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Environmental factors related to the production of a complex set of spicules in a tropical freshwater sponge

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ABSTRACT

Adverse natural conditions will, generally, induce gemmulation in freshwater sponges. Because of this environmental dependence, gemmoscleres are given exceptional value in taxonomic, ecological and paleoenvironmental studies. Other spicules categories such as microscleres and beta megascleres have received little attention with regard to their occurrence and function during the sponge biological cycle. *Metania spinata*, a South American species common to bog waters in the Cerrado biome, produces alpha and beta megascleres, microscleres and gemmoscleres. To detect the environmental factors triggering the production of all these kinds of spicules, the species annual seasonal cycle was studied. Artificial substrates were devised, supplied with gemmules and placed in Lagoa Verde pond which contained a natural population of *M. spinata*. Field monitoring was conducted for eight months in order to observe the growth of sponges and spicules formation. Samples of water were taken monthly for physical and chemical parameters determination. The appearance of the alpha megascleres was sequentially followed by that of microscleres, gemmoscleres and beta megascleres. The first ones built the new sponge skeleton, the last three were involved in keeping inner moisture in the sponge body or its gemmules. The water level, temperature and the silicon (Si) concentration in the pond were the most important factors related to this sequential production of spicules, confirming environmental reconstructions based on the presence or absence of alpha megascleres and gemmoscleres in past sediments.

Key words: field monitoring, *Metania spinata,* paleointerpretations, spicules.

INTRODUCTION

The silicious spicules produced by sponges of the order Demospongiae, which includes all freshwater sponges, show several morphological categories (Volkmer-Ribeiro 1981, Hooper and Van Soest 2002). The most common spicules are the alpha-megascleres, that support the three dimensional structure of the sponges. With regard to freshwater sponges, the exclusive category of spicules that revest the gemmules, i.e. gemmoscleres, may be present. In species of the South-American genus

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Metania Gray, 1867 (Volkmer-Ribeiro and Costa 1992), two categories of megascleres, alpha and beta megascleres, one category of microscleres and one category of gemmoscleres, are present. After Jørgensen´s works (1944, 1947), most attention has been given to the formation of the megascleres of marine and freshwater sponges, as recently reviewed by Uriz et al. (2003). However, microscleres have not received the same attention. Among 119 bibliographic references focusing on worldwide sponge spicules, only one deals with the formation of microscleres in a marine sponge (Custódio et al. 2002). Nine references refer to freshwater sponges and focus on the megasclere formation and the sponge growth in the frame of laboratory experiments (Uriz et al. 2003).

Studies on continental and marine sponges of temperate areas demonstrated that the formation of the spicules depends on the availability of dissolved silicon (Si) (Jørgensen 1944, 1947, Elvin 1971, Pé 1973, Frøhlich and Barthel 1997, Mercurio et al. 2000) and on water temperature (Simpson 1978). The number and size of the megascleres of freshwater sponges, and thus the sponge skeleton structure and strength, strongly depend on dissolved Si availability (Frost 1991).

Very low contents of dissolved Si were shown to prevent the production of microscleres in freshwater sponges (Jewell 1935). It was also an accepted fact that seasonal shifts in water levels and temperatures can induce gemmulation in modern freshwater sponges, therefore, they produce gemmoscleres (Jewell 1935, Simpson 1984, Frost 1991, Buso-Junior et al. 2012).

Because of this environmental dependence the gemmoscleres, that are the silicious spicules produced for gemmules formation, show exceptional value for paleoenvironmental reconstructions (Racek l974, Harrison et al. 1979, Harrison and Warner 1986, Volkmer-Ribeiro and Reitner 1991, Volkmer-Ribeiro and Turcq l996, Turcq et al. 1998, Sifeddine et al. 2001, Volkmer-Ribeiro et al. 2007, Parolin et al. 2007, Machado et al. 2013, 2014).

However, even though several studies dedicated to freshwater sponges gemmulation/growth under lab or natural conditions (Jewell 1935, Simpson l984, Eggers 2001, Dröscher and Waringer 2007, Volkmer-Ribeiro et al. 2012), and particularly on *Metania spinata* Carter 1881 (Melão and Rocha 1998, 1999), reduced attention has been given to the formation of the different spicules categories during the sponge life cycle.

Studies of Brazilian spongillite deposits (Volkmer-Ribeiro and Motta 1995, Volkmer-Ribeiro et al. 1998a, Almeida et al. 2009, Machado et al. 2014) showed that *M. spinata* is the main sponge species that contributed to form the sponge spicules deposits in bogs of the Cerrado Biome. The assumption was put forward that these spongillites were produced in deeper basins developed under cooler climate than the modern ones, favoring reduced fluctuations of the water level and plentifull supply of Si, for a continued sponge spicule production. The sponge spicules of *M. spinata* observed in those deposits are mainly the alpha megascleres and microscleres. Gemmoscleres and beta megascleres occurred only in deposits produced in shallow bogs more subjected to seasonal lowerings of the water level.

This research aimed to detect *in situ* environmental factors responsible for the production of the complex sets of spicules of the *M. spinata* sponge. More specifically, we tested the hypothesis that the arise of different types of spicules of *M. spinata* during its annual life cycle would be related to the seasonal fluctuations in climate and consequently water level and dissolved Si concentration.

MATERIALS AND METHODS

Study Area

The pond Lagoa Verde was chosen during a preliminary survey as it was shown to only contain the sponge species *M. spinata*. In August 2010, which is the period of low water level, abundant specimens were sampled from the sediment and the dry margin of the pond (Volkmer-Ribeiro 1985). Observations with a light microscope evidenced the presence of only *M. spinata* spicules. Voucher specimens were catalogued together with the bulk sediments in the Porifera Collection of Museu de Ciências Naturais da Fundação Zoobotanica do Rio Grande do Sul (MCN-POR), under numbers: MCN-POR 8657, 8661 and 8707.

Lagoa Verde (17°42'16"S; 46°23'32"W; 572 meters a.s.l., Minas Gerais, Brazil) is located in the Municipality of João Pinheiro, on an 85 km^2 karstic plain covered by Cenozoic siliciclastic sediments. The region has many ponds, several contain spongillite deposits and the living community of *M. spinata* (Almeida et al. 2010) (Fig. 1). The pond covers an area of 0.220 km^2 and reaches 671,866 m^3 of water in April, at the end of the rainy season. In 2011, maximum water depth was ~3 m, reduced by at least 1.6 m at the end of the dry season (September). Local vegetation is dominated by the aquatic macrophyte *Eleocharis interstincta* (Vahl) Roem and Schult and shoreline water grasses on which specimens of the *M. spinata* sponges are encrusted (Fig. 1). Regional vegetation is a wooded savanna known as *cerrado* (Veloso et al. 1991) locally subjected to human disturbances (*Eucalyptus* plantation, livestock and mining).

Climate is tropical humid with a five months dry season during autumn and winter (from May to September). Mean annual temperature is 23.2 ºC and mean annual precipitation is 1562 mm (INMET, Climate normals of 1961-1990, Goiânia's data platform). Summer rains are convective and related to the southern shift of the South Atlantic Convergence Zone (SACZ) 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 usually leads to an Indien summer on the continent (*Veranico*). It is characterized by strong reduction or absence of precipitation, low atmospheric humidity and high temperature.

MONITORING AND SAMPLING

Field monitoring was conducted for a total of eight months over the period of a year (August, September and November 2010, and January, February, April, May and June 2011). During that time, monthly samples of water were taken and concomitantly observation of the sponges was performed. Sponges could only be sampled during the six months when they were more robust (August 2010, and January, February, April, May and June 2011). A total of eight months of water samples and a total of six months of sponge samples were obtained.

The water level was directly read from decimal meter scales painted on two PVC pipes placed at the bottom of the pond bottom at the dry period (Fig. 1). Data on precipitation was obtained from the INMET station (Instituto Nacional de Meteorologia, Platform Data Collection #83481, João Pinheiro, www.inmet.gov.br), located 21 km east of the study site.

The most important variables related to the monthly input and output volumes of water in the system, the precipitation (P) and evapotranspiration (ETP) values were obtained from the INMET data. The balance (incorporated) or deficit (loss) of water in the aquatic system was calculated by subtracting P from ETP.

PHYSICAL AND CHEMICAL PARAMETERS

Water samples were obtained in August, September and November 2010, and January, February, April, May and June 2011. Each sample of 30 ml was filtered at 0.45 µm and acidified for concentration analysis of dissolved elements (Si, Ba, Ca, Fe, K, Mg, Mn, Na and Sr) by ICP-OES (Spectro Cirus CCD).

The water temperature, pH, conductivity (COND, uS/cm), resistivity (RES, Ω.cm), total dissolved solids (TDS) and oxidation-reduction potential (ORP, mV) were measured with a portable Ultrameter™ in the field, at midday, 20-30 cm below surface. Three readings at least, were performed during a sampling month and the averages were considered.

Artificial Substrates and Their Placement in the Field

Artificial substrates were used in order to investigate which and when environmental conditions would account for the appearance of each one of the four categories of spicules of *M. spinata* along its seasonal cycle in the pond. Black tulle bags (40x40 cm, 2 mm mesh), were used, each supplied with about three nodules (about 1 cm) of *M. spinata* gemmules inside. The gemmules were taken from the dry specimens sampled during the preliminary survey (August, 2010). Fifteen bags were hung on three nylon lines, each along a transect of 20-25 m extending from the emerged margins towards the immersed center (Fig. 1), and left to lay down on the pond floor. The outer ends of the nylon lines were tied at marginal trees. One of the lines was vandalized so that a total of 30 bags remained for analyses.

The substrates were placed on the pond floor in September 2010, at the end of the dry season. As a

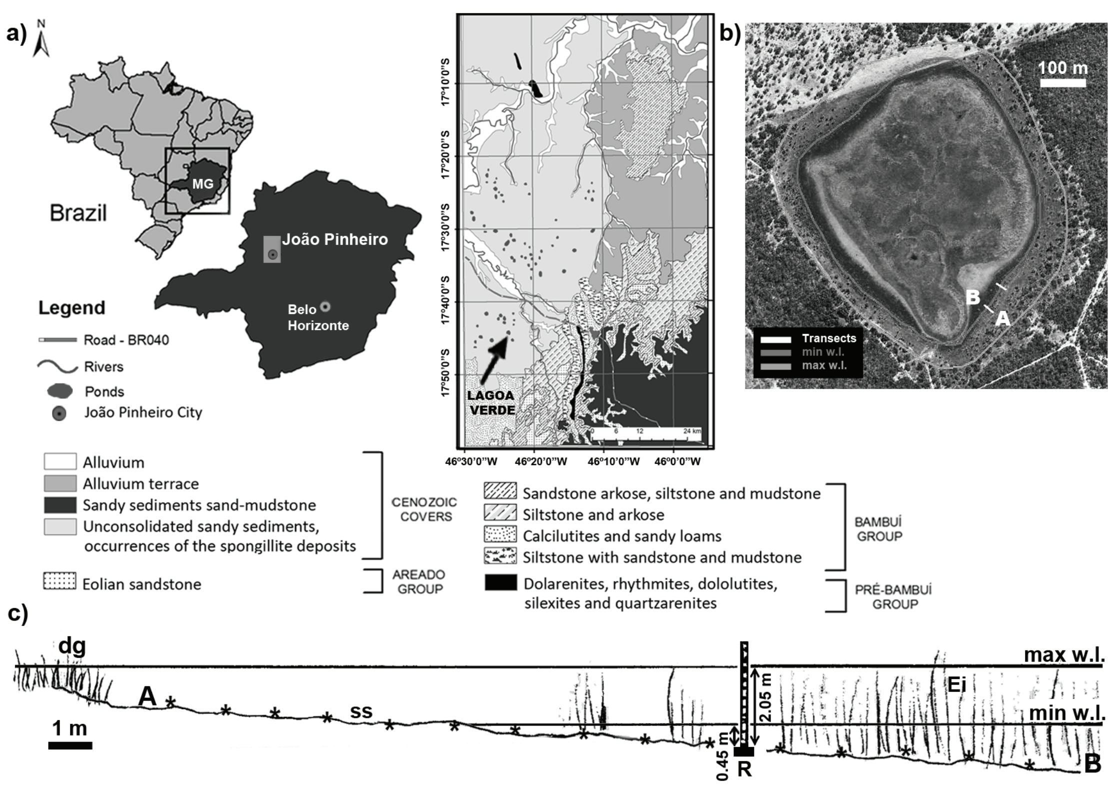

Figure 1- a) Localization area and geological map showing the Lagoa Verde in the João Pinheiro region. Northwestern of the state of Minas Gerais (MG). Brazil. Modified from Oliveira et al. (2002). **b**) Google Earth image on August 24th 2010, showing minimum and maximum water level and the sampling 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*. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

control, three empty bags were placed on the pond floor, each one hanging from a buoy in May 2011 and collected at the beginning of June 2011 (Table I). During the survey of the sponge cycle, all the bags were pulled out of the water for observation of growth and then returned to the pond. Whenever sponges were seen forming crusts on the surface of the bags, one to three bags were scrapped to obtain a sample of sponge for spicules preparation.

Pieces for spicules preparations were also taken from dried sponges standing at the exposed pond margins in August 2010 (Table I), on emergent aquatic macrophyte *Eleocharis interstincta* in May and June 2011 (Table I), and on the pond underwater sediments in May 2011 (Table I).

Obtaining the Spicules

Sponge samples were processed according to Volkmer-Ribeiro (1985) in order to separate clean spicules from organic matter and allow them to be placed on permanent slides thus facilitating their categorization. Three slides were prepared from each sponge sample.

Spicule measurement and statistical analysis

To estimate the proportions of different types of spicules we counted 300 spicules of each sponge sample under an optical microscope at 10x magnification. We made a trial counting the three slides prepared, and observed that confident proportions could be obtained counting 300 spicules in one slide.

To investigate possible differences in the sizes of spicules caused by external factors, the alpha-megascleres were compared since they constitute the only category present during the entire life cycle of the sponge. Using the Kruskal-Wallis statistic method, possible differences of the megascleres sizes were tested taking into account their origin from: 1) substrate type (artificial *vs* natural); 2) different depths.

Redundancy analysis was used to explore the relationship between the composition and frequency of the different types of spicules and the environmental factors measured for the months of August 2010 and January, February, April, May and June 2011. The environmental data were previously standardized and centralized.

Linear regression was used with data from the logarithmized variables (to meet the homoscedastic requirement) to relate the frequency of different categories of spicules with environmental factors. The best model was chosen based on the lowest Akaike Information Criterion (AIC) value.

We used the software R for all analyses. We used function RDA of package Vegan (Oksanen et al. 2013) for redundancy analysis and the function lm of the base package for linear regression.

RESULTS

Environmental Parameters

The water level of Lagoa Verde decreased 1.6 meters during the dry season due to regional decrease in precipitation and increase in evapotranspiration (INMET data). The seasonality of rainfall caused variation in the balance of P-ETP ranging from -84 mm (August 2010) to +324 mm (March 2011). The water temperature ranged from 19.1 °C (August 2010) to 30.5 °C (April 2011) (Table I).

The most of the dissolved elements were concentrated during the dry season and diluted during the rainy season, with a peak in concentration at the end of the dry season in September. Si concentration showed a different pattern with a delayed peak of concentration in November (Table II), probably due to the drainage of the terrestrial siliceous substrate by the first rains.

Regarding the physical and chemical conditions, there is high conductivity, a low resistivity, a high concentration of dissolved solids and higher acidity during the dry season.

TABLE I

Collected data for *Metania spinata* at LagoaVerde.: Month, sampling number, descriptions of samples and substrates, appearance of the spicules categories (αM: alpha megasclere; Mi: microsclere; ßM: beta megasclere; Gm: gemmosclere), their abundance, corresponding data for dissolved silicium (Si mg/L), water temperature (°C), water level (m), monthly pluviometry (mm), precipitation-evapotranspiration (P-ETP), seasonal hydric condition and the growing cycle.

Month of growth	Sample	Artificial *vs* natural substrate	Description	αM	Mi	βM	Gm	Si	Water temperature	Water level	Month precipitation	P-ETP	Environment	The sponges
				(%)	(%)	(%)	(%)	(mg/L)	(°C)	(m)	(mm)	(mm)		
AUG	1	Natural – dried grasses	Full presence of α and β megascleres, microscleres and gemmoscleres	1.6	12.5	2.2	83.7	6.20	19.1	0.65	0	-84	Bog edge exposed	Dry on the edge.
	2	Natural – dried grasses		22.3	24.3	8.9	44.5							
	3	Natural – dried grasses		22.3	24.3	8.9	44.5							
SEP			Placing of artificial substrates					5.90	23.7	0.45	5	-60	Bog edge exposed	Dry on the edge.
OCT			No field trip								202	68		
NOV			Initial observation					10.55	26.5	0.70	312	206	First rains, input of the [Si]	Initial growth on the bags
DEC			No field trip								355	225		
JAN	4	Artificial – Bag4	Only α-megascleres	100	0	0	0	3.92	29.3	1.50	346	226		Sponges with 2-3 cm length on the bag
	5	Artificial – Bag5		100	0	0	0							
	6	Artificial – Bag6		100	0	0	0							
FEB	7	Artificial – Bag7	Only α-megascleres	100	0	0	0	5.82	29.7	1.60	107	-20		Sponges with 4-5 cm length on the bags
	8	Artificial –Bag8		100	0	0	0							
	9	Artificial –Bag9		100	0	0	0							
MAR			No field trip								410	324		
APR	10	Artificial – Bag B8	Only α-megascleres	100	0	0	0	2.83	30.5	2.05	29	-71	High level water	Sponges >7 cm length on the bags
	11	Artificial – Bag A8		100	0	0	0							
	12	Artificial – Bag B13		100	0	0	0							

TABLE I (continuation)

Month of growth	Sample	Artificial *vs* natural substrate	Description	αM (%)	Mi (%)	βM (%)	Gm (%)	Si (mg/L)	Water temperature (°C)	Water level (m)	Month precipitation (mm)	P-ETP (mm)	Environment	The sponges
MAY	13	Natural – *E. interstincta*	Alpha-megascleres, microsleres, and rare gemmoscleres	5.9	91.7	0	2.4	3.36	26.8	1.95	11	-76	Precipitation, water level and temperature start decreasing	No gemmules
	14	Natural – sediment pond		24.6	75.4	0	0							
	15	Natural – *E. interstincta*		24.6	75.4	0	0							
	16	Artificial – Bags A7+A13+A16		19.3	77.2	0	3.5							
JUN	17	Natural – *E. interstincta*	Full presence of α and β megascleres, microscleres and gemmoscleres	14.5	63.4	0.9	21.1	6.11	24.0	1.72	2	-67	Decrease of rainfall, level water and temperature	Gemmules start to form
	18	Natural – *E. interstincta*		3.6	64.9	1.7	29.8							
	19	Artificial - Control Bag		6.3	54	4.5	35.2							

TABLE II

Dissolved elements in water of Lagoa Verde pond along the *Metania spinata* cycle. *n*: sample numbers, SD: standard deviation.

	MONTH	n	Si		Ba		Ca		Fe		K		Mg		Mn		Na		Sr	
			mg/L		µg/L		mg/L		µg/L		mg/L		mg/L		µg/L		mg/L		µg/L	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
2010	AUG	1	6.20		15.30		0.28		412.0		0.73		0.12		27.50		0.49		2.51	
	SEP	2	5.90	± 0.01	73.15	± 19.16	1.25	± 0.30	2069.5	± 821.0	2.30	± 0.05	0.42	± 0.05	89.55	± 14.64	0.77	± 0.01	10.60	± 1.70
	NOV	2	10.55	± 0.07	15.60	± 1.41	0.27	± 0.02	461.5	± 109.6	0.72	± 0.02	0.15	± 0.00	72.70	± 0.00	0.29	± 0.04	2.65	± 0.04
2011	JAN	2	3.92	± 0.01	12.95	± 0.92	0.31	± 0.02	224.0	± 151.3	0.84	± 0.05	0.16	± 0.00	46.65	± 0.92	0.12	± 0.00	2.85	± 0.01
	FEB	2	5.82	± 0.02	10.37	± 1.18	0.18	± 0.01	350.5	± 188.8	0.72	± 0.00	0.15	± 0.01	6.01	± 0.69	0.16	± 0.01	2.07	± 0.07
	APR	4	2.83	± 0.17	5.36	± 0.80	0.18	± 0.04	98.2	± 38.0	1.76	± 1.75	0.17	± 0.04	11.49	± 3.74	0.14	± 0.04	2.63	± 0.35
	MAY	3	3.36	± 0.02	3.81	± 0.42	0.07	± 0.01	26.5	± 3.2	0.81	± 0.09	0.08	± 0.01	2.86	± 0.39	0.16	± 0.02	1.25	± 0.09
	JUN	2	6.11	± 0.11	3.79	± 0.08	0.08	± 0.00	23.5	± 0.6	0.61	± 0.02	0.06	± 0.00	2.64	± 0.11	0.11	± 0.00	0.96	± 0.00

These values indicate a larger concentration of free ions in the water, including the presence of macronutrients during the dry season. The redox potential presented no trends between the different stations, although there were irregular variations, which indicates that throughout the year the pond has a similar capacity of oxidizing the organic and inorganic matters (Table III).

SPONGE MONITORING

The monitoring of the seasonal life cycle of *M. spinata* in Lagoa Verde, started in August 2010 (Figs. 2a, b). The sponge specimens sampled in August 2010 were the most complete they could get to at the site. In September 2010, when the artificial substrates were put in the pond (Fig. 2c), there was no sampling of sponges and the water level was even lower than in August (Table I). In November 2010, sponges were beginning to grow on artificial substrates. Some were still white, while others were green due to their symbiotic association with algae (Melão and Rocha 1997). No sampling was conducted due to the small size of the sponges (Fig. 2d). In January and February 2011, when the water level had risen considerably, the sponges were all green, forming thin and fragile filaments which measured 2-3 cm long in January, and 4-5 cm in February (Fig. 2f, 2g). The examination under the optical microscope of the cleaned spicules showed the exclusive presence of alpha megascleres (Fig. 3, Table I). In April 2011, the water level reached its maximum (2.05 m), and a boat was needed to reach the artificial substrates. The sponges were 7 to 10 cm long, less fragile (Fig. 2e), and the spicular dissociation again showed the exclusive presence of alpha megascleres. In May 2011, when the water level began to decrease, the sponges were over 10 cm in length and some showed branches without any apparent formation of gemmules (Fig. 2h). Lastly, the assembly of spicules changed, with the appearance of microscleres and rare gemmoscleres as well as the existing alpha megascleres (Figs. 3 and 4). In June 2011, the water level continued to recede and the sponges were robust, with branches, and relatively hard nodules containing gemmules (Fig. 2i). In the samples from June 2011 and August 2010, all the categories of spicules were found, with the appearance within their composition of beta megascleres (Table I, Figs. 2 and 3).

In the control samples, the sponges were growing on the artificial substrate, and therefore the use of manufactured substrates in monitoring sponges, without the need to introduce gemmules, was shown to be effective.

TABLE III
Physical and chemical parameters water of Lagoa Verde along the *Metania spinata* cycle. *n*: number lectures; COND: conductivity (µS cm^{-1}); RES: resistivity (KΩ cm^{-1}); TDS: total dissolved solids (mg L^{-1}); ORP: oxidation/reduction potential (mV); pH.

	MONTH	*n*	COND (µS cm^{-1})	RES (KΩ cm^{-1})	TDS (mg L^{-1})	ORP (mV)	pH
2010	AUG	4	-	-	-	-	5.33 ± 0.0
	SEP	3	42.7 ± 0.2	22.9 ± 0.14	27.43 ± 0.15	260.0 ± 17	5.04 ± 0.0
	NOV	5	6.9 ± 0.3	141.8 ± 7.2	4.43 ± 0.2	248.2 ± 12	5.42 ± 0.1
2011	JAN	5	7.5 ± 0.4	103.1 ± 6	4.89 ± 0.3	198.4 ± 5	5.45 ± 0.2
	FEB	5	8.7 ± 2.7	124.2 ± 37.1	5.6 ± 1.9	254.4 ± 8.1	5.40 ± 0.0
	APR	5	6.0 ± 0.2	177.8 ± 8.6	3.80 ± 0.2	186.6 ± 9.3	5.90 ± 0.0
	MAY	6	5.9 ± 0.4	169.6 ± 10.6	3.80 ± 0.2	230.0 ± 17.2	5.50 ± 0.1
	JUN	6	6.2 ± 0.3	159.8 ± 7.5	3.9 ± 0.2	248.3 ± 3.9	5.60 ± 0.1

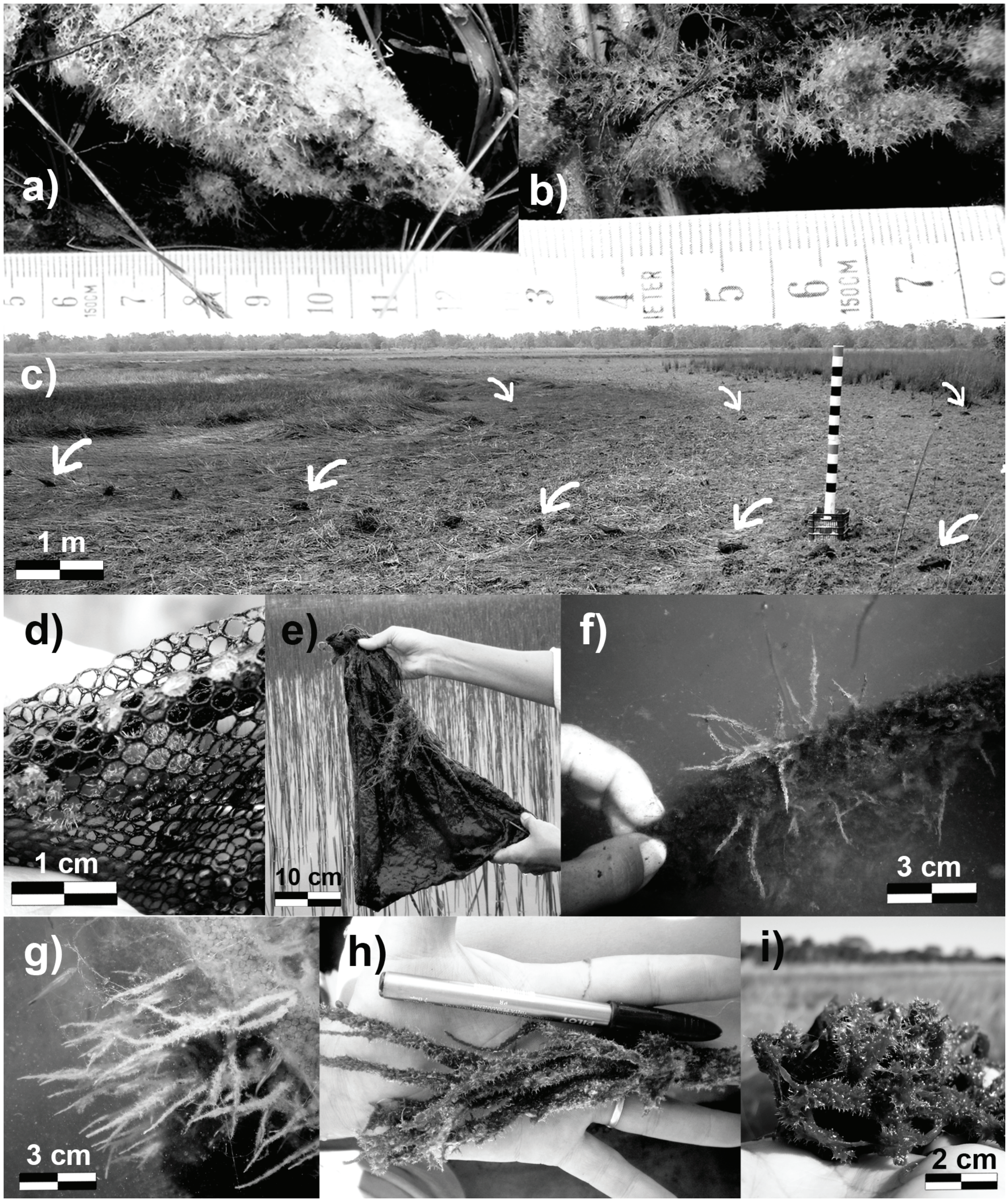

Figure 2 - Field monitoring of *Metania spinata* at Lagoa Verde. **a**) Specimen at the dry exposed pond margin, August 2010. **b**) Dry specimen with conspicuous gemmules at the exposed pond margin, September 2010. **c**) Placing of the tule bags along the two lines on the exposed marginal bottom of the pond. One of the PVC pipes with a 2ms scale to measure the level. **d**) Minute sponges starting their settling and growing on the artificial substrate, November 2010. **e**) Stringy sponge encrusting on the outer side of the tule bag during the cycle monitoring, April 2011. **f**) and **g**) Upright growing of the sponge fibers on the artificial substrate, January and February 2011 respectively. **h**) One thicker branch is detached from the sponge crust on the artificial substrate, May 2011. **i**) Specimen taken from the pond natural substrate bearing the gemmular nodules, June 2011. (See the colors in the online version).

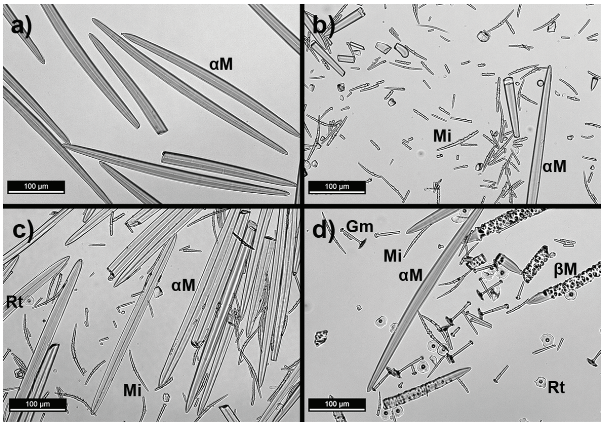

Figure 3 - *Metania spinata*. Images from the Optical Microscope, in their order of appearance, of the spicules from specimens sampled on artificial substrates at Lagoa Verde: **a**) January, February and April. **b**) May. **c**) June. **d**) August. Spicules categories: αM: alpha megasclere; Mi: microsclere; Rt: rotule of broken gemmosclere; βM: beta megasclere; Gm: gemmosclere.

The presence of the alpha megascleres represented 100% of the species spicular set by April, when the peak of high water was reached. On May they came down to 5.9-24.6%, when the microscleres showed up, composing 75.4-91.7% of the sponge spicules, coupling with a first reduction of the water level. The beta megascleres and gemmoscleres appeared in June, the first ones representing 0.9-4.5% and the second ones 21.1-35.2% of the sponge spicular set, whilst alpha megascleres were reduced to 3.6-14.5% and the microscleres to 54-64.9%. In August 2010 the beta megascleres of the dry sponge on the edge represented 2.2-8.9% and the gemmoscleres 44.5-83.7% of the sponge spicules, coupling with the production of the gemmules, the low water level and the higher concentration of the available dissolved Si. At this time alpha megascleres were 1.6-22.3% of the sponge spicular set and microscleres were 12.5-24.3% (Fig. 4, Table I).

The size of the alpha-megascleres ranged from 325 to 495 µm in length and from 13 to 30 µm in thickness. The size of the beta-megascleres varied from 220 to 350 µm in length and from 10 to 25 µm in thickness. The microscleres ranged from 50 to 125 µm in length and from 4 to 6 µm in thickness. The gemmoscleres ranged from 35 to 65 µm in length, 4 to 5 µm in thickness and had rotules with diameters ranging from 15 to 28 µm (Table IV).

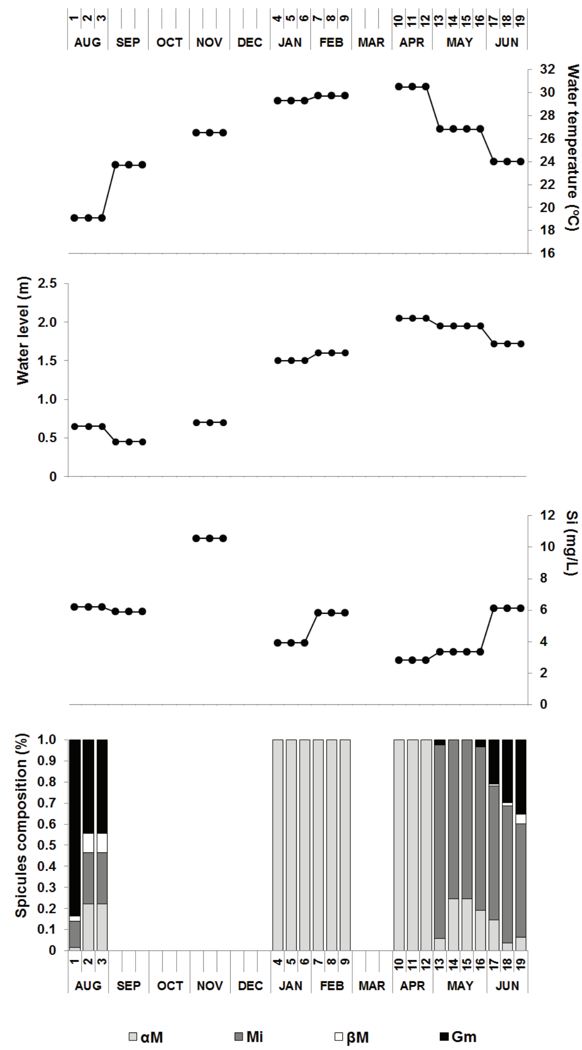

Figure 4 - *Metania spinata*. Bar graph showing the seasonal appearance of the four categories of spicules in Lagoa Verde between August 2010 and June 2011. At the top of graph the aligned points represent the respective seasonal data for dissolved Si(mg/l), water level and water temperature. Samples 1-3: sponges dry on the edge of pond; Samples 4-12, 16, 19: sponges on bags; Samples 13-15, 17-18: sponges on natural substrates. αM: alpha megasclere; Mi: microsclere; ßM: beta megasclere; Gm: gemmosclere.

TABLE IV
Spicules measurements of the *Metania spinata* sponge. Sizes in micrometers (µm). *n*: measurements number, SD: standard deviation, Min: minimal measurements, Max: maximal measurements.

		Alpha Megasclere				Beta Megasclere				Microsclere				Gemmosclere					
Spicules measurements		Length		Width		Length		Width		Length		Width		Length		Width		Rotule diameter	
	n	**Mean (µm)**	SD	**Mean (µm)**	SD	**Mean (µm)**	SD	**Mean (µm)**	SD	**Mean (µm)**	SD	**Mean (µm)**	SD	**Mean (µm)**	SD	**Mean (µm)**	SD	**Mean (µm)**	SD
MEAN	**465**	**420**	**31**	**20**	**3**	**270**	**24**	**19**	**3**	**84**	**11**	**5**	**1**	**49**	**5**	**4**	**1**	**23**	**2**
Min		325	-	13	-	220	-	10	-	50	-	4	-	35	-	4	-	15	-
Max		495	-	30	-	350	-	25	-	125	-	6	-	65	-	5	-	28	-

No significant differences were found between the lengths of the sponge alpha-megascleres, whether located on natural or artificial substrates ($p>0.05$). Moreover, there was no difference in the measurements of spicules obtained from different depths ($p>0.05$). Thus the different substrates or depths at which the sponges developed did not affect the size of the alpha-megascleres.

The first two axes of the RDA (Redundancy Analysis) cumulatively explained 80.7% of the variations in the composition and frequency of spicules over the six sampling periods. The water level, water temperature and dissolved Si were the most important parameters (Fig. 5). Precipitation is the most important factor related with the presence of alpha-megascleres, and dissolved Si is the most important factor related to the presence of gemmoscleres (Fig. 5).

Additionally, in the linear regression regarding the specific proportionality of spicular categories with the environmental variables that influence their composition, the simple models were the most robust (lowest AIC value, Table V).This suggests that the presence of alpha-megascleres are directly related to higher precipitation, microscleres are inversely related to precipitation, and that the presence of beta-megascleres and gemoscleres are directly related to the high concentration of Si in the water.

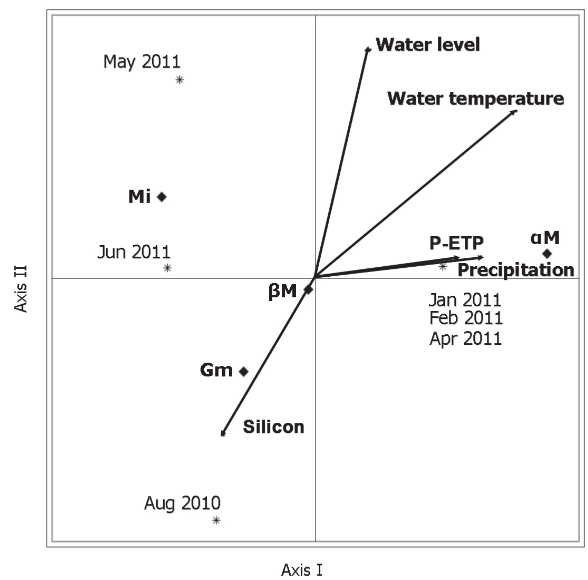

Figure 5 - *Metania spinata*. Redundancy Analysis (RDA) evidences the most probable relationship of the seasonal production of the four categories of spicules with the corresponding environmental parameters in Lagoa Verde (precipitation, P-ETP, water temperature, water level and Silicon). Spicules categories: αM: alpha megasclere; Mi: microsclere; βM: beta megasclere; Gm: gemmosclere.

DISCUSSION AND CONCLUSIONS

A first item deserving attention refers to the function of the artificial substrate. The empty bags placed as control showed the same progressive external encrusting by *M. spinata* as the bags with planted gemmules inside, evidencing a substrate occupation from outside. It could arise either from arqueocytes liberated from the past season gemmules at the pond

TABLE V
Best regression models of the influence of environment variables on the percentage of spicules categories of *Metania spinata* in LagoaVerde pond between August 2010 and June 2011 (*n* = 14). SE = Standard Error.

Coefficients	Estimates ± SE	p
Alpha-megascleres		
Intercept	1.439±0.081	<0.001
log(Precipitation)	0.201±0.056	<0.004
Beta-megascleres		
Intercept	-2.723±0.365	<0.001
log(Silicon)	4.446±0.490	<0.001
Gemmoscleres		
Intercept	-2.711±0.347	<0.001
log(Silicon)	4.553±0.466	<0.001
Microscleres		
Intercept	1.648±0.195	<0.001
log(Precipitation)	-0.435±0.135	<0.007

bottom, or from swimming larvae from sponges encrusting the pond natural substrates and thus able to perform an early sexual reproduction (Frost 1991), with the summer regional rains. The alpha megasclere category of spicule showed the same range of size found by Volkmer-Ribeiro and Costa (1992). However, the length range was superior to those reported by Ezcurra de Drago (1975) and Machado et al. (2012), and the average thickness was much larger than that found by Machado et al. (2012). Regarding the other spicules types, the beta-megascleres presented larger sizes than the only ones available for comparison, described by Ezcurra de Drago (1975) while both the microscleres and gemmoscleres were very similar, although slightly larger. Alpha megascleres studied from sponges sampled at differing levels in the natural substrates had the same length as the ones from the artificial substrates showing that the natural biological cycle was correctly monitored using the artifical substrates.

Investigations on sponge biomass and growth rates were carried out by Frost et al. (1982) and Melão and Rocha (1998) respectively. Artificial substrates were placed in the natural habitat containing weighted and measured pieces of *Spongilla lacustris* and *M. spinata* respectively. These results raised the issue as to whether these studies were not affected by the encrusting of new sponges around the ones originally placed in the cages.

The *M. spinata* annual cycle conforms however, to the biological seasonal sequence of growing, gemmule formation and decaying, detected by Frost et al. (1982) in a population of *Spongilla lacustris,* the only species inhabiting a northern sphagnum pond. Of particular interest is the fact that *S. lacustris* as well as *M. spinata* naturally encrusted the respective ponds muddy bottoms besides its aquatic macrophytes.

High Si concentration measured in Lagoa Verde at the beginning of the rainy season suggested that a significant input of Si comes either from the drainage of the silica of geological origin in the watershed, or from the contribution of sponge spicules detached from the lakeside vegetation by the voluminous rainfalls. The increase of Si in water favors the provision of monosilicic acid ($Si(OH)_4$), available for sponge uptake. This event coincides with the eclosion of the gemmules and the development of new specimens, which were observed to grow naturally on the vegetation, in the lake sediment and on the artificial substrates.

The RDA allowed identifying the main environmental factors associated with the different types of spicules (presence and absence). For January, February and April, a match was found between the highest values of pH, water temperature, P-ETP and less directly with high water level and the absence of the beta megascleres, microscleres and gemmoscleres. In May, a rapid production of microscleres, and the beginning of gemmosclere production was found to occur, resulting in a mixture of alpha megascleres, microscleres and rare gemmoscleres, probably due to the increase of nutriment (including Si) concentrations. In June and August the abundance of microscleres and gemmoscleres, including beta megasclere, which are auxiliary in the formation of gemmules, can be related to the increasing concentration in dissolved Si and the lowest water levels due to low P-ETP values.

The appearance of the four categories of spicules of *M. spinata* was seen to be sequential. Alpha megascleres were the only ones to appear during the initial steps of the sponge encrusting and growing (January-April 2011) thus conforming to the traditionally accepted supporting role of this spicule category. The production of this spicule category continued along the sponge seasonal cycle, but decreased after the initial phase, then giving place to a microsclere burst.

However, the production of the microscleres preceeding that of gemmoscleres and beta megascleres is intriguing. Their complementary role into the strengthening of sponge structures as the canals or the ectoderm/pinacoderm (Bergquist 1978, Uriz et al. 2003) is apparent in *M. spinata,* a species which has stringy delicate growths. The microscleres appear as an important silicious component, being reduced in numbers only when the gemmoscleres attained their full appearance, as can be inferred from spicular analysis of the dry sponges sampled at the pond margins in August 2010. The massive presence of microscleres preceeding the formation of gemmules, poses a new question. The immediate reversal of the pinacoderm porocytes in a freshwater sponge with a whole life cycle subjected to a very short period of immersion (Buso-Junior et al. 2012) raises the suspicion that microscleres, which have a preference for encrusting the sponges pinacoderm, may also contribute with an extra sealing of the animal´s outermost boundary. Thus, porocytes reversal and the microclere armature would act together as the first device towards the saving of the sponge´s inner moisture against any reduction in the habitat water level. This suspicion is strengthened by the fact that as long as the *M. spinata* microscleres started to appear, a minimum amount of its gemmoscleres was also detected.

On the other hand, the beta megascleres appeared when the gemmulation process was firmly established. These megascleres conform thus two extra function of spicules: the one to provide for the retention of the gemmules into the skeleton and the other to provide for an extra gemmullar sealing. However, one more assumption has to be presented: that beta megascleres, which are the more robust, heavily spined spicules produced by this sponge, may also act into the anchoring of the gemmulles in the bottom mud, at the same time providing for an immediate silica source at the eclosion time.

As a matter of fact new green crusts of the sponge were detected not only on the natural and the artificial substrates but also on the pond bottom as also observed by Frost et al. (1982) for *Spongilla lacustris* in a northern bog. In spite of the fact that the monitoring experiment was not carried throughout a whole year, the spicular spectrum from the samples taken in June 2011, couples well with the one obtained in August 2010, indicating what must be the climax of the sponge seasonal biological cycle. At that moment microscleres and alpha megascleres attained their minimal abundance, the silica being taken in favor of the production of gemmoscleres and beta megascleres.

M. spinata is the only neotropical species of the genus to exhibit such long, thin and spiny microscleres and long, thick and spiny beta megascleres. If habitats are compared among *M. spinata, M. reticulata, M. fittkaui and M. kiliani,* all species with beta megascleres, (Volkmer-Ribeiro and Costa 1992) the first one is the only with a fleshy skeleton and only one to live in shallow lentic environments, whilst the other three have hard skeletons permanently adhering to branches and leaves of the seasonal flooded forests along Amazonian rivers. In such species the gemmules are retained in the hard dried sponge by the capsules of beta megascleres and ecclode in the next flooding period forming a new thin layer of living sponges on top of the previous skeleton thus producing remarkable silicious encrustings on the substrates (Volkmer-Ribeiro et al. 2012).

The diversity of the regional climatic conditions was the unexpected factor which certainly brought out the didactic sequence of appearance of all the spicules of *M. spinata* as an answer to the play between the two most impacting factors affecting the sponge biological cycle at Lagoa Verde, i.e. dissolved Si and water level.

The results allow us to perceive the different composition of spicules that *M. spinata*, present in pond waters from north (Ezcurra de Drago 1975) to southeast South America (Volkmer-Ribeiro 1999) may offer, whenever its speciments/spicules are detected.

M. spinata occurs with only megascleres and reduced amount of microscleres encrusting all the marginal vegetation along Lago do Caçó, a past dune area at Maranhão State (Volkmer-Ribeiro et al. 2001). The place, in spite of its name, corresponds to the damming of a permanent brook with an inlet and an outlet. The species was identified from quite rare gemmoscleres present in some specimens. The bottom sediments contained only alpha megascleres and microscleres (Volkmer-Ribeiro et al. 2001).

A quite different lentic habitat was studied in Maraca several strongly seasonal ponds at Roraima State, northern most Brazil (Volkmer-Ribeiro et al. 1998b). Living *M. spinata* occurred together with also living specimens of the other sponges which have spicules composing spongillite deposits in Midwestern Brazil. *M. spinata*, as well as the other species formed abundant gemmules and contained all the four spicules categories seen in Lagoa Verde. However, no spicules of any of the sponge species were found in the bog bottom sediments. The lake had very poor seasonal supply of silica and thus all the available biosilica (from degrading vegetation, diatoms, and previous season sponge spicules) was certainly being retaken from the bog pond sediment.

M. spinata was detected in sediments from Central Brazil, dated from at least 50.000 yr BP (Machado et al. 2014). When detected in past lentic sediments its spicules have been used as proxy data to support paleoenvironmental interpretations (Cândido et al. 2000, Parolin et al. 2007, Almeida et al. 2009, 2010, Machado et al. 2014). However, these interpretations were mostly based on the available knowledge indicating that gemmoscleres/beta megascleres would stand for water reduction in seasonal climates, whilst megascleres and microscleres taken both as supporters of the skeleton structures, allied to absence of gemmoscleres, would indicate absence of marked seasonality and sustained water input. The results presented here support and increase the confidence on the use of these spicules as proxy data. The microscleres stand up now with the remarkable value of indicating water level shifts of lower magnitude, followed or not by drought periods. The study now dedicated to *M. spinata* indicates that any of the other five species composing the sponge assemblies of these Cerrado Biome bogs are entitled to an also particular search for the functions their spicules play along their biological cycle and corresponding entrusting as proxy data.

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RESUMO

Condições naturais adversas, geralmente, induzirão gemulação nas esponjas de água doce. Devido a essa dependência ambiental, é atribuído excepcional valor às gemoscleras, em estudos taxonômicos, ecológicos e paleoambientais. Outras categorias de espículas, tais como as microscleras e beta megascleras, têm recebido pouca atenção com respeito à sua ocorrência e função durante o ciclo biológico da esponja. *Metania spinata*, uma espécie sul-americana comum em águas de turfeira no Bioma Cerrado, produz alfa e beta megascleras, microscleras e gemoscleras. Para detectar os fatores ambientais indutores da produção de todas essas categorias de espículas, foi estudado o ciclo sazonal anual da espécie. Substratos artificiais foram criados, supridos com gêmulas e colocados na Lagoa Verde, que continha uma população natural de *M. spinata.* Monitoramento em campo foi conduzido por oito meses visando observar o crescimento de esponjas e a formação de espículas. Amostras da água foram colhidas mensalmente, para determinação de parâmetros físicos e químicos. O aparecimento das megascleras alfa foi seguido sequencialmente pelo das microscleras, gemoscleras e beta megascleras. As primeiras constroem o novo esqueleto da esponja, as últimas três estavam envolvidas na manutenção da umidade interior no corpo da esponja ou de suas gêmulas. O nível da água, temperatura e a concentração de sílica (Si) na lagoa foram os fatores mais importantes, relacionados a essa produção sequencial de espículas, confirmando reconstruções paleoambientais baseadas na presença ou ausência de megascleras alfa e gemoscleras em sedimentos passados.

Palavras-chave: monitoramento em campo, *Metania spinata*, paleointerpretações, espículas.

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