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Microhabitat selection of a Sicilian subterranean woodlouse and its implications for cave management

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Abstract: Human activities in subterranean environments can affect different ecosystem components, including the resident fauna. Subterranean terrestrial invertebrates are particularly sensitive to environmental change, especially microclimatic variations. For instance, microclimate modifications caused by the visitors may directly affect local fauna in caves opened to the public. However, since numerous factors act synergistically in modulating the distribution and abundance of subterranean species, it remains challenging to differentiate the impact of human intervention from that of other factors. Therefore, evidence of the impact of tourism on cave invertebrate fauna remains scarce. Over a year and with approximately two visits a month, we investigated the effects of the presence of visitors on the subterranean endemic woodlouse *Armadillidium lagrecai* in the strict natural reserve of Monello Cave (Sicily, Italy). We found that natural microclimatic fluctuations, and not direct human disturbance, were the main factors driving the distribution of *A. lagrecai*. Specifically, *A. lagrecai* select for more climatically stable areas of the cave, where the temperature was constantly warm and the relative humidity close to saturation. We also observed a significant temporal effect, with a greater abundance of *A. lagrecai* in summer and a gradual decrease during the winter months. The number of visitors in the Monello cave had no effect on the abundance and distribution of *A. lagrecai*. However, considering the high sensitivity of the species to microclimatic variations, it seems likely that a significant increase in the number of visitors to the cave could indirectly affect this species by altering local microclimate. Constant monitoring of the environmental parameters within the cave is therefore recommended.

Keywords: Armadillidium, biodiversity conservation, cave arthropods, human impacts, subterranean fauna

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INTRODUCTION

Subterranean ecosystems are highly stable relative to their surrounding surface environments (Poulson & White, 1969; Howarth, 1980; 1993). The permanent darkness, scarce energy inputs, constantly high relative humidity, and temperature stability exert a selective pressures driving the evolution of specific physiological, morphological, and behavioral traits on resident organisms (Culver & Pipan, 2010). Consequently, subterranean species often show pronounced sensitivity to even small changes in the

environmental conditions (e.g., Barr & Kuehne, 1971; Howarth, 1980; Culver, 2005). Temperature is one of the major abiotic factors affecting subterranean species and their life history (Bernabò et al., 2011; Novak et al., 2014; Rizzo et al., 2015; Mammola et al., 2019b; Pallarés et al., 2019, 2020); most specialized subterranean species select microhabitats where the temperature is generally higher and stable, such as the innermost areas of caves. The presence of subterranean fauna is similarly correlated with stable and high relative humidity, as their thin cuticles of cave adapted organisms render them vulnerable

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to desiccation (Howarth, 1980) which they would encounter in the drier areas of the cave.

Owing to this sensitivity of the specialized subterranean fauna, there is nowadays a growing awareness of a need for protection of subterranean ecosystems (Mammola et al., 2019a).

As fragile and confined environments, cave ecosystems are susceptible to numerous impacts related to human activities (Culver & Pipan 2019; Romero, 2009). One of the most studied among these impacts is the effect of touristic activities in caves opened to visitors (Cigna, 2016). Human-induced impacts associated to tourist caves can affect different ecosystem components (Mammola, 2019), but our understanding of the nuances of these effects remains scarce (Mammola et al., 2020). A massive presence of tourist directly alters the underground microclimate resulting in seasonal variations in temperature and relative humidity (Cigna, 2004). At the same time, the joint effect of visitors and artificial lights affects the presence of microorganism and their distribution (Mulec, 2014). For example, organic materials passively transferred into the caves by visitors may enhance the growth of bacterial and fungal species alien to the cave community (Chelius et al., 2009; Mammola et al., 2017; Pfendler et al., 2018), whereas artificial lights lead to the development of photosynthetic biofilms (*lampenflora*) on rocky surfaces (Falasco et al., 2014; Piano et al., 2015; Baquedano Estévez et al., 2019). While an influence of tourism on the resident terrestrial fauna could also be expected, evidence related to the impact of direct anthropic disturbance on cave dwelling arthropods remains scarce (Isaia et al., 2011; Pellegrini & Ferreira, 2012; Pacheco et al., 2020).

Being specialized animals, subterranean invertebrates are theoretically vulnerable even to minor changes in their environment, including human-induced alterations. For instance, trampling of visitors and the subsequent modification to the substrate might cause a direct disturbance (Spate & Hamilton-Smith, 1991). The growth of *lampenflora* as well as the introduction of external opportunistic species of microbes favor the proliferation of more competitive species, which threaten the local cave-dwelling fauna (Mulec et al., 2008; Mulec & Kosi, 2009; Castello, 2014). Also, microclimate modifications associated with tourism could potentially cause the disappearance or migration of the local fauna (Slaney & Weinstein, 1997; Juberthie, 2000; Krajick, 2001; van Beynen & Townsend, 2005). A greater knowledge of the cave's main physical parameters inside the cave (in particular temperature, humidity, and CO₂) is essential for the conservation of the cave environment and its fauna. Moreover, is necessary to distinguish if environmental changes are due to natural causes or human activities in order to possibly define criteria for their conservation (Huppert et al., 1993; Fernandez-Cortes et al., 2006). However, due to the lack of long time series of data and several sampling impediments, it is generally difficult to tease apart the influence of these two factors in driving diversity patterns in caves (Eberhard, 2001).

The aim of our study was to explore the relative influence of microclimatic variations and the presence of visitors on the spatio-temporal dynamics of specialized subterranean fauna. We used the Monello Cave as a model system. It is located in a Strict Natural Reserve in eastern Sicily, and is renowned for its peculiar subterranean terrestrial fauna (Caruso, 1994). In particular, we focused on the specialized subterranean woodlouse *Armadillidium lagrecai* Vandel, 1969 (Malacostraca: Isopoda: Armadillidiidae) and its relation to both natural and anthropogenic factors related to cave tourism.

MATERIAL AND METHODS

Study area

The Monello Cave (cadastral number SiSr7007, Latitude 37°01'04.00" N; Longitude 15°09'57" E, altitude 10 m) is located in the municipality of Siracusa, in southeastern Sicily (Italy). The cave is included in the Strict Nature Reserve "Grotta Monello" (Fig. 1). The reserve was established in 1998 in order to protect the cave, its speleothems and its invertebrate fauna (see Caruso, 1994; Nicolosi et al., 2019; Sendra et al., 2019; Sabella et al., 2020). The cave is also part of the Special Area of Conservation (SAC) ITA090011 "Grotta Monello" and is formally recognized as a Geosite of regional interest. The site is managed by the Centro Universitario per la Tutela e la Gestione degli Ambienti Naturali e degli Agroecosistemi of the University of Catania (CUTGAN).

The cave is located in the eastern sector of the Hyblean Plateau, within the NW-SE trending Late Quaternary Florida Basin (Ghisetti & Vezzani, 1980), a 12 km wide and 20 km long tectonic depression characterizing the northeastern sector of the Hyblean Plateau. It is located in the Early-Middle Miocene calcarenites and calcirudites with fragments of bryozoa, echinoderms, algae, and bivalves that constitute the upper member (or Siracusa Member) of the Climiti Mounts Formation (Pedley, 1981).

The cave has a total planimetric development of about 540 m. The first part consists of an artificially excavated horizontal gallery, 110 m in length, accessible through a small iron gate that prevents any unauthorized access to the cave. The gallery leads to the main room of the cave ("Sala Grande"). From there, several secondary passages originate, developing along different levels and showing a remarkable concentration of speleothems.

The cave was heavily modified to make it accessible for tourism. In the 80s, the Province of Siracusa excavated a pathway across the cave, which destroyed a huge number of speleothems (Fig. 2a).

Before the official opening, a study conducted by the University of Catania led to the discovery of an invertebrate assemblage of considerable interest (Caruso, 1994). This assemblage included specialized subterranean species such as the diplopod *Glomeris dionysii* (Strasser 1961) (Diplopoda: Glomerida: Glomeridae) and the isopod *Armadillidium lagrecai* (Fig. 2b), so far uniquely found in the Monello Cave. The peculiarity of the local fauna prompted the

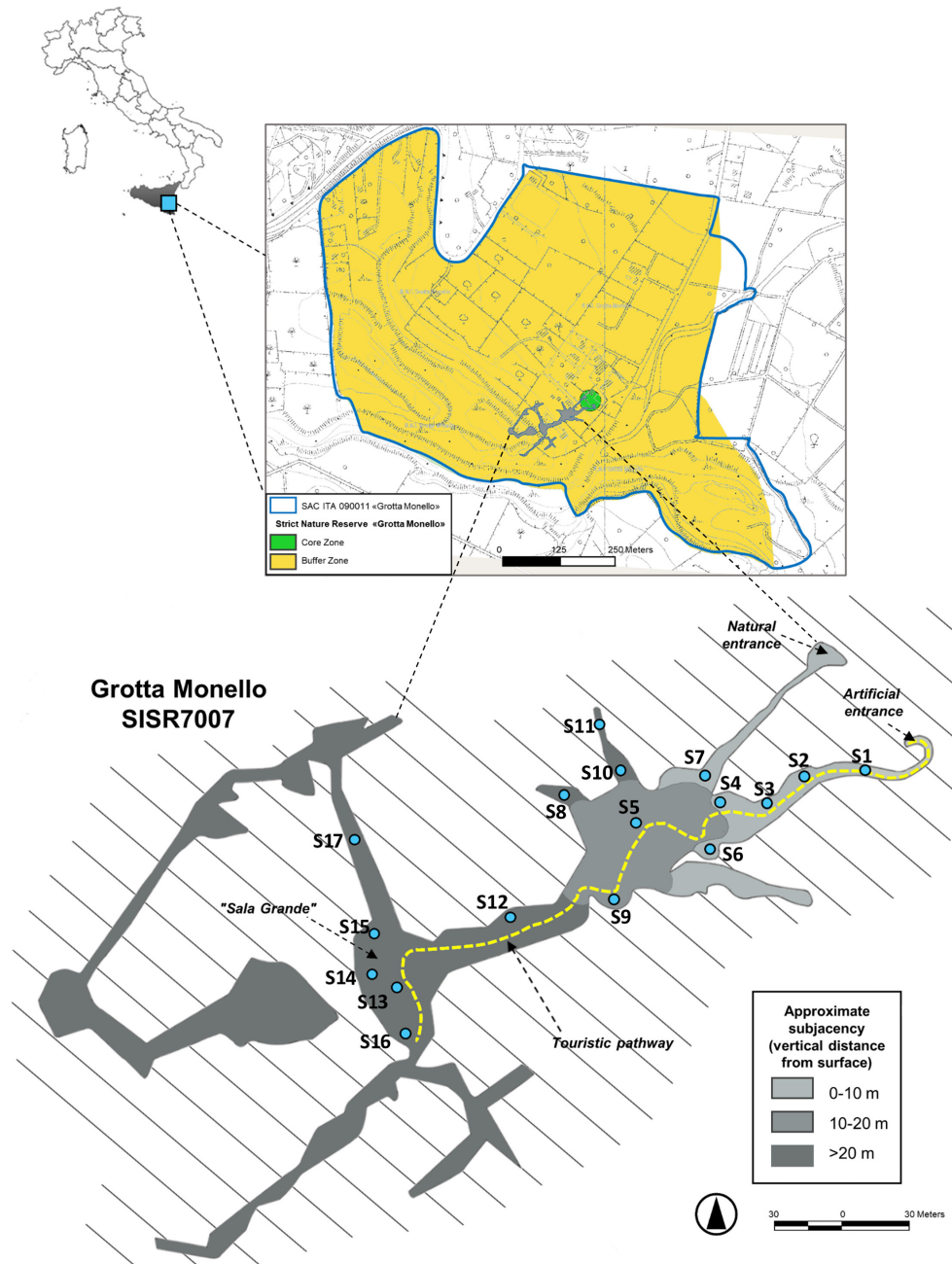


Fig. 1. Map of the study area. The sampling sites are represented by blue dots, located at 16, 24, 43, 47, 52, 62, 63, 73, 83, 84, 92, 96, 120, 123, 131, 136, and 143 m from the cave entrance. The different grey shades represent the subajacency (i.e., vertical distance from the surface). Topography of the Monello Cave is from the original drawings of Ruggieri & Amore (2000).

establishment of the Strict Nature Reserve, preventing uncontrolled touristic use.

Management of the Monello Cave

From its establishment in 1988 until 2018, access to the Monello Cave had been regulated by the managing body (CUTGANA) and its usage limited to approved scientific research.

Starting in 2018, the cave was opened to the public for a scientific investigation to evaluate the impact of visitors on the subterranean ecosystem. As of 2020, visitors were allowed in the cave under strict regulation: 3 visits were permitted per month and each visitor group was limited to twenty-four people, which in turn were divided in two subgroups of twelve, excluding one guide and one assistant. Visitors were not allowed to spend more than 40 minutes inside the cave, and each wore a helmet with a flashlight dimmer than 100

lumens. There was no built-in lighting system and visitors were required to stay on the visitor path.

Sampling design

We conducted the study from December 2018 to February 2020. We visited the cave 27 times over 14 months (approximately two visits/month).

Based on previous studies (Faille et al., 2014, Piano et al., 2015, Mammola et al., 2017), we considered the number of visitors and the distance from the tourist path as proxies for the human disturbance. We assumed these to be the only perturbations currently present in the Monello cave that are potentially capable of causing a measurable impact. In particular, we assigned to each sampling event the total number of visitors to the cave in the previous two weeks. In this regard, we selected nine sampling points along the visitor pathway (sampling points number 1, 2, 4,



Fig. 2. a) The corridor leading to the “Sala Grande” in the Monello Cave; b) The subterranean woodlouse *Armadillidium lagrecai* Vandel 1969; c) The cardboard used for sampling the fauna in the Monello Cave.

7, 9, 12, 13, 14, and 16), and eight further points away from the path and/or in the lateral branches not opened to public (sampling points number 3, 6, 5, 8, 10, 11, 15, and 17, see Fig. 1). We measured the distance from the cave entrance and from the pathway with a Leica DISTO (TM) laser distance meter.

In order to minimize impact on the fauna and avoid killing, we monitored the fauna using three sheets of corrugated cardboard, which were glued together using a starch-based glue to form single sheets of 15 cm height x 30 cm length. The presence of three sheets creates a thicker and longer lasting surface, otherwise, the high humidity of the cave would flake it quickly. The corrugated side was then placed on the ground (Fig. 2c). According to Caruso (1994), such a surface creates a microhabitat that attracts invertebrates, acting as a refuge, facilitating their count and eventual collection. We placed seventeen cardboard sheets within the cave.

The abundance of *Armadillidium lagrecai* under each cardboard was recorded at each sampling session. We also recorded the presence of *Glomeris dionysii*, *Chthonius* (*Chthonius*) *multidentatus* Beier 1963 (Arachnida: Pseudoscorpiones: Chthoniidae), *Roncus siculus* Beier 1963 (Arachnida: Pseudoscorpiones: Neobisiidae), *Tychobythinus inopinatus* Sabella, Costanzo and Nicolosi 2020 (Insecta: Coleoptera: Staphylinidae), and *Plusiocampa* (*Plusiocampa*)

tinomorei Sendra & Nicolosi 2019 (Entognatha: Diplura: Campodeidae) (see Table S1 and Fig. S1).

Being the main climatic factors known to affect the distribution of subterranean species (Tobin et al., 2013; Mammola et al., 2015; Kozel et al., 2019), at each sampling session, we used an Indoor Air Quality Monitor Delta Ohm instrument to measure instant temperature and relative humidity at each cardboard site. The outside air temperature was provided by SIAS (Servizio Informativo Agrometeorologico Siciliano).

Statistical analyses

We performed all statistical analyses in R (R Core Team, 2018). We explored the factors driving the spatial and temporal abundance of *Armadillidium lagrecai* within the cave using regression-type analyses (Zuur & Ieno, 2016). We calculated the standard deviation of temperature values during the whole sampling period and derived the subjacency (vertical distance from the surface) of each sampling point. We used abundance of individuals of *A. lagrecai* at each site as dependent variables. Given their low abundances, we could not fit regression models for the other species recorded in this study (Fig. S1).

We carried out data exploration following the protocol by Zuur et al. (2010). We evaluated the presence of outliers in the independent variables using Cleveland dotplots. We explored collinearity among

the covariates using pairwise Pearson's r correlations, setting the threshold for collinearity at $|r| \geq 0.7$. We explored non-linearity in the variable distributions and used coplots to evaluate the existence of potential interactions among covariates.

As a result of data exploration, we log-transformed the distance from the visitor pathway and square-root-transformed the total number of visitors in the previous two weeks to homogenize their distribution and cope with a few outliers. Pairwise Pearson's correlations revealed that the temperature was collinear with relative humidity ($r = 0.76$), standard deviation of temperature ($r = -0.73$), distance from entrance ($r = 0.67$) and subjacency ($r = 0.62$). The subjacency was also collinear with standard deviation of temperature ($r = -0.89$), and distance from the entrance ($r = 0.88$). Therefore, we kept temperature as the only independent variable in the analysis.

During data exploration, we found a non-linear response of the abundance of *A. lagrecai* to the sampling week (cumulative counting), reflecting a seasonal variability in the abundance of the species. In light of this seasonal variability and the relative association of the sampling week with temperature, we decided to i) model the temporal distribution of *A. lagrecai* separately using a generalized additive model; and ii) to include the sampling week as a random term in the main regression model, in order to study the effect of microclimatic conditions and tourism on the abundance of *A. lagrecai* avoiding the confounding effect of seasonality.

With respect to i), we fitted the generalized additive model using the *gam* command in the R package *gam* (Hastie, 1991). The structure of the model (in R notation) was:

$$y \sim s(\text{Sampling week})$$

where y = abundance of *A. lagrecai* and s = smooth term.

With respect to ii), we modelled the abundance of *A. lagrecai* with a Poisson generalized linear mixed model (Zuur et al., 2009). We fitted the model in R using the *glmer* command in the R package *lme4* (Bates et al., 2015). The structure of the initial model (in R notation) was:

$$y \sim \text{Temperature} + \text{Number of visitors} + \text{Distance from the path} + (1 | \text{Sampling week})$$

where y = abundance of *A. lagrecai* and $(1 | \text{Sampling week})$ is a random term to deal with repeated observations and measurements at each sampling site.

Once we fitted the initial models, we performed model selection with the R package *MuMIn* (Bartón, 2016) to select a best performing model the minimized AIC and maximized Aikake weight values (Zuur et al., 2009). We carried out model validation on the final model using the R package *Performance* (Lüdecke et al., 2020).

RESULTS

Presence of visitors

During the monitoring period, the cave was visited by 953 visitors with an average of 68 visitors/month. Visitors were more abundant in spring between April and June 2019, with a peak of 159 visitors in June. Visitors were also abundant in November 2019 and January 2020. In contrast, the number of visitors was low in August and December, with 29 and 32 visitors, respectively (Fig. 3).

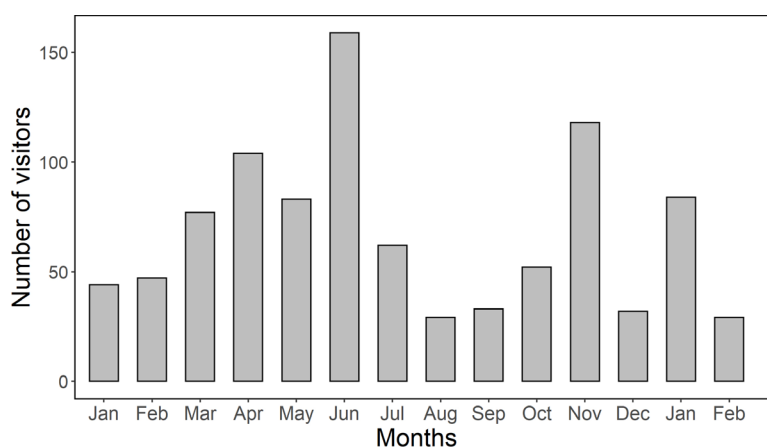


Fig. 3. Number of visitors/months during the monitoring activity.

Cave temperature and relative humidity

With regard to temperature, max-min ranges were attenuated with increasing distance from the cave entrance, and delayed compared to the values recorded outside the caves. The mean annual temperature values deep inside the cave (sites S13-S17) showed little variation over the year (mean \pm SD = 18.4 ± 0.29). Conversely, temperature in the outermost sector (sites S1-S3) showed greater variability (mean \pm SD = 14.8 ± 2.2). Here, temperature was relatively stable in summer, spring and autumn, while in winter

temperature aligned with the outside temperature. The lowest temperatures were recorded in January (12.7°C). The annual trend of the average temperature for each site is shown in Fig. 4.

Relative humidity was constantly close to saturation in the deeper part of the cave, with values ranging from 98 to 100% during the whole sampling period. In the entrance zone, the relative humidity measured was around 80% and showed greater variability (annual range of 20%) (Fig. 5).

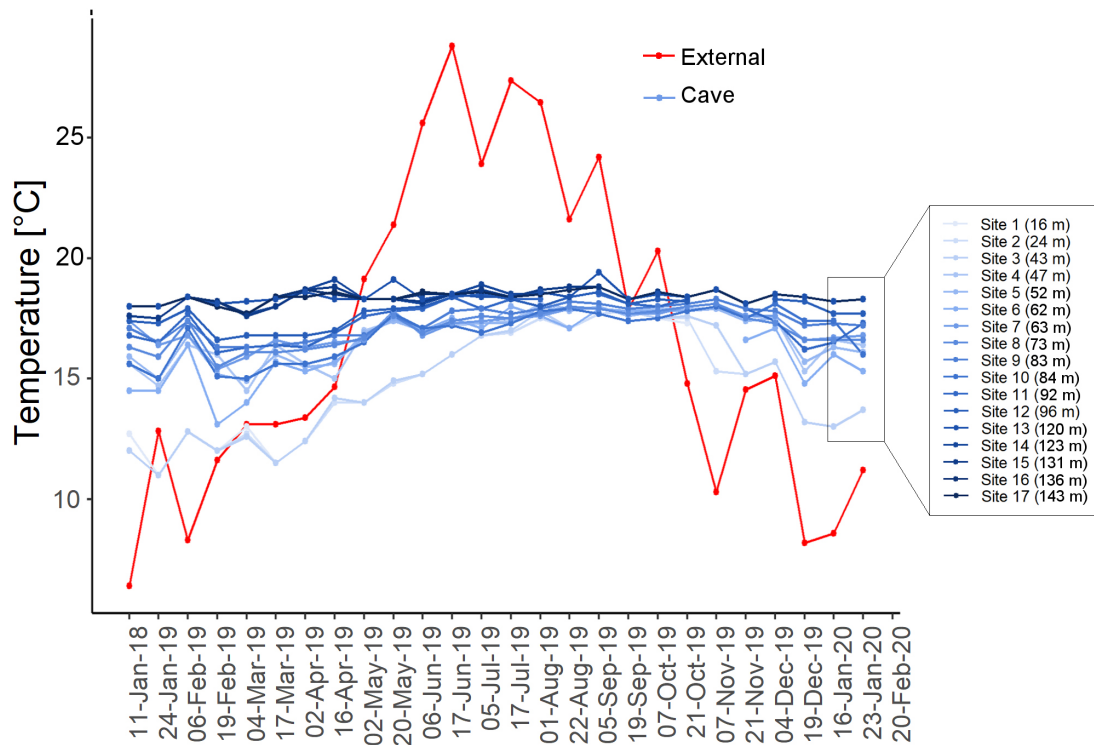


Fig. 4. Temperature of the Monello Cave. Annual trend of external temperature (red line) and temperatures inside the cave measured at each sampling site during the survey (blue lines). The shades of blues indicate the position of the sampling site relative to the cave entrance, from the outermost (lighter blues) to the innermost sites (darker blues).

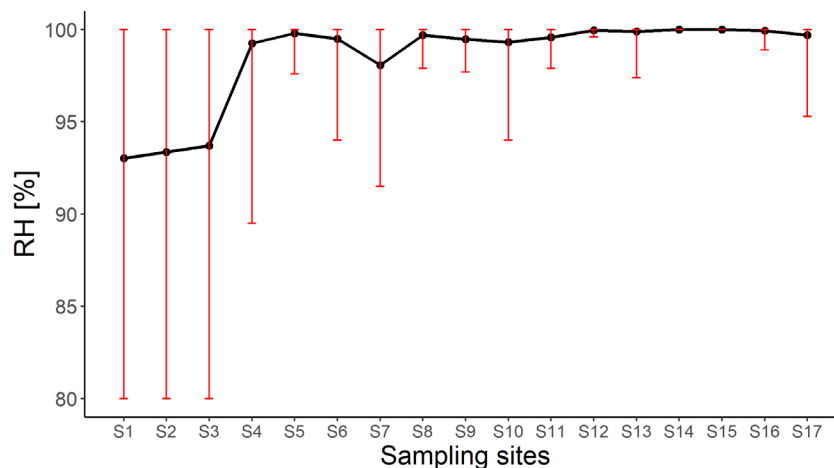


Fig. 5. Annual mean values of relative humidity with max-min ranges (red bars) for each sampling site.

Temporal distribution of *Armadillidium lagrecai*

The generalised additive model indicated that the abundance of *Armadillidium lagrecai* varied significantly throughout the sampling period (edf = 5.14; $\chi^2 = 46.15$, $p < 0.001$), with a decrease from March to June and from November to January. In contrast, abundance increased in June and peaked in October (Fig. 6a).

Factors affecting the abundance of *Armadillidium lagrecai*

Results of model selection are shown in Table 1. The best performing model (Marginal $R^2 = 0.77$; Random effect variance \pm s.d. = 0.11 ± 0.33) included the temperature and the distance from the visitor path as a fixed term. According to the model, species abundance significantly increased with increasing temperature, with the maximum abundance around 18°C (Fig. 6b). Given that temperature was positively

correlated with relative humidity ($r = 0.8$), we recovered the same trend when we built the model by using the relative humidity instead of temperature (Fig. 6c), with *Armadillidium lagrecai* being more abundant in the sampling sites with relative humidity approaching or reaching saturation. None of the other factors had a significant influence on abundance, including the number of visitors and the distance from the tourist path. However, the latter was kept in the final model even though it was not significant based on the z-test ($p = 0.06$).

It is worth noting that the final model was slightly overdispersed (dispersion ratio = 2.1; Pearson's $\chi^2 = 902.612$; $p < 0.001$). However, in light of the model validation (Fig. S2) and given that overdispersion ratio was rather low, we determined that the Poisson distribution was adequate and we did not switch to a quasi-Poisson or negative binomial distribution.

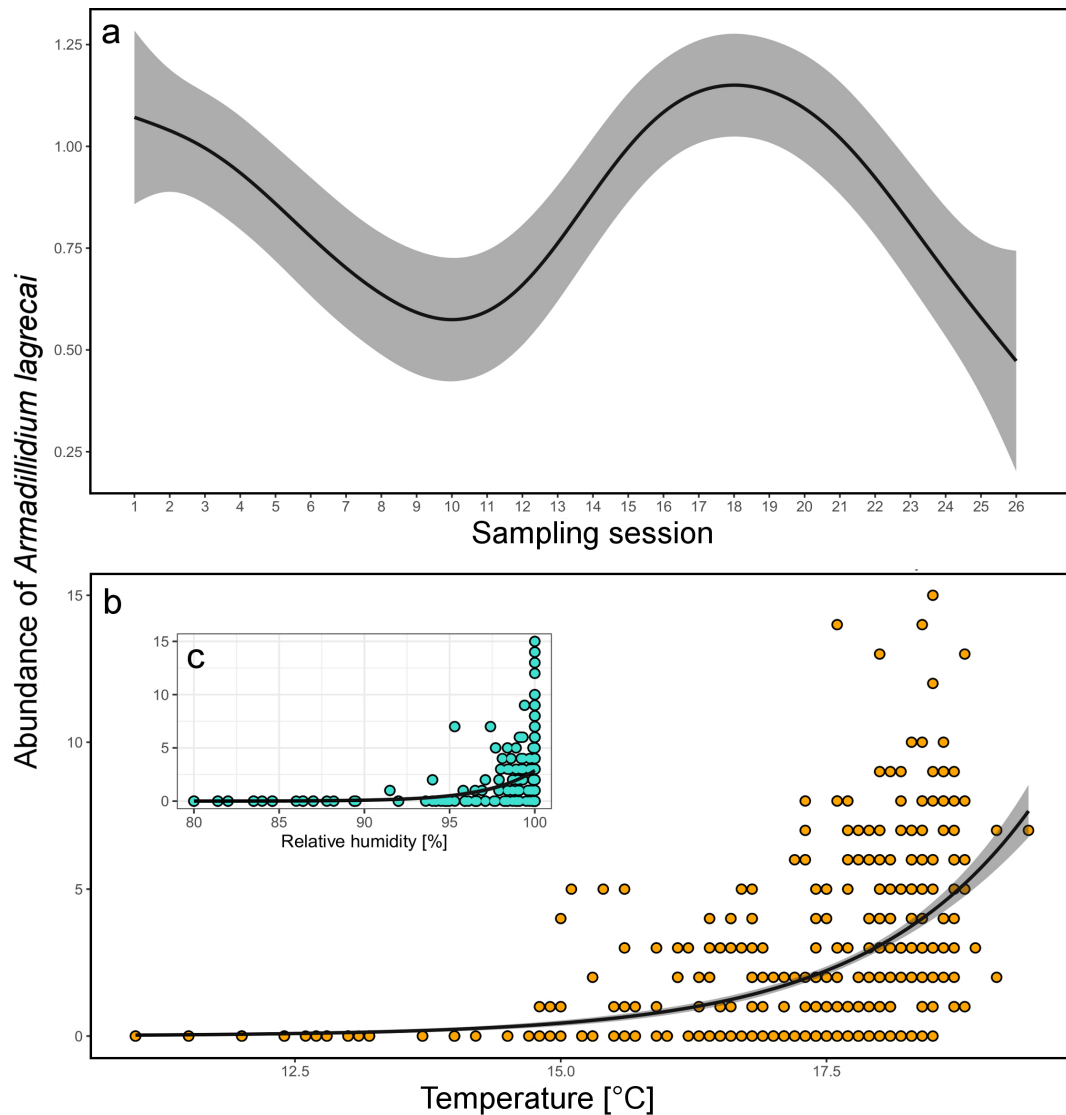


Fig. 6. a) Abundance trends of *Armadillidium lagrecai* in the Monello Cave. Generalized additive model analysis-predicted values (black line) and 95% confidence intervals (grey area) of abundance over time (smoother); b) Effect of temperature on the abundance according to a generalized linear mixed model. The inset graph (c) shows the trend obtained using relative humidity instead of temperature as explanatory variables in the model.

Table 1. Results of model selection and estimated regression parameters. Estimated regression parameters (Estimated $\beta \pm$ S.E.) for the fixed term are given only for the selected model. AIC = Akaike Information Criterion; Δ AIC = (AIC of the model – AIC of the best model); df = Degrees of freedom; w_i = Akaike weights.

Model structure	Variables	Estimated $\beta \pm$ S.E.	p-value	d.f.	AIC	Δ AIC	w_i
y ~ Temperature + Distance from the path + (1 Sampling week)	Intercept	-12.32 \pm 0.77	-	4	1776.56	0.00	0.67
	Temperature	0.74 \pm 0.04	<0.001				
	Distance from the path (log-transformed)	0.05 \pm 0.03	0.06				
y ~ Temperature + (1 Sampling week)		-	-	3	1777.95	1.39	0.33
y ~ Temperature + Number of visitors + Distance from the path + (1 Sampling week)		-	-	6	2239.73	463.16	0.00

DISCUSSION

The opening of a cave to tourism activities impacts the cave ecosystem in several ways. The presence of visitors can lead the environmental alterations in terms of heat, airflow, humidity, and moisture (De Freitas, 2010), which potentially affects the resident fauna. Despite subterranean arthropod diversity in show caves could be high (see Culver & Sket, 2002), investigations aiming at unravelling the effect of tourism on cave arthropods

remain scarce (e.g., Casale et al., 2008; Barcirov et al., 2010; Fong, 2011; Isaia et al., 2011; Pellegrini & Ferreira, 2012; Faille et al., 2014; Pacheco et al., 2020).

By monitoring tourist access, it is possible to assess their effect on cave climatic stability, and eventually modify management strategies (Faille et al., 2014). For instance, monitoring activities should be carried out to evaluate the impact of human visitors on subterranean fauna and microclimate, especially if the cave falls within protected areas.

Although caves are stable environments, they exhibit seasonal changes in their microclimate which may modify the spatial and temporal distribution of subterranean species (Barr & Kuene, 1971; Peck, 1976; Howarth, 1980). These microclimate fluctuations are usually greater near the entrance, where the cave is most affected by external conditions, and drastically decrease in the deep areas where air temperatures remain stable, even when the external air temperature changes (Badino, 2010). Consequently, as in the Monello Cave, deep cave sectors emerge as optimal habitat for specialized subterranean fauna (Tobin et al., 2013; Mammola et al., 2015).

Based on the results of regression analysis, temperature and relative humidity seems to be more important than direct human impact in driving the distribution of *Armadillidium lagrecai* in the Monello Cave. Our data suggest that microclimate influences the spatial distribution of *A. lagrecai*, confirming its preference for the warmest and most stable cave sections, as well as an apparent seasonality effect on abundance variation which is possibly related to reproduction (Caruso, 1994).

The high correlation between temperature and relative humidity suggests the two factors play equally important roles in determining *A. lagrecai* abundance. The species showed a preference for areas of the cave where relative humidity approached or reached saturation (Fig. 6c). Relative humidity is considered to be one of the most important ecological limiting factors in the subterranean domain (Howarth, 1980; 1983), since even small variations can double the evaporation rate (Buecher, 1999) and have direct consequences on the resident fauna (Howarth, 1980). For example, subterranean woodlice possess cuticles with disproportionately thinner and more numerous endocuticular lamellae compared to their surface counterparts; the mechanisms preventing the evaporative loss of water are therefore reduced (Vittori et al., 2017).

Armadillidium lagrecai abundance increased in summer (July–September) when temperature increased within the cave, and gradually decreased during the coldest months (October–January). This corroborates findings from Caruso (1994), who described a reproductive peak in May for the *A. lagrecai* when the warm season begins and cave temperatures gradually rise.

Armadillidium lagrecai demonstrated a clear preference for the innermost sampling sites of the cave (>96 m) where microclimate fluctuations were minimal. Moreover, considering the high correlation between the subjacency and both temperature and the distance from the cave entrance, we can infer that the abundance of *A. lagrecai* increased in the deeper zones with greater subjacency (>20 m). However, it was also in these innermost sections of the cave that we counted a lower number of specimens in the coldest months. This would suggest migrations toward the deeper voids and fissures of the cave, which according to Badino (2010) are characterized by even greater stability in terms of temperature fluctuation.

The distance from the visitor path was the only factor besides temperature that we kept in the analysis; however, its effect was not found to be statistically significant.

In accordance with previous studies (Faille et al., 2014; Pacheco et al., 2020), our analysis has not detected a strong direct effect of the presence of visitors on the abundance of our model species. In other words, with a moderate regime of 68 visitors per month, the current management of the Monello Cave does not seem to harm this subterranean species. The number of visitors present during the sampling activities, as well as the rigorous path delimitation, appears to minimize human disturbance. However, given the effect that a larger number of visitors may have on the cave microclimate, any departure from the current visitors regime we observed should be carefully assessed for the proper management of the cave.

CONCLUSIONS

The study investigated the factors affecting the microhabitat selection of *Armadillidium lagrecai*.

Under current management, it appears that the existing visitors regime in the Monello Cave has no effect on the abundance and distribution of *A. lagrecai*. Our data suggested that the species preferably select the areas of the cave where the microclimate factors were mostly constant during the sampling activities. The observed abundance fluctuations were determined by the microclimatic regime that occurs naturally inside the cave rather than by any disturbances caused by visitors.

However, any increase in visitor trampling could potentially cause the death of cave arthropods crossing the path or hiding under stones. Considering the moderate size of the cave, the number of visitors along the route should therefore continue to be scheduled, also limiting the permanence time in the “Sala Grande”, which correspond to the most microclimatically suitable area for the species.

Finally, it cannot be excluded that greater number of visitors could alter the cave microclimate in terms of temperature and humidity (Baker & Genty, 1998; Dominguez-Villar et al., 2010). Considering the high sensitivity of *A. lagrecai* to microclimate variations, and the fact that Monello Cave is one of the richest areas of troglobitic species in Sicily while being relatively small in size, the presence of visitors should continue to be accurately monitored with microclimatic surveys and visitor limitations maintained.

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Authorship statement: GN and MI set the lines of enquiry, designed the study and led the writing of the paper. GN wrote the first draft of the paper and prepared figures and tables. GN, SC, and GS set the sampling activities and collected the data. SM and GN analyzed the data. All authors reviewed the first draft of the paper and provided important improvements to the original text. This study is framed into a scientific investigation conceived by RC, SC, and GS aimed at evaluating the impact of visitors on the Monello Cave.

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