Temporal and spatial dynamics of a Brazilian cave-restricted freshwater sponge

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Resumo

As esponjas de água doce da ordem Spongillida colonizaram diversos ambientes, incluindo subterrâneos, onde registros são raros. Este trabalho investigou fatores ambientais que influenciam a distribuição de *Racekiela cavernicola* na caverna Brejões I (Bahia, Brasil). Durante a estação chuvosa (01/2024), a espécie foi registrada apenas em poças freáticas subterrâneas, enquanto na seca (07/2024), predominou ao longo do rio Jacaré. Fatores como temperatura, sazonalidade e condutividade apresentaram influência significativa, sendo a abundância maior em temperaturas até 23°C e condutividade maior que 2,0 mS/cm. Metais pesados foram detectados nos sedimentos dos ambientes aquáticos e podem ter afetado os padrões observados, portanto, é fundamental implementar iniciativas que promovam o monitoramento da qualidade da água em Brejões e a análise detalhada da distribuição e formato da fase filtradora ativa dessa espécie, a fim de compreender seu status de conservação e aprofundar o conhecimento sobre sua biologia.

Abstract

Freshwater sponges of the order Spongillida have colonized various environments, including subterranean ones, where records are rare. This study investigated the environmental factors influencing the distribution of *Racekiela cavernicola* in the Brejões I cave (Bahia, Brazil). During the rainy season (01/2024), the species was found only in subterranean phreatic pools, while in the dry season (07/2024), it predominated along the Jacaré River. Factors such as temperature, seasonality, and conductivity significantly influenced, with higher abundance recorded at temperatures up to 23°C and conductivity more significant than 2 mS/cm. Heavy metals were detected in the sediments of aquatic environments, which may have influenced the observed patterns. Thus, it is crucial to implement initiatives focused on monitoring water quality in Brejões and conducting detailed analyses of the distribution and morphology of the active filtering phase of this species, to understand its conservation status better and expand knowledge of its biology.

1. Introduction

There are over 9,000 known species of sponges (Porifera) (DE VONGD et al., 2025), but only 3% inhabit freshwater environments, all of which belong to the order Spongillida (MANCONI & PRONZATO, 2016). This group has successfully colonized a wide range of aquatic habitats, including lotic and lentic systems in both perennial and temporary regimes, spanning coastal islands, high plains, mountains, and even subterranean environments (MANCONI & PRONZATO, 2016). A key factor in their colonization success is the presence of gemmules, resilient asexual reproductive structures capable of cryptobiosis, which also aid in dispersal processes (MANCONI & PRONZATO, 2016).

Records of freshwater sponges in subterranean environments are rare, partly due to the logistical challenges of exploring and studying such habitats (MAMMOLA et al., 2019). These occurrences fall into two categories: epigean sponges that enter subterranean environments accidentally and sponges that are restricted to these habitats, exhibiting adaptations associated with isolation (stygobionts) (Volkmer-Ribeiro et al., 2010).

To date, four cave-restricted freshwater sponge species have been documented. The first is *Eunapius subterraneus*, which includes two subspecies, *E. s. subterraneus* and *E. s. mollisparspanis* (Sket & Velikonja, 1984). This species, recorded in the temperate Ogulin Karst region of Croatia, requires taxonomic revision, as molecular data suggest it does not belong to the genus *Eunapius* (Harcet et al., 2010). The other three species are from tropical regions: one potentially from the genus *Racekiela*, found in the Cueva de Los Sabinos in San Luis Potosí, Mexico (Legendre et al., 2023); *Arinosaster patriciae* (Volkmer-Ribeiro et al., 2021), discovered in a sandstone sinkhole in the Arinos River Basin, Mato Grosso, Brazil; and *Racekiela cavernicola* (Volkmer-Ribeiro et al., 2010), recorded in a large cave system in the semi-arid Bahia state of Brazil. The latter site, due to its size and variety of aquatic habitats, provides a valuable opportunity to study distribution patterns, as water characteristics influence the dispersal of freshwater sponges (Evans, 2016), offering important insights into the conservation status of *Racekiela cavernicola*.

The initial objective of this study was to assess the environmental factors influencing the distribution of *Racekiela cavernicola* during a single sampling event. However, no evidence of the species was found in the Jacaré River during the rainy season (January 2024), with specimens observed only in subterranean water pools. Concerned about potential environmental contamination, we collected water and sediment samples for contaminant analysis. A second sampling effort was conducted in the dry season (July 2024) to further investigate the distribution of this species.

2. Materials and methods

2.1. 2.1. Study Site

The study was conducted in the Brejões I cave, located in the municipality of São Gabriel, Bahia state (Figure 1A), within the boundaries of the Gruta dos Brejões/Veredas do Romão Gramacho Environmental Protection Area (APA). The region's climate is classified as semi-arid (BSh), with annual rainfall below 700 mm, according to the Köppen--Geiger classification (ALVARES et al., 2013). The dominant vegetation is seasonally dry tropical forest, part of the Caatinga biome, within the biogeographic Irecê District. This district is surrounded by the Southern Sertaneja Depression and the Chapada Diamantina, characterized by limestone outcrops and caves, though heavily impacted by agricultural expansion (MORO et al., 2024).

Brejões I is part of the Brejões Karst System, which develops along the Jacaré River valley, a tributary of the São Francisco River. The cave is approximately 6.5 km long and features a monumental entrance about 106 meters high. It has two elevation levels: the upper level, predominantly dry, with large skylights and speleothems, and the lower level, more humid due to the Jacaré River (Figure 1C), which resurfaces among blocks about 750 meters from the main entrance (BERT-BORN & KARMANN, 2002; CARDOSO et al., 2020). There are also lagoons near the river's drainage areas with floating calcite deposits and three subterranean water pools (Figure 1B).

2.2. Sampling Design

Biotic and abiotic data were collected in shallow areas along the Jacaré River and in three subterranean water pools during two sampling events: one in the rainy season (01/2024) and another in the dry season (07/2024). At each sampling point, six 1 m² quadrats (Figure 1B) were aligned and spaced 1 meter apart to count the sponges' filtering structures, characterize and quantify substrates (fine sediment, rocks, pebbles, and rafts), and measure the water's physicochemical parameters. These included temperature (°C), pH, conductivity (mS/cm), oxidation-reduction potential (mV), dissolved oxygen (mg/l), turbidity (NTU), salinity, and total dissolved solids (g/l), using a Horiba U-50 multi-parameter probe.

Additionally, water and sediment samples were collected from aquatic environments at points 8 and 7 (Figure 1A) and a reference sample outside the cave to evaluate potential anthropogenic impacts. Cr, Cd, Pb, Zn, P, and N concentrations were analyzed.

2.3. Data Analysis

To evaluate seasonal and spatial differences in the water's physicochemical parameters throughout the cave, we used ANOVA for models meeting the assumptions of normality and homogeneity of variances. When these assumptions were violated, we applied the Kruskal-Wallis test.

To identify which water parameters influence the distribution of *Racekiela cavernicola*, we employed Generalized Additive Models (GAM) using the gam function from the mgcv package. These models are widely used to estimate smooth nonlinear relationships between predictors and response variables (PEDERSEN et al., 2019).

Initially, we assessed correlations between variables, selecting only one from each redundant group (Spearman index ≥ ±70%) for analysis. Next, we built a model with all possible variables. We used the dredge function from the MuMIn package for model selection, prioritizing the lowest AICc and ranking them by the highest weight. It is important to note that this approach does not account for concurvity among predictors (similar to multicollinearity), which can be a limitation. While more robust algorithms exist for selecting multiple GAM models to address this issue, their application is less intuitive. All analyses were performed using R software (Development Core R Core Team, 2019)

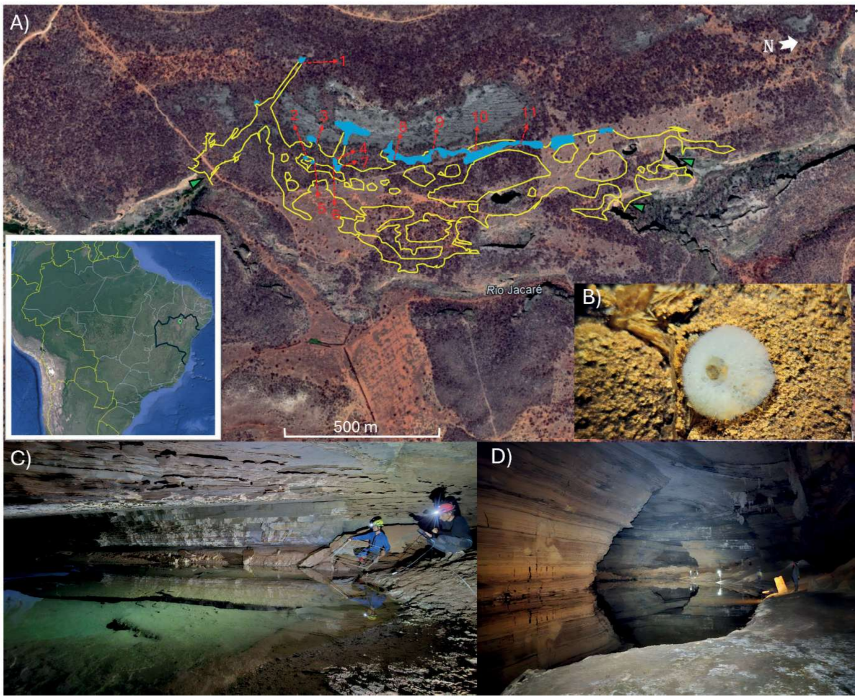

Figure 1: *A) Location and map of the Brejões I cave, showing sampling points (Jacaré River – points 1, 3, 8, 9, 10, and 11; subterranean pools – points 2, 4, 5, 6, and 7). Green triangles indicate the cave entrances.B) Racekiela cavernicola. C) Data collection in the subterranean pool at point 5; D) Jacaré River (flow direction towards the north).*

3. Results

3.1. Seasonal and Habitat Effects on Water Physicochemical Parameters

We observed that some physicochemical parameters of the cave's aquatic environment varied between seasons and habitat types. Temperature was higher during the rainy season (rain = 23.9 ± 0.52; dry = 21.5 ± 1.83; F (1,2) = 172.7; p < 0.001). However, during this period, there was no significant temperature difference between the river and pools (river = 24.33 ± 0.39; pool = 23.49 ± 0.18; p = 0.446), whereas in the dry season, a significant difference was observed (river = 23.03 ± 0.99; pool = 19.80 ± 0.14; p < 0.001). pH was higher during the rainy season (rain = 8.60 ± 0.512; dry = 8.39 ± 0.45; F (1,2) = 27.10; p < 0.01), and pools exhibited higher averages of pH (river = 8.08 ± 0.16; pool = 8.94 ± 0.22; F (1,2) = 34.75; p < 0.01) and dissolved oxygen (river = 1.09 ± 1.20; pool = 2.69 ± 2.26; Kruskal-Wallis chi-square = 10.49; p < 0.01) compared to the river.

Turbidity varied seasonally in pools (rain = 1.47 ± 2.21; dry = 4.24 ± 2.15; Kruskal-Wallis chi-square = 3.93; p = 0.04). The other parameters did not show significant variation between seasons or habitats.

3.2. Contaminant Analysis

We were unable to determine the concentrations of elements in the water samples, but we successfully quantified the elements in sediments, as shown in Table 1.

Table 1: *Concentrations of some chemical elements in the sediment of the aquatic environment in the Brejões I cave. Cr, Cd, P, Pb, and Zn in mg/kg (ppm); N in g/kg.*

Site	Cr	Cd	N	P	Pb	Zn
out of the cave	10,1	0,3	1,25	736,8	5,6	17,2
7	15,4	0,9	0,83	544,4	19,8	25
8	17,1	1,2	2	621,2	20,1	38,3

3.3. Factors Affecting the Distribution of *Racekiela cavernicola*

We counted 98 sponges during the rainy season, all in subterranean pools, and 1,101 during the dry season, with 1,028 in the river and 73 in the subterranean pools. These results indicate a possible seasonal effect on the species' abundance.

When analyzing data from both seasons combined, some variables showed positive redundancy (pH and dissolved oxygen = 0.78; conductivity, salinity, and total dissolved solids = 0.99). Two models showed the same AICc weight (0.0072), differing only in random factors. We chose the model that considered the nested structure for our study. This model explained 98% of the data variation and presented the following structure: fixed variables (season, temperature, pH, ORP, and conductivity) and random factors (collection points, season, and habitat). Temperature (edf = 5.0; ref.def = 8; chi.sq = 37.6; p < 0.0001), conductivity (edf = 5.6; ref.def = 6.5; chi.sq = 58.0; p < 0.0001), and season (z value = 3.36; p < 0.001) were significant.

The species abundance showed a non-linear relationship with temperature, increasing up to approximately 23°C and then decreasing to around 25°C (Figure 2). The increase in conductivity also raised the number of sponges, although the intensity of this relationship varied across intervals (Figure 2).

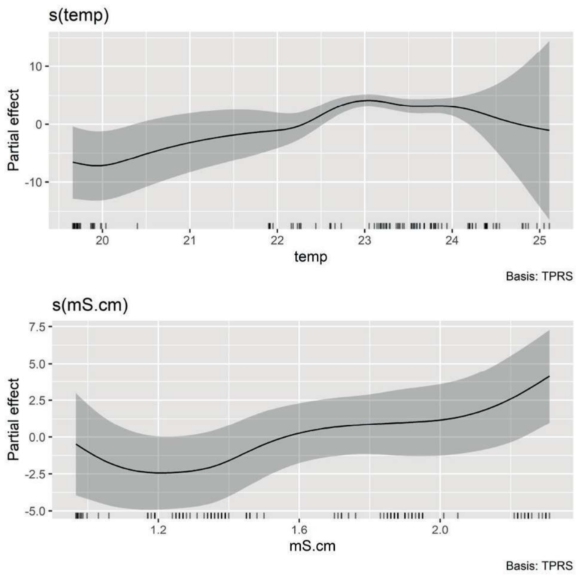

Figure 2: *Factors affecting the distribution of the species Racekiela cavernicola. Temperature (temp), conductivity (mS.cm).*

4. Discussion

Sessile benthic invertebrates are particularly vulnerable to heavy metal pollution due to their feeding habits (suspension or filtration) and limited mobility (ROSENBERG et al., 2004). Sponges, for example, process large volumes of water and, even at low contaminant concentrations, can exhibit morphological, physiological, and behavioral changes (CEBRIAN et al., 2006; CEBRIAN & URIZ, 2007). However, these responses vary depending on exposure duration, species, and life stage (CEBRIAN et al., 2006). Some sponge species have even been proposed as biomonitors for heavy metals due to their bioaccumulation capacity (CALHEIRA, 2020; CEBRIAN et al., 2006).

Our findings indicate that the species *Racekiela cavernicola* inhabits an environment where heavy metals are present. The concentrations of Cr, Cd, Pb, and Zn found in the sediments are mostly below the Quality Reference Values (VRQ) or between the VRQ and Prevention Values (VP) established by the São Paulo State Environmental Agency for soils and groundwater (VRQ: 40, 0.5, 17, 60; VP: 75, 1.3, 72, 86, respectively). However, at sampling point 8, Cd levels were twice the VRQ, approaching the prevention threshold (1.3).

These findings suggest potential agricultural impacts on the Brejões Karst System. Continuous environmental monitoring in the region and further studies to identify the source of elevated heavy metal concentrations are crucial. Monitoring *R. cavernicola* populations and investigating potential morphological changes in the species could provide valuable insights into environmental quality.

Regarding our initial objective of identifying factors influencing the species' distribution, we cannot exclude the potential impact of heavy metals on the observed patterns. Previous studies have shown that heavy metals, even at sublethal concentrations, can interfere with gemmule hatching and the development of freshwater sponges, thereby affecting their life cycles and distribution (CALHEIRA, 2020; MYSIN-GUBALA & PORRIER, 1981; RICHELLE et al., 1995).

Although the observed effects were not lethal, the abundance (or at least the number of sponges in the active filtering phase) of this species appears to exhibit seasonality. Most taxa within the order *Spongillida* have life cycles synchronized with seasonal hydroperiod rhythms, whether short- or long-term (MANCONI & PRONZATO, 2016). The typical life cycle includes four annual phases: active filtering (associated with sexual reproduction), metamorphosis via gemmulation, dormancy as a gemmule mat, and gemmule hatching followed by sponge regeneration (MANCONI & PRONZATO, 2016).

However, this species seems to deviate from this standard pattern, maintaining an active filtering phase year-round, with a marked increase in abundance during the dry season. Habitat-specific factors may influence this behavior (CALHEIRA et al., 2020), as certain species, such as *Ephydatia fluviatilis*, demonstrate plasticity in their life cycles (MANCONI & PRONZATO, 2016). Seasonal temperature fluctuations between the rainy and dry seasons in the semi-arid Caatinga biome may serve as a primary driver of phase transitions in this species' life cycle, as suggested by its distribution model.

Another factor influencing the species' distribution was conductivity (mS/cm), which could correlate with cave sites offering greater availability of nutrients and organic particles. Given that cave environments are generally oligotrophic (CULVER & PIPANCulver & Pipan, 2013), the higher prevalence of filtering structures in such locations may reflect an adaptive strategy to maximize the capture of scarce food resources.

5. Conclusion

We conclude that seasonality, temperature, and conductivity likely influence the distribution and abundance of filtering structures in the cave-dwelling species *Racekiela cavernicola*. However, the presence of heavy metals in the environment may also have contributed to the observed patterns. Efforts to monitor water quality in the Brejões region, along with studies on the distribution and morphology of this species, are crucial for understanding its conservation status and enhancing knowledge of its biology and the processes driving isolation in freshwater sponges.

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