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Effectiveness of quadrat sampling on terrestrial cave fauna survey - a case study in a Neotropical cave

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ABSTRACT. Quadrat sampling is a method used for a long time in plant ecology studies but only recently it has been used with focus on fauna. For the cave fauna samplings, there are rare works applying this methodology. The present study compared the methods of quadrat sampling with direct search qualitative for terrestrial cave fauna. For this, we conducted five sampling collections in a limestone cave in central Brazil. Quadrat sampling contributed with 121 exclusive species and 716 specimens and direct search qualitative method contributed with 91 exclusive species and 355 specimens. Mann-Whitney test evidenced significant differences between the two methods. We demonstrated that quadrat sampling method was slightly more efficient to analyze the species richness and much more effective to assess the abundance than the use of only direct search qualitative method, mainly considering tiny and/or cryptobiotic invertebrates (e.g., earth worms, symphylans, psocopterans, trichopterans, dipterans, small spiders, and small isopods). We recommend the association of different methods to test patterns in cave fauna, since incomplete sampling may lead to erroneous estimates and equivocated decisions about management, impact studies and cave conservation.

Keywords: cave fauna, terrestrial invertebrates, central Brazil, sampling methodology.

A eficácia do método de amostragem por quadrados em levantamentos da fauna terrestre cavernícola - um estudo de caso em uma caverna Neotropical

RESUMO. Amostragem por quadrados é um método utilizado há muito tempo em estudos ecológicos botânicos e apenas recentemente tem sido utilizado com foco em estudos faunísticos. Para amostragens em cavernas, raros trabalhos aplicaram esta metodologia. Nosso trabalho compara o método de amostragem por quadrados com o de busca ativa qualitativa sobre a fauna cavernícola terrestre. Para tal, realizamos cinco eventos de coleta em uma caverna calcária do Brasil central. A amostragem, utilizando o método de quadrados, contribuiu com 121 espécies exclusivas e 716 espécimes. O método de busca ativa contribuiu com 91 espécies exclusivas e 355 espécimes. O teste de Mann-Whitney mostrou diferenças significativas entre os dois métodos. Demonstramos que o método de amostragem de quadrados foi sutilmente mais eficiente para acessar a riqueza de espécies e muito mais efetivo para acessar a abundância do que apenas a utilização do método qualitativo de busca ativa, principalmente para invertebrados diminutos e/ou criptobióticos (por exemplo, vermes, sínfilos, psocópteros, tricópteros, dípteros, pequenas aranhas e pequenos isópodes). Recomendamos o uso de métodos combinados para o teste de padrões da fauna cavernícola, uma vez que amostragens incompletas podem levar a estimativas erradas e decisões equivocadas acerca de manejo, estudos de impacto e conservação de cavernas.

Palavras-chave: fauna cavernícola, invertebrados terrestres, Brasil central, metodologia de amostragem.

Introduction

Caves are one of the subterranean habitats (among others like interstitial, hyporheic, mesovoid shallow substratum/*milieu souterrain superficiel* – MSS, and a recent hypogean habitat, named alluvial mesovoid shallow substratum) (JUBERTHIE; DECU, 1994; ORTUÑO et al., 2013). These habitats are formed by natural openings in solid rocks with completely dark spaces, from few millimeters in diameter to large scales such as conduits and galleries with kilometers of extension (CULVER; PIPAN, 2009).

This subterranean realm, also known as hypogean, is quite different from the epigean (surface) environment, without primary production by photosynthesis and high humidity of air, for example (POULSON; WHITE, 1969). These particularities impose a special selective regime and only organisms with character-states useful in these conditions (such as permanent darkness and food scarce) can survive therein.

Sampling techniques commonly used in different terrestrial epigean habitats are not necessarily suitable in the subterranean habitats (WEINSTEIN; SLANEY, 1995). For example, sampling by quadrat method has been used for a long time in ecological studies, mainly in plant ecology (Por exemplo, WEAVER, 1918; GLEASON, 1920). This method consists of a square frame to delimit an area in which species are counted and/or collected (HENDERSON, 2003). In this context of biology, this kind of sampling is consolidated, since it is one of the most robust methods to assess abundance and species richness (KREBS, 1999).

In caves, the main sampling method for terrestrial fauna is hand collecting by visual inspection of all possible habitats, without the use of any specific device (por exemplo, TRAJANO; GNASPINI-NETTO, 1991; HUNT; MILLAR, 2001). Pitfall traps are also usually employed in caves (with or without bait), but this method should be used with caution since it can impact the whole terrestrial fauna, oversampling some taxonomic groups, such as collembolans, orthopterans and cockroaches (SHARRATT et al., 2000). Moreover, the rock type or the substrate could hamper or preclude the installation of the trap and undermine the design of the project. Terrestrial leaf litter packs, aspirators, Berlese and Winkler extractors are other methods used for cave fauna sampling, however these devices are employed less often.

There is only one study comparing sampling methods in caves (WEINSTEIN; SLANEY, 1995) and, in this case, the quadrat sampling method has not been assessed. Weinstein and Slaney (1995) compared six methods of surveys in an Australian cave (pitfall alone, pitfall with bait, dry leaf litter, wet leaf litter, direct search quantitative and direct search qualitative). The authors considered the wet leaf litter trap as the most efficient to investigate the abundance, and they considered the wet leaf litter trap and direct search qualitative equally robust in the assessment of species richness.

Considering the use of the quadrat method, only two works used this methodology in caves, without comparison with other methods (SHARRATT et al., 2000; BICHUETTE; TRAJANO, 2003). These authors found a high faunal richness, with rare organisms, many of them troglobitic (restricted and differentiated subterranean fauna).

In this work, we investigated the effectiveness of the quadrat sampling method in analyzing the species richness and abundance comparing with the direct search qualitative method of terrestrial subterranean fauna from a Neotropical cave. For this, we used the most common sampling in caves, the direct search qualitative method combined with the quadrat sampling method.

Material and methods

Study site

The study was conducted in the Angélica cave (13°31’29” S and 46°23’07” W; 562 m altitude) located in the Parque Estadual de Terra Ronca (PETER)/ Terra Ronca State Park, municipality of São Domingos, northeastern Goiás State, central Brazil (Figure 1a). This limestone cave is one of the largest caves in Brazil with an extension of ca. 14 km and is part of a huge cave-system with subterranean drainage named Angélica-Bezerra. We performed the collections in a reach close to the sinkhole with approximately 100 m length with five bases (treatments) (Figure 1b).

Survey

We collected the invertebrate terrestrial fauna in five occasions (replicas) along 15 months. We established five monitoring bases, each one with 20 m length and 20 m width, along the 100 m studied. Our total sampling area was 2000 m^2 (100 x 20 m) with each base of approximately 400 m^2 (20 x 20 m). We divided the total sampling area to assure that the collections were performed covering the same areas in all occasions (replicas).

Considering the quadrat sampling method (QuS), we distributed the quadrats arbitrarily, always respecting the limits defined for each base, independently of the substrates. All observed fauna was collected through this method, which comprised an area of 0.25 m^2 per square (Figure 2). Each collector established 20 quadrats for each base, totaling 40 quadrats per base, 200 quadrats per occasion (replica) and 1000 quadrats in total.

Considering the direct search qualitative sampling method (DSQm), we intensively searched for species in areas in which they were deemed most likely to be found (WEINSTEIN; SLANEY, 1995). We established 60 minutes for the DSQm for each base.

We applied both sampling methods as follows: in the same time, while two collectors applied the quadrat sampling method in half of the base (400 m^2), another two applied the direct search qualitative method (DSQm) in the other half (the other 400 m^2). In this way, there were always four collectors (two on QuS and two on DSQm) conducting the samplings.

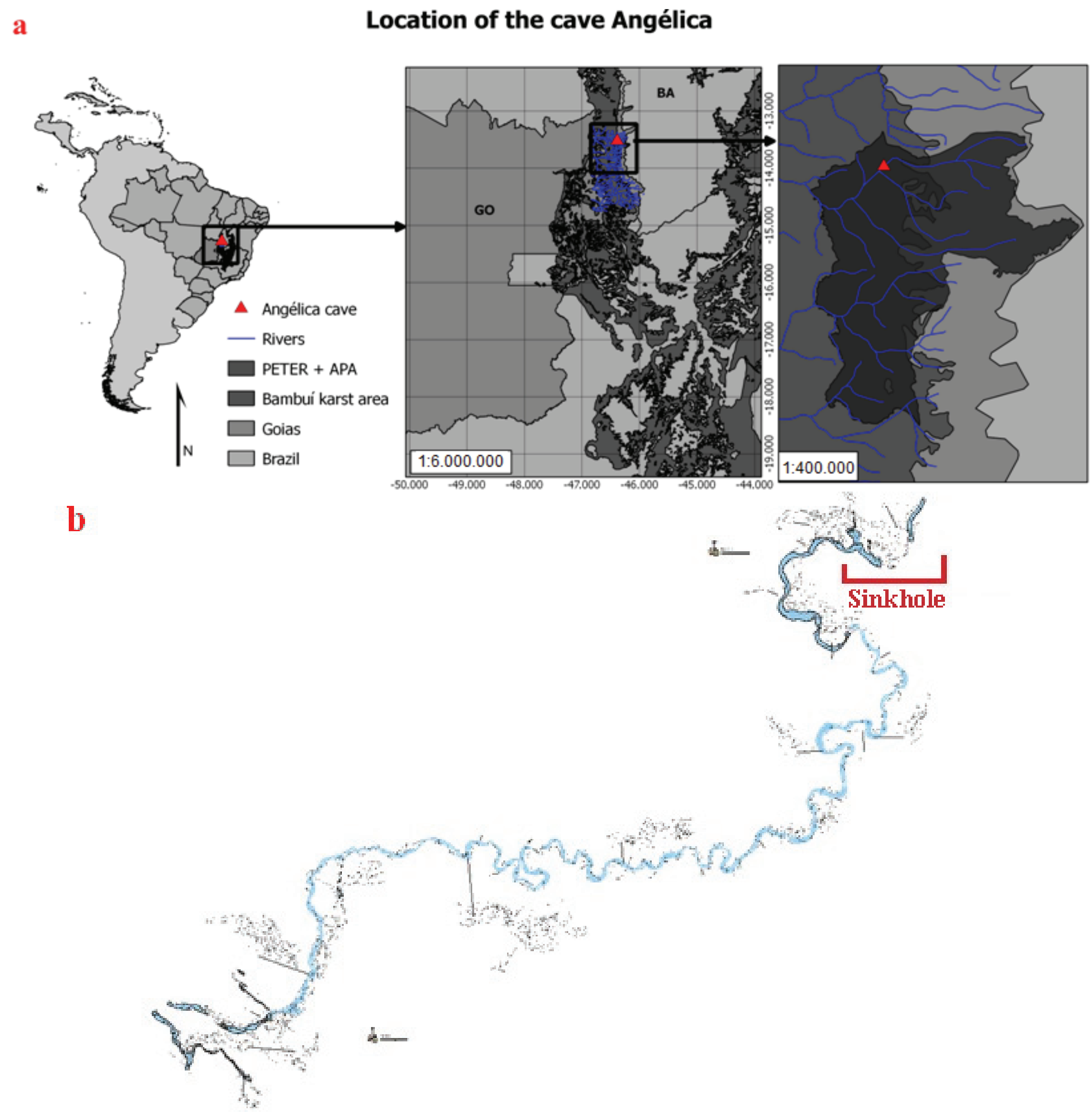

Figure 1. a. Study area - Terra Ronca State Park, Goiás State, central Brazil); b. Map of the Angélica cave with sinkhole highlighted. Source: Grupo Bambuí de Pesquisas Espeleológicas (GBPE).

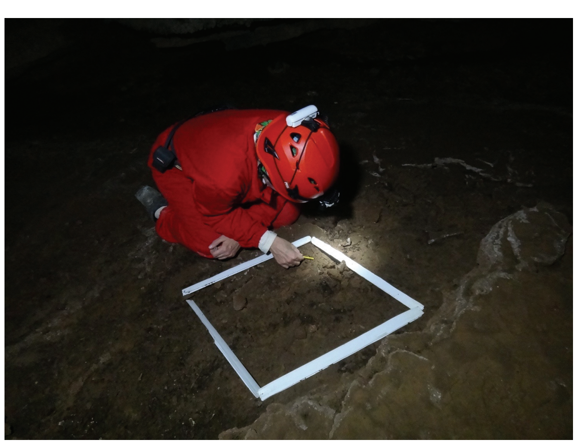

Figure 2. Collection in caves using the quadrat-sampling method. Photography: Grupo Pierre Martin de Espeleologia (GPME).

We covered different substrates, including rock substrate, soil, logs, guano piles, under rocks, sand, among others, from the twilight zone (places with light influence) to aphotic zone (places with permanent darkness).

All material collected during the samplings were fixed in 70% ethanol and then identified in laboratory with specific literature, scientific collection reference consults and confirmation with experts on different recorded taxa, and deposited at the Laboratório de Estudos Subterrâneos of Universidade Federal de São Carlos (LES/UFSCar) and other repositories. Immature individuals collected that could not be clearly identified based on the adult specimens were excluded from analysis to prevent overestimation of some taxa.

Analysis: For statistical comparison of both methods, we applied the non-parametric Mann-Whitney test ($\alpha = 0.05$), using the software PAST 2.13 (HAMMER et al., 2001). In addition, Mao-tau sample-based rarefaction curves were constructed considering each sampling method (QuS and DSQm) as well as Jackknife 1 and Chao 2 estimators for both methods. All curves were constructed in the software EstimateS 9.1 (COWELL, 2013).

Results

We distributed 40 quadrats in each base (two collectors), totaling 200 quadrats in each sampling occasion (40 quadrats *x* five bases) and 1000 quadrats for total all experiment in the Angélica cave (200 quadrats *x* five occasions). As the quadrat was 0.25 m^2, we covered a total area of 250 m^2 (1000 x 0.25 m^2), from a total of 1000 m^2, as well as, we covered 10 m^2 in each base. This represents exactly 25% of sampled area with Qus.

The time spent in DSQm was 25 hours for the 1000 m^2 covered by the direct search qualitative method (60 minutes in each base x five bases x five replicas).

For both sampling methods, we recorded 257 morphospecies and 1,071 specimens (Table 1). The collections using the QuS contributed exclusively with 121 morphospecies (47.1%), and DSQm contributed exclusively with 91 morphospecies (35.4%) and another 45 morphospecies (17.5%) was achieved from both methods (Table 1). Considering the abundance, the QuS achieved 716 specimens (66.9%) of the total subterranean fauna in the Angélica cave and DSQm contributed with 355 specimens (33.1%) (Table 1). Mann-Whitney test showed significant differences for abundances ($p = 0.001$), with the quadrat method exhibiting the higher ones (Table 1, Figure 3).

Table 1. Richness, Exclusive richness and Mann-Whitney test for abundance. QuS - quadrat sampling method, DSQm - direct search qualitative method.

	QuS	DSQm
Richness	166 (121 + 45*)	136 (91 + 45*)
Exclusive richness	121 (47.1%)	91 (35.4%)
Abundance	716 (66.9%)	355 (33.1%)
Mann-Whitney	91.05	60.45
p-value	0.001	

*This number (45) represents the number of species collected by both methods independently.

Approximately 29 Orders of subterranean fauna were sampled in the Angélica cave, DSQm was the most efficient method for abundance in six of them (Amblypygi, Collembola, Orthoptera, Opiliones, Scolopendromorpha and Scutigeromorpha). Pseudoscorpiones showed the same abundance in both methods (Figure 3). All other Orders (Acari, Araneae, Geophilomorpha, Spirostreptida, Symphyla, Diplura, Blattaria, Coleoptera, Diptera, Ephemeroptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Neuroptera, Plecoptera, Psocoptera, Thysanoptera, Trichoptera, Pulmonata, Isopoda and Haplotaxida) were better sampled by QuS. Amongst these, Araneae, Coleoptera and Blattaria exhibited the greatest differences in abundance (Figure 3).

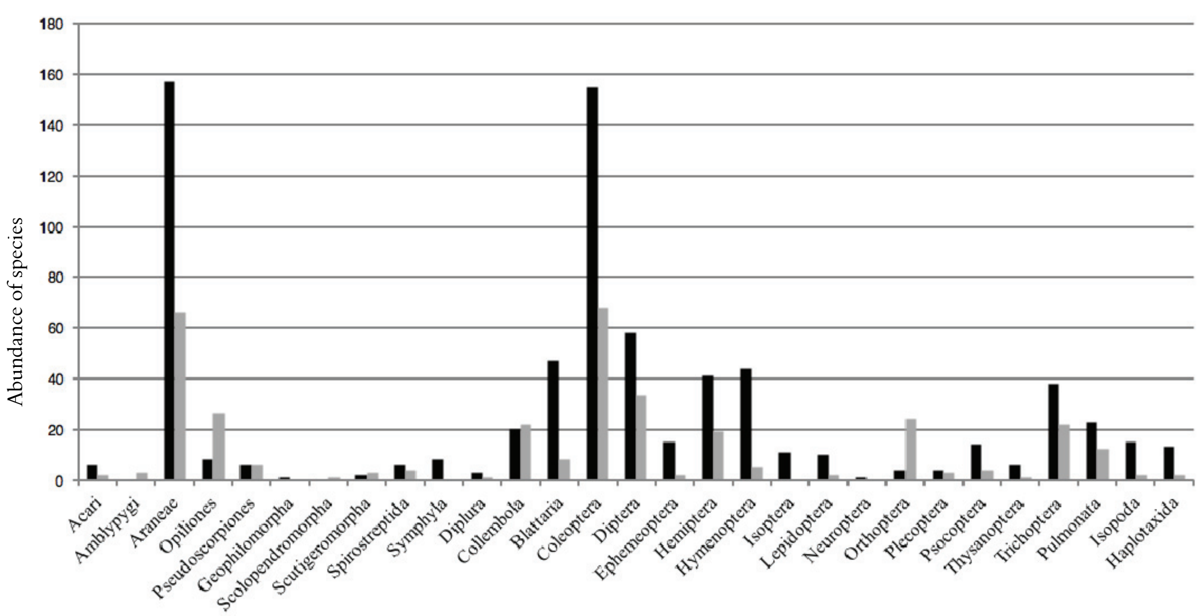

Figure 3. Abundance of species recorded by Orders captured by both sampling methods. Black columns - quadrat sampling method; grey columns - direct search qualitative method.

Comparing the effective richness between the methods, QuS was somewhat better for sampling in contrast with DSQm (Figure 4), as well as when comparing both methods by the Jackknife 1 estimator. However, Chao 2 estimator curves for both methods were practically the same (Figure 4). The Chao 2 estimator confidence interval for Qus was 300.48 to 545.66 and for DSQm was 277.76 to 585.47.

Discussion

Quadrat sampling method is a specialized type of visual inspection, wherein it is delimited sample areas in surveys, optimizing collection of subterranean fauna. In this sense, we emphasized that this method allows the measurement of the sampling effort, since the exact area sampled is known. Thus, comparisons among subterranean systems with ecological focus are plausible, since the assumptions of the collection methods are respected, namely: number of individuals in each quadrat is collected and/or counted; size of quadrat is known; quadrat samples are representative of study area as a whole (KREBS, 1999).

Considering studies drawing comparisons between sampling methods in caves, this is the first one using the quadrat sampling method. Moreover, the application of replicas along 15 months is essential to avoid possible sampling bias over time (*sensu* TRAJANO et al., 2012).

The use of QuS proved to be efficient, since it was responsible for the most part of abundance and also demonstrated a slightly better performance to assess the species richness collected in comparison with DSQm. The quadrat sampling method was responsible for 121 species (47.1%) and 716 specimens (66.9%) exclusively.

According to the Mann-Whitney test, there was a statistically significant difference between the QuS and DSQm abundances, evidencing the collection efficiency by quadrat sampling method relative to the other method. This effectiveness of abundances in the quadrat sampling method is due to the accurate delimitation of the study area and sampling effort when all fauna is collected inside the quadrat.

In the comparison of the sample-rarefaction curves between the methods, QuS achieved a little better result than DSQm, with 30 species collected exclusively by QuS (Table 1, Figure 4). Indeed, Jackknife 1 (affected by unique species) estimator curve followed the same trend. However, Chao 2 (affected by unique and duplicate species) was almost the same for both sampling methods. Thus, both methods were efficient to sample the area, considering species richness.

The size and behavior of the organisms influence the effectiveness of each sampling method. In this way, the quadrat sampling method could be less effective for larger and more active organisms like crickets, cockroaches and amblypygids and also some tiny organisms with rapid scape, like collembolans. Nevertheless, these taxa were well sampled using DSQm, which corroborates the advantage of using combined methods.

The quadrat sampling method ensures the exploration of microhabitats that are often neglected, enhancing the capture of these barely visible organisms (e. g., edaphic organisms inside caves or with cryptobiotic habits). In fact, we observed higher efficiency in the collection of small and tiny fauna using the quadrat sampling method with a huge diversity (Figure 4).

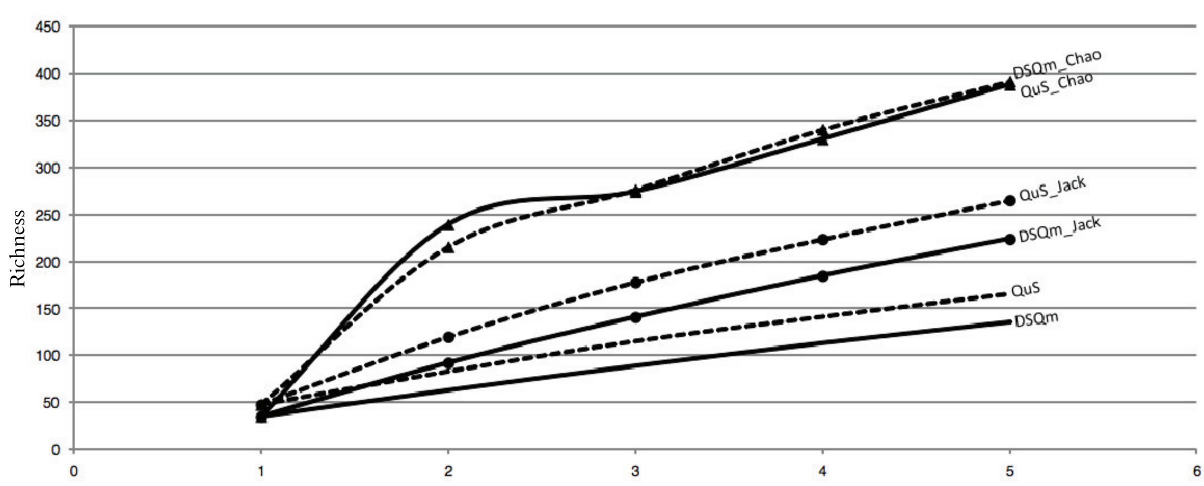

Figure 4. Mao-tau sample-based rarefaction curves for both sampling methods and Jackknife 1 and Chao 2 estimators. Solid lines - Direct Search Qualitative method; Dashed lines - Quadrat method.

The use of pitfall or vulcan traps has been suggested in Brazilian caves, in many faunistic inventories for huge mining and hydroelectric projects, for being the most efficient method (unpubl. data); however, these methods are considered unsuitable in fragile cave habitats, since they can cause disturbances to the cave community with risk of overcollection of some groups (WEINSTEIN; SLANEY, 1995; SHARRATT et al., 2000; TRAJANO et al., 2012). Moreover, as it could occur in DSQm, many tiny species and/or with low locomotion ability were not collected in an efficient way using pitfall or vulcan traps.

Some authors agree that the combination of sampling methods, as used herein, is essential to investigate the richness of a community (CULVER, 1982; CULVER; PIPAN, 2009). Our results corroborate this idea. We conclude that a more effective cave survey of terrestrial fauna can be reached by a combination of different methods and we suggest that quadrat sampling method should be one of those.

Other methods used in surveys of terrestrial subterranean fauna should be employed for higher accuracy, such as general extractors (por exemplo, WEISTEIN; SLANEY, 1995), since incomplete sampling may lead to erroneous estimates. Thus, in an application way, it may result in equivocated decisions about management, impact studies and conservation actions for caves.

Conclusion

Quadrat sampling method is more efficient to analyze the species richness and abundance than the use of only direct search qualitative method;

Tiny invertebrates, including the cryptobiotic (such as symphylans, psocopterans, small isopods and small spiders) are better sampled (quantitatively) with the quadrat method.

The combination of methods in surveys to test patterns in cave fauna are essential to avoid the cascade errors in decisions about conservation of subterranean habitats and should be employed in huge projects such as mining and hydroelectric plants.

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References

BICHUETTE, M. E.; TRAJANO, E. A population study of epigean and subterranean *Potamolithus* snails from southeast Brazil (Mollusca: Gastropoda: Hydrobiidae). **Hydrobiologia**, v. 505, n. 1-3, p. 107-117, 2003.

COWELL, R. K. **EstimateS**. Statiscal estimation of species richness and shared species from samples. Version 9, 2013. Available from: <purl.oclc.org/estimates>. Access on: June 25, 2014.

CULVER, D. C. **Cave life** - evolution and ecology. Cambridge: Harvard University Press, 1982.

CULVER, D. C.; PIPAN, T. **Biology of caves and other subterranean habitats**. Oxford: Oxford University Press, 2009.

GLEASON, H. A. Some applications of the quadrat method. **Bulletin of the Torrey Botanical Club**, v. 47, n. 1, p. 21-33, 1920.

HAMMER, O.; HARPER, D.; RYAN, P. D. PAST. Paleontological statistics software package for education and data analysis. **Paleontologia Electronica**, v. 4, n. 1, p. 1-9, 2001.

HENDERSON, P. A. **Practical methods in ecology**. Oxford: Blackwell Publishing, 2003.

HUNT, M.; MILLAR, I. **Cave invertebrate collecting guide**. Wellington: Department of Conservation Technical, 2001. v. 26.

JUBERTHIE, C.; DECU, V. Structure et diversité du domaine souterrain; particularities des habitats et adaptations des espèces. In: JUBERTHIE, C.; DECU, V. (Ed.). **Encyclopaedia biospeologica I**. Bucarest, Moulis: Société de Biospéologie, 1994. p. 5-22.

KREBS, C. J. **Ecological methodology**. New York: Addison-Welsey Educational Publishing, 1999.

ORTUÑO, V. M.; GILGADO, J. D.; JIMÉNES-VALVERDE, A.; SENDRA, A.; PÉREZ-SUÁREZ, G.; HERRERO-BORGOÑÓN, J. J. The "Alluvial Mesovoid Shallow Substratum", a new subterranean habitat. **Plos One**, v. 8, n. 10, p. 1-16, 2013.

POULSON, T. L.; WHITE, W. B. The cave environment. **Science**, v. 165, n. 3897, p. 971-981, 1969.

SHARRATT, N. J.; PICKER, M. D.; SAMWAYS, M. J. The invertebrate fauna of the sandstone caves of the Cape Peninsula (South Africa): patterns of endemism and conservation priorities. **Biodiversity and Conservation**, v. 9, n. 1, p. 107-143, 2000.

TRAJANO, E.; GNASPINI-NETTO, P. Composição da fauna cavernícola Brasileira, com uma análise preliminar da distribuição dos táxons. **Revista Brasileira de Zoologia**, v. 7, n. 3, p. 383-407, 1991.

TRAJANO, E.; BICHUETTE, M. E.; BATALHA, M. A. Environmental studies in caves: the problems of sampling, identification, inclusion, and indices. **Espeleo-Tema**, v. 23, n. 1, p. 13-22, 2012.

WEAVER, J. E. The quadrat method in teaching ecology. **The plant world**, v. 21, n. 11, p. 267-283, 1918.

WEINSTEIN, P.; SLANEY, D. Invertebrate faunal survey of Rope Ladder Cave, Northern Queensland: a comparative study of sampling methods. **Australian Journal of Entomology**, v. 34, n. 3, p. 233-236, 1995.

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