were produced during the degeneration phase in the dry season.

The measured and reconstructed values of the water level, water temperature, and $\delta^{18}\text{O}_{\text{water}}$ 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 2011. This finding is consistent with meteorological conditions: 2010 was drier, warmer, and thus more evaporative than 2011. As they are based on water level variations, the $\delta^{18}\text{O}_{\text{water}}$ estimates are higher in 2010 than in 2011. Although both the reconstructed and measured $\delta^{18}\text{O}_{\text{water}}$ 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, 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 $\delta^{18}\mathrm{O}_{\mathrm{water}}$ reconstructions reproduce the seasonal trends. 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 the day, INMET database 2010–2011, João Pinheiro), significant variations in the pond water temperature can be expected, which explains the difference between the averaged temperatures and those measured at 12.00 p.m. 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).

For a given month, the $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ 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 $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ 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 $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ values increase with the latest $\delta^{18}\mathrm{O}_{\mathrm{water}}$ values, either measured ($R^2 = 0.80$; Fig. 5a), reconstructed ($R^2 = 0.75$; Fig. 5b), or reconstructed and weighted ($R^2 = 0.65$; Fig. 5c). The $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ values decrease with the latest water temperature values, either measured ($R^2 = 0.77$; Fig. 6a), reconstructed ($R^2 = 0.57$; Fig. 6b), or reconstructed and weighted ($R^2 = 0.79$; Fig. 6c). The $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ values also increase with dissolved Si concentration, although the correlation is moderate ($R^2 = 0.56$). When measured data are considered, the $\Delta^{18}\mathrm{O}_{\mathrm{silica-water}}$ values display a positive correlation with water temperature ($R^2 = 0.63$).

The associated coefficient is 0.3 ‰ °C^{-1} (Fig. 7a). No correlation is observed when reconstructed or reconstructed and weighted values are considered (Fig. 7b and c). When measured $\delta^{18}O_{\text{water}}$ values are considered, the $\Delta^{18}O_{\text{silica-water}}$ values display a moderate negative correlation with the dissolved Si concentration ($R^2 = 0.48$; Fig. 8). Again, no correlation is observed when reconstructed or reconstructed and weighted $\delta^{18}O_{\text{water}}$ values are considered ($R^2 < 0.1$).

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). Conversely, our results clearly indicate the absence of an oxygen isotopic equilibrium between the silica in the sponge spicules and the pond water.

4 Discussion

There is a single correlation between the $\Delta^{18}O_{\text{silica-water}}$ value and water temperature when the data from the time of sample collection are considered. This result 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 $\delta^{18}O_{\text{water}}$ value and temperature averaged over the latest month or the entire period of spicule formation may influence the $\delta^{18}O_{\text{silica}}$ signature (Figs. 5b, c, and 6b, c) but in opposite ways that cancel each other out (Fig. 7b and c).

The biologically controlled mineralisation of spicules has been described in detail (Schröder et al., 2003, 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 specialised 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, localised 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 polymerise and depolymerise silica, and the reorganisation of the silica sheath in the forming spicule may contribute to give the spicule assemblage a late δ^{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 polymerisation. 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 $\mathrm{mg\,L^{-1}}$ 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 $\mathrm{mg\,L^{-1}}$, 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.

5 Conclusions

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 $\delta^{18}\mathrm{O}_{\mathrm{silica}}$ values in sponges as a direct proxy for past $\delta^{18}\mathrm{O}_{\mathrm{water}}$ and temperature changes. However, a clear decreasing trend in $\Delta^{18}\mathrm{O}_{\mathrm{silica-water}}$ values from the rainy summer season to the dry winter season is observed. Several kinetic fractionations may occur during enzymatically controlled Si uptake, polymerisation, depolymerisation, and reorganisation 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 ‰ $^{\circ}\mathrm{C}^{-1}$. However, 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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Table 1. Characterisation 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 $\delta^{18}O_{\text{water}}$	Sample	Substrate		$\delta^{18}O_{\text{measured1}}$			$\delta^{18}O_{\text{measured2}}$			X	$\delta^{18}O_{\text{silica}}$	Corrected* $\delta^{18}O_{\text{measured silica}}$			$\Delta^{18}O_{\text{silica−measured water}}$	$\Delta^{18}O_{\text{silica−reconstructed water}}$
	(cm)	(°C)	(‰ vs. VSMOW)		Artificial vs. natural	Description	(‰ vs. VSMOW)			(‰ vs. VSMOW)				(‰ vs. VSMOW)		(‰ vs. VSMOW)		(‰ vs. VSMOW)	(‰ vs. VSMOW)
							Average	SD	n	Average	SD	n				Average	SD		
Aug 2010	65	19.1	3.3	LV01	Natural	dried grasses	30.4	0.0	2	33.5	0.2	2	0.074	27.6	30.4	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			25.3	25.8
				LV03	Natural	dried grasses	30.0	0.1	2	32.6	0.2	2	0.063	27.0	29.8			26.6	27.0
Mar 2011	205	30.5	−6.0	LV04	Artificial	bag C8	23.3	0.3	2	25.4	0.4	2	0.059	27.5	22.4	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			29.9	28.9
				LV06	Artificial	bag C13	26.6	0.0	2	29.0	0.1	2	0.057	31.1	26.0			32.0	31.1
Apr 2011	195	26.8	−4.0	LV07	Natural	*E. interstincta*	26.0	0.1	2	28.3	0.1	2	0.053	29.9	25.4	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			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			28.4	29.0
				LV12	Natural	*E. interstincta*	26.0	0.2	2	29.2	0.0	1	0.077	29.6	25.1			29.0	29.6
May 2011	172	24.0	−2.0	LV10	Natural	*E. interstincta*	26.8	0.1	2	29.4	0.2	2	0.061	30.1	26.2	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			29.7	31.6
Control May 2011	172	24.0	−2.0	LV13	Artificial	1 month control bag	25.8	0.0	2	28.8	0.2	2	0.072	28.8	24.9	–	–	26.9	28.8

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[*] Corrected for methodological bias (Alexandre et al., 2012).

Table 2. Regional meteorological data, pond water parameters, and sponge growth coefficient calculated after Melão and Rocha (1999) for the months of sponge growth. $\Delta^{18}O_{silica-reconstructed\ water}$ 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 characterisation									
Sampling date		Last month of growth	Precipitation (mm)	ETP (mm)	P-ETP (mm)	Monthly mean t_{atm} (°C)	t_{atm} at the time of collection 12 h (°C)	Volume (m^3)	Measured level (cm)	Reconstructed level[a] (cm)	Measured t_{water} (°C)	Reconstructed monthly mean t_{water}[b] (°C)	Dissolved Si ($mg\,L^{-1}$)	Measured $\delta^{18}O_{water}$ (‰ vs. VSMOW)	Reconstructed $\delta^{18}O_{water}$[c] (‰ vs. VSMOW)	Sponge growth coefficient[d]	$\Delta^{18}O_{silica-reconstructed\ water}$ (‰ vs. VSMOW)
	1	Nov 2009	**85**	**133**	**−48**	**26.2**		670 837		48		30.1			3.324	2	
	2	Dec 2009	**413**	**113**	**301**	**24.3**		671 137		94		28.0			0.876	3	
	3	Jan 2010	**125**	**116**	**9**	**26.2**		671 146		95		30.1			0.801	6	
	4	Feb 2010	**83**	**129**	**−46**	**26.8**		671 101		88		30.8			1.174	10	
	5	Mar 2010	**180**	**119**	**61**	**25.4**		671 162		98		29.2			0.677	18	
	6	Apr 2010	**77**	**99**	**−22**	**24.2**		671 140		94		27.9			0.855	32	
	7	May 2010	**61**	**90**	**−29**	**23.9**		671 111		90		27.6			1.092	57	
	8	Jun 2010	**5**	**63**	**−58**	**21.2**		671 053		81		24.6			1.565	101	
	9	Jul 2010	**0**	**73**	**−73**	**22.1**		670 980		70		25.6			2.157	179	
16 Aug 2010		**Aug 2010**	**0**	**84**	**−84**	**22.9**	**17.6**	670 896	**65**	57	**19.1**	26.5	**6.2**	**3.250**	2.838	179	26.8
29 Sep 2010		**Sep 2010**	**5**	**64**	**−60**	**25.0**	**21.8**	670 837	**45**	48	**23.7**	28.8	**5.9**	**4.380**	3.325		
		Oct 2010	**202**	**134**	**68**	**26.4**		670 905		59		30.3			2.768		
23 Nov 2010	1	**Nov 2010**	**312**	**106**	**206**	**24.3**	**22.4**	671 111	**70**	90	**26.5**	28.0	**10.6**	**0.390**	1.088	2	
	2	Dec 2010	**355**	**131**	**224**	**24.9**		671 335		124		28.7			−0.738	3	
8 Jan 2011	3	**Jan 2011**	**346**	**121**	**226**	**25.0**	**24.8**	671 561	**150**	158	**29.3**	28.8	**3.9**	**−5.060**	−2.575	6	
15 Feb 2011	4	**Feb 2011**	**107**	**126**	**−19**	**26.5**	**27.2**	671 541	**160**	155	**29.7**	30.4	**5.8**	**−0.660**	−2.418	10	
3 Apr 2011	5	**Mar 2011**	**410**	**85**	**325**	**24.4**	**22.4**	**671 866**	**205**	205	**30.5**	28.1	**2.8**	**−6.030**	−5.063	18	29.1
1 May 2011	6	**Apr 2011**	**29**	**100**	**−71**	**24.6**	**24.4**	671 795	**195**	194	**26.8**	28.3	**3.4**	**−3.980**	−4.484	32	29.7
5 Jun 2011	7	**May 2011**	**11**	**87**	**−76**	**23.2**	**20.8**	671 719	**172**	182	**24.0**	26.8	**6.1**	**−2.020**	−3.866	57	30.8

[a] According to Eq. (1).

[b] According to Eq. (2).

[c] According to Eq. (3).

[d] According to Eq. (4) (see text for further details).

Table 3. Abundance of spicules of various categories: alpha megasclere (αM); beta megasclere (βM); microsclere (Mi); gemmosclere (Gm). *n*: sum of counted particles.

Last month of growth	Sample	*n*	αM (%)	βM (%)	Mi (%)	Gm (%)
Aug 2010	LV01	300	11	15	17	57
	LV02	300	55	22	12	11
	LV03	300	55	22	12	11
Mar 2011	LV04	300	100	0	0	0
	LV05	300	100	0	0	0
	LV06	300	100	0	0	0
Apr 2011	LV07	300	24	0	75	1
	LV08	300	62	0	38	0
	LV12	300	62	0	38	0
	LV09	300	55	0	44	1
May 2011	LV10	300	48	3	42	7
	LV11	300	17	8	61	14
Control May 2011	LV13	300	25	18	43	14

Table 4. Weighted averages of $\delta^{18}O_{water}$ and water temperature since the start of sponge growth and associated $\Delta^{18}O_{silica-weighted\ water}$ values.

Last month of growth	Spicule growth weighted values $\delta^{18}O_{water}$ (‰ vs. V-SMOW)	Monthly mean t_{water} (°C)	$\Delta^{18}O_{silica-weighted\ water}$ (‰ vs. V-SMOW)
Aug 2010	2.012	26.2	28.388 26.588 27.788
Mar 2011	−3.368	28.9	25.768 27.168 29.368
Apr 2011	−3.873	28.6	29.273 29.573 28.373 28.973
May 2011	−3.870	27.8	30.070 31.570

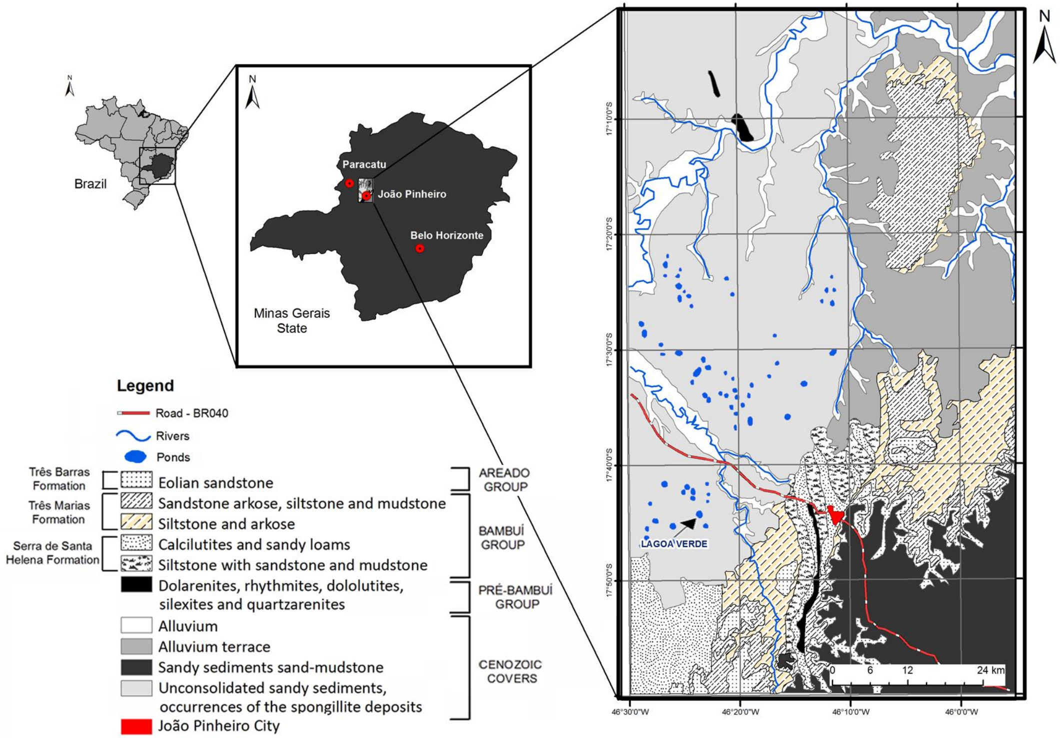

Fig. 1. Geological description of the area and location of the 80 ponds and spongillite deposits reported by Oliveira et al. (2002) in northwestern Minas Gerais, Brazil. Lagoa Verde is indicated by the small arrow. Figure adapted from Oliveira et al. (2002).

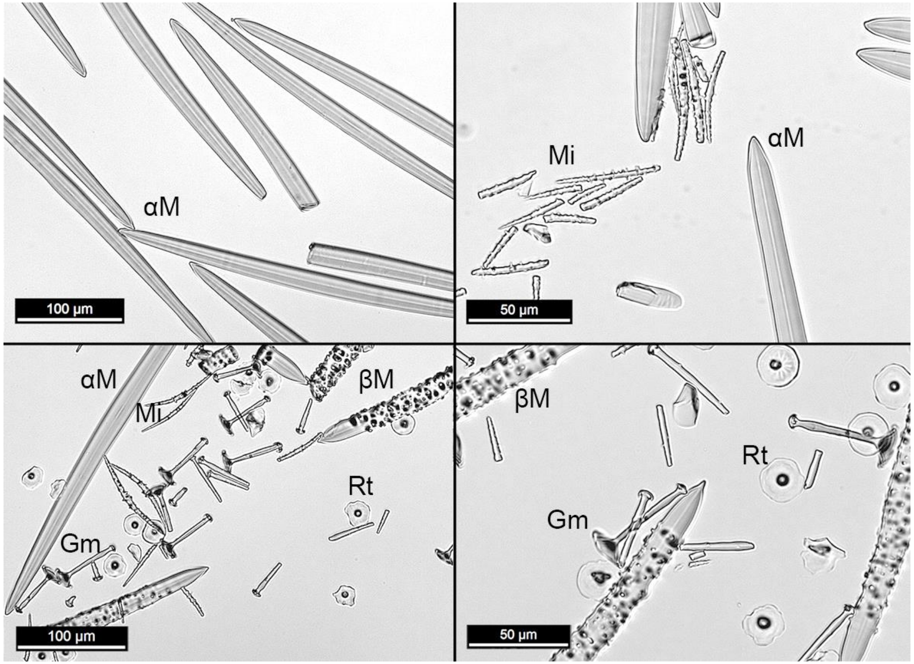

Fig. 2. Optical microscopy images of spicules of *Metania spinata* extracted from Lagoa Verde sponge samples. αM: alpha megasclere; βM: beta megasclere. Mi: microsclere; Gm: gemmosclere; Rt: broken rotule of the gemmosclere.

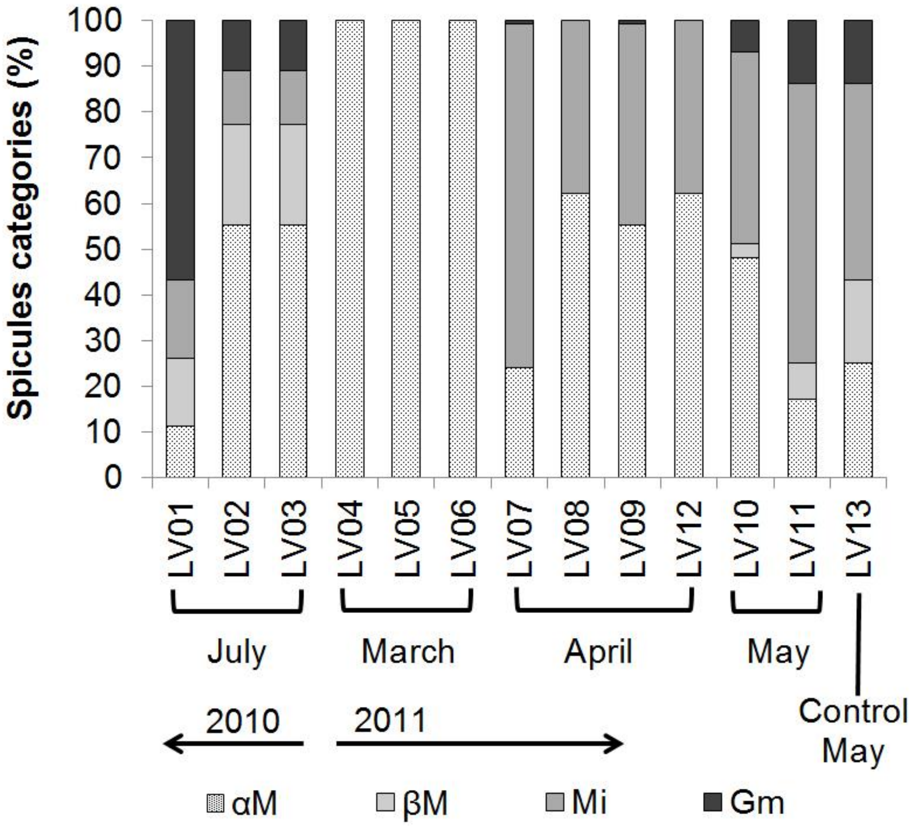

Fig. 3. Percentages of spicule categories per sample. *α*M: alpha megasclere; *β*M: beta megasclere. Mi: microsclere; Gm: gemmosclere.

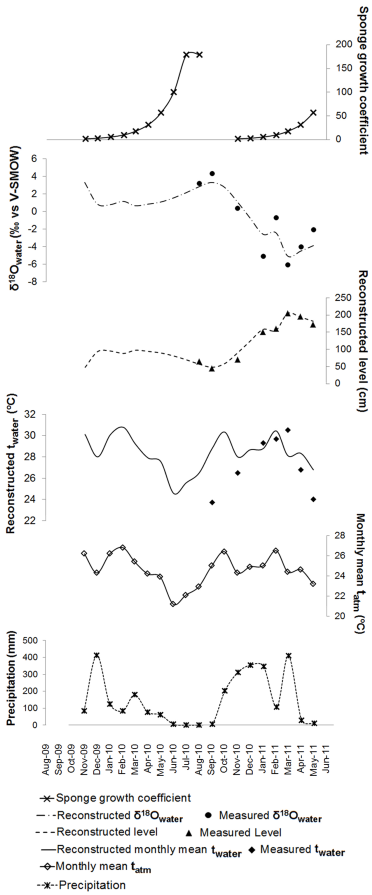

Fig. 4. Reconstructed and/or measured values of monthly precipitation, monthly mean atmospheric temperature (t_{atm}), water temperature (t_{water}), pond level, $\delta^{18}\mathrm{O}_{\mathrm{water}}$, and sponge growth coefficient over the investigated sponge growth period.

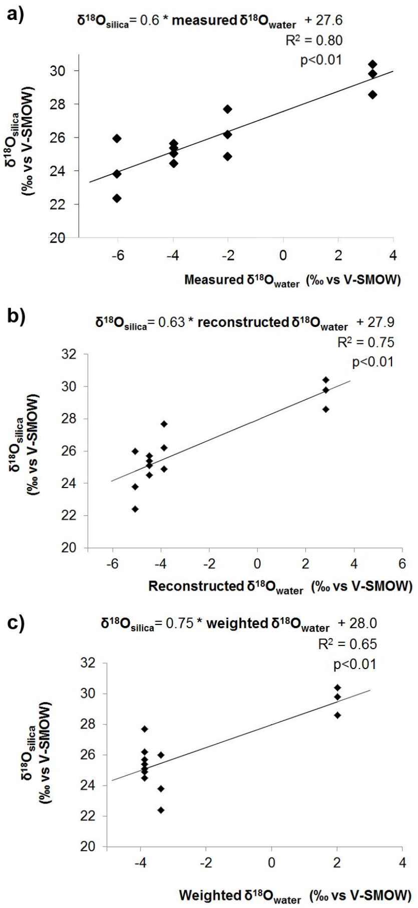

Fig. 5. $\delta^{18}O_{silica}$ values plotted vs. **(a)** measured $\delta^{18}O_{water}$ values at the time of collection, **(b)** reconstructed $\delta^{18}O_{water}$ values, and **(c)** average of weighted $\delta^{18}O_{water}$ estimates since the start of sponge growth.

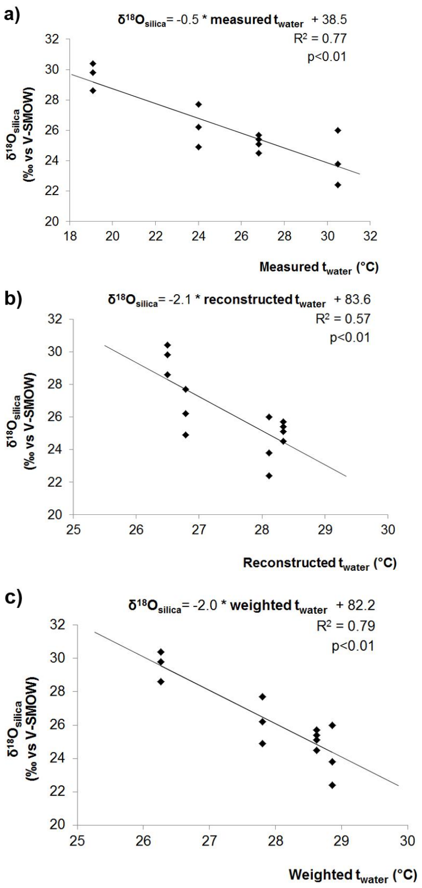

Fig. 6. $\delta^{18}O_{silica}$ values vs. **(a)** measured temperature at the time of collection, **(b)** reconstructed monthly mean water temperature during the latest month of growth, and **(c)** average of weighted monthly mean water temperature estimates since the start of sponge growth.

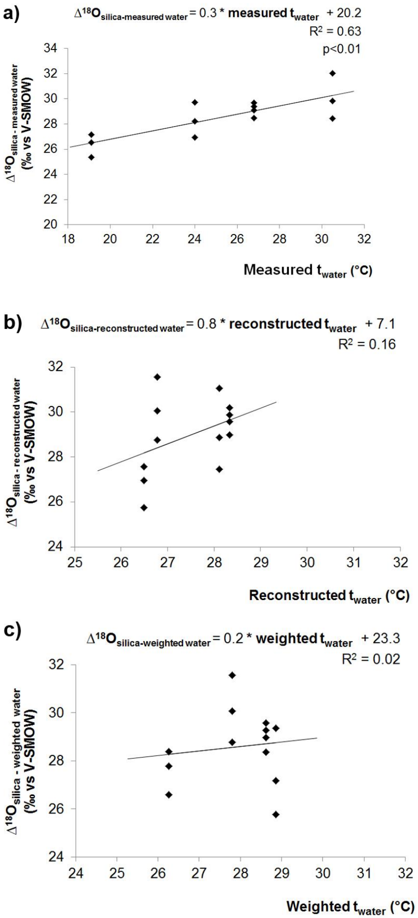

Fig. 7. $\Delta^{18}O_{silica-water}$ values vs. water temperature determined as the **(a)** measured water temperature, **(b)** reconstructed monthly mean water temperature, and **(c)** average of weighted monthly mean water temperature.

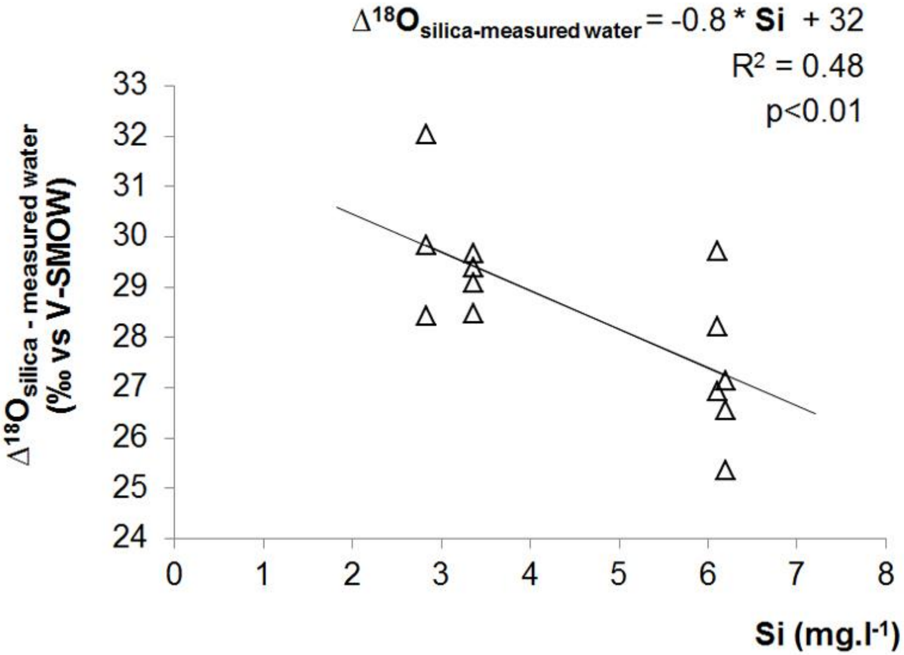

Fig. 8. $\Delta^{18}O_{silica-measured\ water}$ values vs. dissolved Si concentration.