

# Day–night and seasonal variations of a subterranean invertebrate community in the twilight zone

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## Abstract

Being characterized by the absence of light and a reduced environmental cyclicity, the subterranean domain is generally regarded as temporally stable. Yet, in the proximity of cave entrances (twilight zones), patterns of sunlight and darkness can be detected within the 24-hour day–night cycle. In parallel, changes in the abiotic and biotic conditions are expected; however, these patterns have been rarely explored in animal communities dwelling in the twilight zone. We performed a biological investigation in a small abandoned mine in the Western Alps, monitoring it once per season, both during the day and at night. At each survey, we collected data on the spatial distribution of the resident species, their activity patterns, and the main microclimatic parameters. We observed significant daily variations in the environmental conditions during winter and spring, namely higher temperature, relative humidity and availability of trophic resources at night. In conjunction with these disparate nocturnal conditions, the abundance of troglophile species was also higher, as well as the activity patterns of one of the most frequent species inhabiting the entrance area – the orb-weaver spider *Meta menardi*. We further documented temporal changes in the composition of the parietal community, due to species using the mine as a diurnal, nocturnal or overwintering shelter. Overall, our results suggest that the communities of the twilight zone are not temporally stable and we highlight the importance of taking into account not only their seasonal, but also their daily variations.

## Keywords

arthropods, seasonality, disphotoc zone, spatial dynamics, day night, cave cricket, cave spiders, activity patterns, mine

## Introduction

Light availability plays a crucial ecological role for organisms on the earth surface (e.g., Panda et al. 2002, Bradshaw and Holzapfel 2010, Gaston et al. 2013, Tierney et al. 2017). Alternatively, the absence of light, at least from a biological point of view (see Badino 2000), represents the most crucial factor conditioning the ecology of subsurface habitats and the evolution of subterranean inhabitants (Pipan and Culver 2012, Battiston and Marzotto 2015, Culver and Pipan 2015, Konec et al. 2015, Fišer et al. 2016, Tierney et al. 2017). As a direct consequence of the lack of solar irradiation, primary phototrophic producers are absent in the deep parts of the caves, with the exception of plant roots growing from the soil into sub-superficial subterranean habitats (Gibert and Deharveng 2002). The permanent darkness below the ground also determines the absence of daily dark–light cycles and a reduced influence of the environmental cyclicality (Poulson and White 1969, Howarth 1980, 1983), two factors which seemingly triggered the reduction in the circadian components of activity of numerous cave-limited species over their evolutionary history (Trajano and Menna-Barreto 1995, Hervant et al. 2000, Trajano et al. 2005, Moran et al. 2014, Espinasa et al. 2016). As such, the subterranean domain is generally regarded as temporally stable.

However, evidences have accumulated testifying that subterranean habitats are not entirely aseasonal (Poulson and Culver 1969, Tobin et al. 2013), and that their inhabitants are not entirely arrhythmic (Friedrich 2013, Abhilash et al. 2017). Most subterranean habitats are indeed characterized by a constant flux of invaders and migrants (Chapman 1993, Culver and Sket 2002, Culver and Pipan 2009, Romero 2009, and there can be a temporal variability in the presence and distribution of vertebrates (e.g., Reichard et al. 2009, Krofel 2010, Biswas 2014, Baker 2015, Lunghi et al. 2015, 2017, Ineich and Bourgoïn 2016, Balogová et al. 2017, Lunghi et al. 2018) and invertebrates (e.g., Crouau-Roy et al. 1992, Carchini et al. 1994, Gnaspini et al. 2003, Novak et al. 2004, Papi and Pipan 2011, Tobin et al. 2013, Mammola and Isaia 2014, Bento et al. 2016, Mammola et al. 2015, 2016a, 2017, Bichuette et al. 2017, Lunghi et al. 2017, Plăiașu et al. 2017) found in caves. For instance, invertebrates may move in search of food in larger cave chambers, and move back to the more stable fissures connected with caves in response to any physiological stress (Juberthie 1969, Chapman 1985). Besides, numerous species commonly exploit subterranean habitats in certain phases of their life cycle, e.g. as seasonal shelters (Novak et al. 2010a, Chelini et al. 2011, Lipovšek et al. 2016, Balogová et al. 2017) or as breeding sites (Diesel et al. 1995, Glover et al. 2008, Barzaghi et al. 2017). As a direct consequence, there are seasonal turnovers in the species composition in a typical subterranean community.

In several subterranean habitats, there are transitional photic zones such as cave entrances, where changes in light availability can be detected during the day (but see Mejía-Ortíz et al. 2018 for a peculiar example of a cave with almost no dark zone). In these habitats, theoretically, variations in the composition of the animal community and the abundance and activity of individual species should occur within the 24-hour day–night cycle. Daily spatial and temporal dynamics have been seldom documented

in cave-dwelling species (e.g. Weber et al. 1995, Taylor et al. 2005, Polseela et al. 2011, Havird et al. 2013). In this respect, certain species of bats represent classical examples, resting inside the cave during the day and leaving it at night to feed (e.g., Rodríguez-Durán and Lewis 1987, Agosta et al. 2005, Berková and Zúkal 2006). Similarly, other organisms such as nocturnal species taking shelter in caves during the day display analogous activity patterns. For example, Ineich and Bourgoin (2016) recently reported about the peculiar case of a nocturnal Madagascar gekkonid, *Paroedura tanjaka* Nussbaum & Raxworthy, 2000 (Squamata: Gekkonidae), which exploit the cave habitat during daytime to find food and as a reproductive site, meanwhile avoiding most epigean predators. However, to the best of our knowledge, such daily dynamics have never been explored at the level of the entire invertebrate community inhabiting the twilight zone.

We performed a pilot study in a small subterranean site in the Western Italian Alps, in order to unravel the existence of diurnal–nocturnal and seasonal dynamics in the abundance and patterns of activity of resident species. We hypothesized that i) there are variations in the environmental conditions at the twilight zone (e.g. microclimate, trophic resources) both seasonally and within a day–night cycle. We further hypothesized that ii) in parallel to these daily and seasonal environmental variations, there are changes in the species composition and in the abundance of the resident species. Finally, we hypothesized that iii) there are different activity patterns in the resident species during day- and night-time.

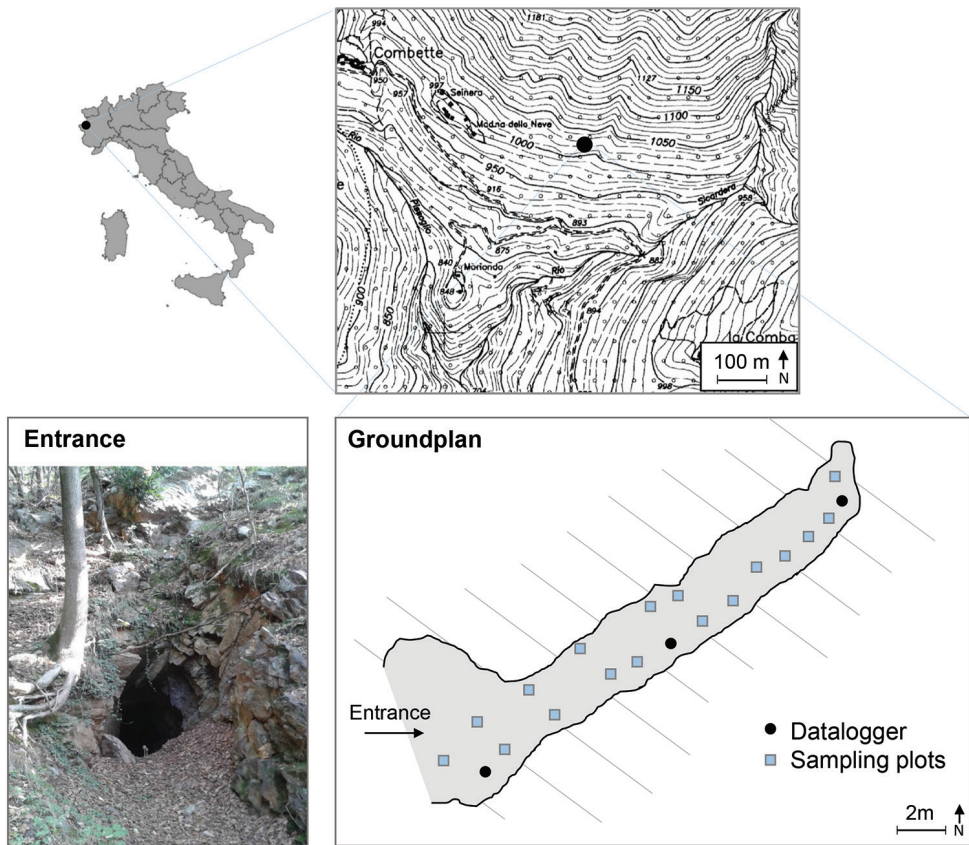
## Methods

### Study site

The study was performed in an abandoned mine near the hamlet of Seiner, municipality of Bruzolo, Susa Valley, Cottian Alps, Italy [entrance at 1007 m asl; 7.201E, 45.136N (WGS84 reference system)]. We chose an artificial site rather than a natural cave due to its linear shape and low ceiling, allowing us to measure the environmental parameters with high accuracy and to access resident animals more easily (Mammola 2018). The Seiner mine represents a typical subterranean habitat at the epigean/hypogean ecotone (*sensu* Prous et al. 2004). It has a horizontal length of only 22 m and the passage cross-section of about  $2.5 \times 2$  m (Figure 1). The mine is in a mixed deciduous forest of chestnuts, oaks, maples and hornbeams, and opens in micaschist rocks used for talc extraction during the first half of the XX century.

### Sampling design

We used a sampling-square methodology to monitor the study site, as it has been shown to be an effective, non-invasive means for investigating the annual dynamics of



**Figure 1.** Map of the study area and groundplan of the Seineria mine, with indication of sampling plots and dataloggers.

invertebrates in both artificial and natural subterranean environments (Bourne 1976, Mammola and Isaia 2014, 2016).

Twenty-two sampling plots of  $1 \times 1$  m were positioned from the entrance up to the end of the mine (Figure 1). We randomly distributed the sampling plots among the mine walls and roof (i.e. parietal habitats; Moseley 2009a). The mine floor was not investigated, because it was impossible to obtain a reliable visual census of the organism due to the presence of leaf litter and debris, and because the floor is not an elective microhabitat for the species considered in our analysis (Mammola and Isaia 2014, Mammola et al. 2016a; see section ‘activity pattern’).

We visited the study area once per season, between November 2016 and June 2017. In each season, the day of the sampling session was arbitrarily chosen to correspond to the day of new Moon closest to the solstice (summer, winter) or the equinox (autumn, spring). During each sampling session, we conducted one visit around 12:00 am, and we repeated the monitoring approximately four hours after sunset.

During each visit, we censused individuals of each invertebrate species occurring at each sampling plot. Species were identified in the field up to the lowest recognized

taxonomic rank, given the reliability of their in-field identification and our taxonomical expertise. We grouped each species into one of the most common ecological categories (troglobiont, troglophile, troglaxene, accidental organisms) used in subterranean biology (Schiner-Racovitz's categories; Sket 2008, Trajano and Carvalho 2017). Each taxon was attributed to one of these categories on the basis of the ecological information available in literature on its natural history (Lana 2001, Smithers 2005a, Isaia et al. 2011, Allegrucci et al. 2014, Mammola and Isaia 2014, Mammola et al. 2016a, 2016b, 2017). We used such information to calculate the abundance of troglophiles and troglaxenes for each sampling plot – no troglobionts were present in the study site.

We characterized the annual climatic conditions in correspondence of the entrance, 10 m inside and 20 m inside by three EL-USB-2 dataloggers (Lascar Electronics; accuracy of  $\pm 0.5$  °C for temperature and  $\pm 2.0\%$  for relative humidity), placed at 1 meter height on the wall and programmed to record temperature and relative humidity every 12 h for the whole sampling period – one measurement at 12:00 am and one at 12:00 pm. During each survey, at the centre of each sampling plot we also measured: i) illuminance (lux; lx) using a photometric probe LP 471 Phot (Delta OHM S.r.l.; accuracy of 0.2%) pointed toward the entrance; ii) air flow velocity (WS; m/s) by a Testo 425 telescopic flow velocity/temperature probe (Testo SE and Co, KGaA; accuracy of  $\pm 0.03$  m/s); and iii) temperature (T; °C) and relative humidity (RH; %) by EL-USB-2 dataloggers (Lascar Electronics; accuracy of  $\pm 0.5$  °C for temperature and  $\pm 2.0$  % for relative humidity).

### Activity patterns

In order to obtain an estimation of the activity patterns of the three troglophile species inhabiting the mine, during each survey we recorded the diurnal and/or nocturnal movements of the spiders *Meta menardi* (Latreille, 1804) (Araneae: Tetragnathidae) and *Pimoida graphitica* Mammola, Hormiga & Isaia, 2016 (Araneae: Pimoidae), and the cave-dwelling cricket *Dolichopoda azami* Saulcy, 1893 (Orthoptera: Rhaphidophoridae). These species were chosen for this analysis owing to i) their high identification reliability in the field (Mammola and Isaia 2014, Mammola et al. 2016a); ii) their high abundance within the study site (pers. obs.); and iii) their large body size – adults of centimetric length, making a visual monitoring in the field possible.

During each diurnal and nocturnal sampling session, we monitored the activity of each individual of the three species occurring within the sampling plots. The activity was expressed as the number of seconds while the animal was moving, using a stoppable chronometer during observation sessions of one minute. We considered any movement of the animals, with or without spatial displacements. During each session, we set the LED light of our speleological helmet to the red spectrum, in order to minimize disturbance to the animal – in accordance with the general chromatic visual spectrum of most arthropods (Briscoe and Chittka 2001). For each plot and each species, we calculated the total species activity, as the sum of the individual activities divided by the number of individuals within the plot.

## Statistical analysis

All statistical analyses were performed in R (R development team 2017). Differences in the environmental conditions at the twilight zone between day and night and across seasons were evaluated graphically (*Graphics* and *Lattice* R packages; R development team 2017, Sarkar 2008) and by means of standard statistical metrics. Wherever appropriate, we tested statistical differences by means of factorial linear regressions (ANOVA) or generalized linear regressions (generalized linear models; GLMs).

To analyse the day–night differences in the abundance, and seasonality of the abundance of troglonexes and troglophiles, we used a mixed-design analysis of variance with Poisson distributed data (generalized linear mixed models; GLMMs). For the activity pattern of the three species, we used linear mixed models (LMM). GLMM and LMM were fitted with the R packages *lme4* (Bates et al. 2014) and *nlme* (Pinheiro et al. 2014), respectively. Regression-type analyses were conducted following the general protocol of Zuur and Ieno (2016).

Counts of troglonexes, counts of troglophiles and total activity values for each plot represented the dependent variables. Environmental features (distance from the entrance, temperature, humidity, airflow and illuminance) and their relative interaction with the sampling period, either diurnal (day–night) or seasonal (winter, spring, autumn, summer), represented the independent covariates (i.e., explanatory variables). In order to capture potential non-linear trends in the response of the dependent variables, we allowed up to second order polynomial for the continuous independent variables, when appropriate. The mixed procedure accounted for multiple observations from the same sampling plot, by specifying the sampling plot within the seasonal sampling session as a random-intercept nested structure.

Prior to fitting the models, we explored the datasets following the standard protocol for data exploration proposed by Zuur et al. (2010). Indeed, the inclusion of outliers and highly correlated predictors in a regression analysis may lead to incorrect results (type I and II statistical errors). Thus, we used Cleveland dotplots to assess the presence of outliers in dependent and independent variables. We investigated multicollinearity among continuous covariates via Pearson correlation tests ( $r$ ) and variance inflation factors values (VIFs), setting the threshold for collinearity at  $r > |0.7|$  and  $VIF > 3.0$ . The collinearity between continuous and categorical variables was graphically evaluated with boxplots.

Once we fitted the initial models, including all covariates and interactions of interest, we applied model selection via backward elimination (Johnson and Omland 2004). Models were simplified by sequentially deleting covariates and/or interactions according to AICc values (Hurvich and Tsai 1989). The process was repeated until all remaining variables were statistically significant. In turn, validation plots were constructed using model residuals, and Poisson GLMMs were checked for over-dispersion.



## Results

### Environmental conditions

During the day, illuminance ranged from 900 lx in the vicinity of the entrance, to 0 lx at the end of the mineshaft (mean $\pm$ sd= 22.68 $\pm$ 103.56). Illuminance was always null at night. Airflows ranged from 0 to 0.61 m/s (mean $\pm$ sd= 0.08 $\pm$ 0.48). There were no daily or seasonal variations in the intensity of the airflows (ANOVA;  $R^2$ = 0.08,  $p$ = 0.26 n.s.).

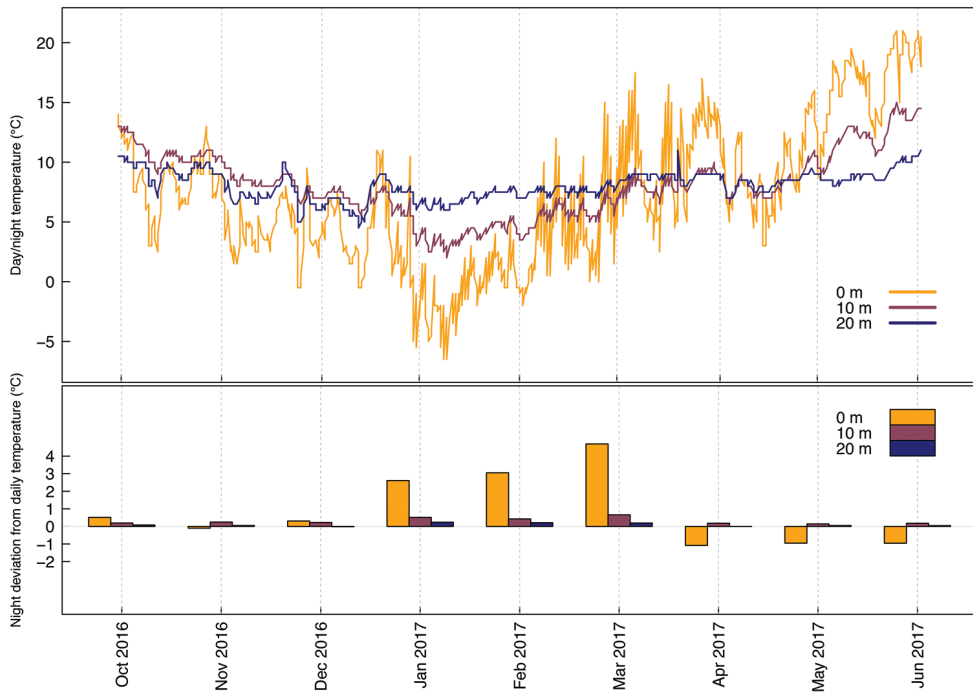
The mean annual temperature at the entrance, at 10 m and at 20 m inside was comparable, however, values at the entrance showed higher seasonal variability (mean $\pm$ s.d. 0 m= 7.19 $\pm$ 5.92 °C; 10 m= 8.08 $\pm$ 2.76 °C; 20 m= 8.09 $\pm$ 1.24 °C). Overall, the amplitude of changes and min–max ranges were progressively attenuated with increasing distance from the entrance (Figure 2). There were no significant thermal variations between day and night inside the mine (ANOVA;  $R^2$ = 0.12,  $p$ =0.17 n.s.). At the entrance (0 m), significant variations between day and night were observed in winter and spring (Figure 2). In particular, night temperature was significantly higher in winter (LM; Winter\*Night: estimated  $\beta$  $\pm$ SE= 0.595 $\pm$ 0.318,  $p$ < 0.001), and significantly lower in spring (LM; Spring\*Night: estimated  $\beta$  $\pm$ SE= –0.708 $\pm$ 0.318,  $p$ = 0.02), with respect to diurnal temperature.

Relative humidity ranged daily and seasonally between 70% and 100% (mean $\pm$ sd= 88.85 $\pm$ 5.65). Difference between day and night were more pronounced in winter and spring (Figure 3), with significantly higher humidity values at night (beta-GLM; Winter\*Night, estimated  $\beta$  $\pm$ SE= 0.550 $\pm$ 0.133,  $p$ < 0.001; Spring\*Night, estimated  $\beta$  $\pm$ SE= 0.384 $\pm$ 0.141,  $p$ = 0.006).

### Animal community and abundance models

The mineshaft hosted a diversified subterranean biocoenosis, including rich populations of arthropods typical of the twilight zone of Western Alpine caves (Table 1). Over the year, we found 27 taxa within the study area. The most abundant predators [*Meta menardi*, *Metellina merianae* (Scopoli, 1763), *Pimoa graphitica*, *Dolichopoda azami*, *Tegenaria* cf. *silvestris* (Araneae: Agelenidae)] were recorded during all surveys (Table 1). Some taxa were exclusively recorded either during the day (e.g. geometrid moths) or at night (e.g. centipedes and millipedes). There were also seasonal variations in the animal community, with species found in either one [*Eupolybothrus* sp. (Lithobiomorpha: Lithobiidae), *Callipus* cf. *foetidissimus* (Nematophora: Callipodidae)] or more seasons [e.g. *Troglohyphantes lucifer* Isaia, Mammola & Pantini, 2017 (Araneae: Linyphiidae)].

Regression models were performed to identify the most important factors driving the abundance of both troglloxenes and trogllophiles. Data exploration revealed that the variable temperature was collinear with the categorical variable season, and therefore



**Figure 2.** Temperature variation in the study area. Data refer to record of temperature and relative humidity measured every 12 h (one measurement at midday and one at midnight). Top panel: annual trends of temperatures measured at the entrance (0 m; orange line) and inside the mine (10 and 20 m; purple and blue lines, respectively). Bottom panel: mean of monthly positive and negative temperature deviations at night, with respect to the daily temperature recorded during the same period.

it was not further considered. The variable light intensity and relative humidity were collinear with the categorical variable day–night, given that illuminance was always null at night and that the relative humidity higher at night (see Figure 3). Thus, these variables were not introduced in the regression analyses.

Best AICc models and model estimated parameters are reported in Table 2. With respect to the abundance of troglonexes, there was a significant interaction between the day–night cycle and the seasonality (Figure 4). Overall, the abundance of troglonexes within the mineshaft was significantly higher at night, in summer and autumn. In autumn, there was the highest discrepancy between day and night, with a higher abundance at night. With respect to autumn, in the other seasons the day–night differences were significantly lower (Table 2).

Higher abundance of troglaphiles were observed at night across all seasons (Table 2). Their abundance also varied seasonally with the distance from the entrance (Figure 5). In winter and spring, the highest abundance of troglaphiles was predicted in the inner section of the mine, whereas during summertime closer to the entrance. In autumn, the predicted abundance of troglaphiles peaked at intermediate distances (Figure 5).



**Table 1.** Checklist of the taxa recorded within the study site. For each taxon we report the ecological classification (Adapt; TF= Troglophile, TX= Troglaxene, AC= Accidental), the diurnal/nocturnal occurrence (D/N; D= day, N= night) and the seasonal abundance.

Species (Family)	Adapt	D/N	Autumn	Winter	Spring	Summer
<b>ARACHNIDA: ACARINA</b>						
Gen. sp.	AC	D	–	–	–	1
<b>ARACHNIDA: ARANEAE</b>						
<i>Tegenaria</i> cf. <i>silvestris</i> (Agelenidae)	TF	D/N	12	22	27	6
<i>Amaurobius</i> sp. (Amaurobiidae)	TX	N	–	–	2	5
<i>Araneus diadematus</i> Clerck, 1757 (Araneidae)	AC	D/N	7	2	4	3
<i>Drassodes</i> sp. (Gnaphosidae)	AC	N	–	–	–	1
<i>Troglohyphantes lucifer</i> Isaia et al., 2017 (Linyphiidae)	TF	D/N	–	8	3	5
<i>Labulla thoracica</i> (Wider, 1834) (Linyphiidae)	AC	N	–	3	6	–
<i>Pimoida graphitica</i> Mammola et al., 2016 (Pimoidae)	TF	D/N	39	45	22	10
<i>Meta menardi</i> (Latreille, 1804) (Tetragnathidae)	TF	D/N	90	100	135	74
<i>Metellina merianae</i> (Scopoli, 1763) (Tetragnathidae)	TF	D/N	38	32	42	60
<i>Episinus</i> sp. (Theridiidae)	AC	N	1	–	–	–
<b>ARACHNIDA: OPILIONES</b>						
<i>Amilenus</i> sp. (Phalangiidae)	AC	D/N	–	–	–	4
<b>MYRIAPODA: CHILOPODA</b>						
<i>Eupolybothrus</i> sp. (Lithobiidae)	TF	N	–	–	1	–
<b>MYRIAPODA: DIPLOPODA</b>						
<i>Callipus</i> sp. (Callipodidae)	TX	N	–	–	3	1
<b>INSECTA: DIPTERA</b>						
Gen. sp.	AC	D/N	569	252	223	377
<i>Musca</i> cf. <i>domestica</i> (Muscidae)	AC	N	2	–	–	–
<i>Limonia</i> cf. <i>nubeculosa</i> (Limoniidae)	TX	D/N	67	44	48	88
<b>INSECTA: HYMENOPTERA</b>						
Gen. sp. (Formicidae)	AC	D	–	–	1	1
<b>INSECTA: RHYNCHOTA</b>						
<i>Pentatoma</i> cf. <i>rufipes</i> (Pentatomidae)	AC	N	–	–	–	1
<b>INSECTA: LEPIDOPTERA</b>						
<i>Scoliopteryx libatrix</i> (Linnaeus, 1758) (Noctuidae)	TX	D/N	–	6	–	–
Gen. sp. (Geometridae)	AC	D	–	–	–	2
<i>Triphosa</i> cf. <i>dubitata</i> (Geometridae)	TX	D	–	–	–	8
<b>INSECTA: ORTHOPTERA</b>						
<i>Dolichopoda azami</i> Saulcy, 1893 (Rhaphidophoridae)	TF	D/N	28	12	28	18
<b>INSECTA: TYSANURA</b>						
<i>Lepisma</i> sp. (Lepismatidae)	AC	D/N	3	2	9	1
<b>MOLLUSCA: GASTROPODA</b>						
<i>Oxychilus</i> sp. (Oxychilidae)	TX	D	–	1	1	–
<i>Limax</i> sp. (Limacidae)	AC	D/N	–	–	–	1

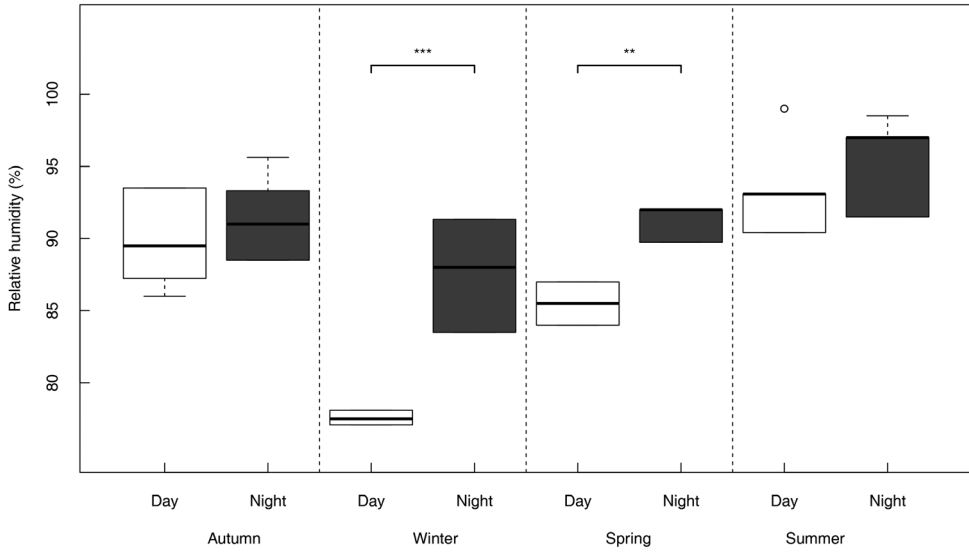
**Table 2.** For each model are shown significant variables included in the relative best AICc model. For the categorical variable Day–Night, the baseline is ‘day’. For the categorical variable Season, the baseline is ‘Autumn’. Variables excluded due to model selection or in the initial data exploration (collinearity analysis) are not shown. The symbol asterisk (\*) indicate an interaction.

Dependent variable	Independent variables (baseline)	Estimated $\beta \pm \text{SE}$	p-value
Abundance of external elements	Intercept	0.085 $\pm$ 0.257	–
	Day–Night (Night)	1.945 $\pm$ 0.180	<0.001
	Season (Winter)	1.870 $\pm$ 0.334	<0.001
	Season (Spring)	1.890 $\pm$ 0.327	<0.001
	Season (Summer)	0.534 $\pm$ 0.352	0.130
	Season (Winter) * Day–Night (Night)	-2.212 $\pm$ 0.210	<0.001
	Season (Spring) * Day–Night (Night)	-2.117 $\pm$ 0.212	<0.001
	Season (Summer) * Day–Night (Night)	-1.250 $\pm$ 0.256	<0.001
Abundance of troglophiles	Intercept	0.079 $\pm$ 0.374	–
	Day–Night (Night)	0.243 $\pm$ 0.078	0.002
	Distance	0.480 $\pm$ 0.098	<0.001
	Distance <sup>2</sup>	-0.029 $\pm$ 0.005	<0.001
	Season (Winter)	0.335 $\pm$ 0.5150	0.515
	Season (Spring)	-0.318 $\pm$ 0.553	0.565
	Season (Summer)	1.356 $\pm$ 0.496	0.006
	Season (Winter) * Distance	-0.358 $\pm$ 0.130	0.005
	Season (Spring) * Distance	-0.213 $\pm$ 0.136	0.116
	Season (Summer) * Distance	-0.380 $\pm$ 0.136	0.005
	Season (Winter) * Distance <sup>2</sup>	0.026 $\pm$ 0.007	<0.001
	Season (Spring) * Distance <sup>2</sup>	0.018 $\pm$ 0.007	0.001
	Season (Summer) * Distance <sup>2</sup>	0.016 $\pm$ 0.006	0.003
Activity of <i>Meta menardi</i>	Intercept	0.834 $\pm$ 0.364	–
	Day–Night (Night)	1.279 $\pm$ 0.517	0.015

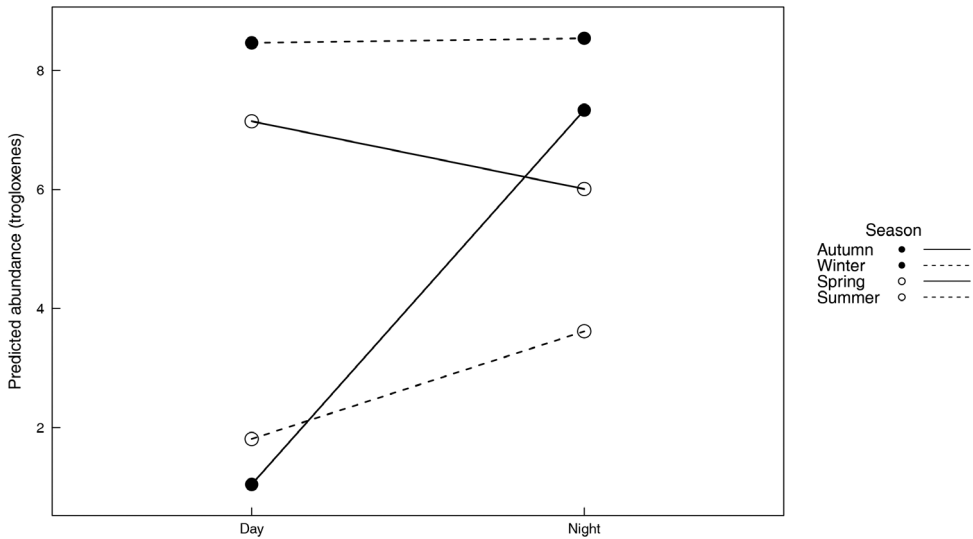
## Activity patterns

Overall, we recorded the activity patterns of *Pimoida graphitica* 116-times, of *Meta menardi* 399-times and of *Dolichopoda azami* 86-times. The three species showed contrasting activity patterns. The total activity of *P. graphitica* on the web and of *D. azami* on the walls were extremely reduced both during the day (*P. graphitica*, day mean activity $\pm$ sd= 0.01 $\pm$ 0.11; *D. azami*= 0.06 $\pm$ 0.55) and at night (*P. graphitica*, night mean activity $\pm$ sd= 0.80 $\pm$ 6.71; *D. azami*= 0.17 $\pm$ 0.58). Although overall activity was slightly higher at night in both species, the variable activity was highly zero-inflated (over 90% of observations were zero), meaning that individuals were mostly inactive. It was thus not possible to construct meaningful regression models with the available data, even using specific statistical techniques designed to deal with zero inflation (zero-inflated regression model did not converged; Zuur et al. 2012).

Conversely, *M. menardi* was in general more active, enabling to fit a robust model for this species. Model selection procedure revealed that the best model supported by observation included only the categorical variable day-night (Table 2). The total activ-

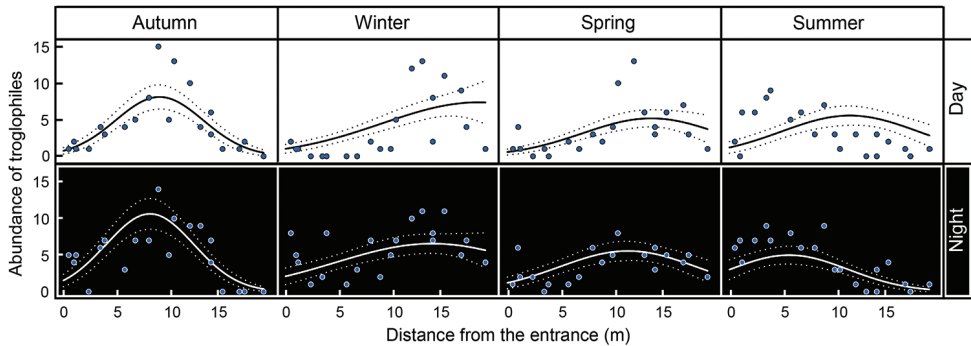


**Figure 3.** Boxplots showing the difference between relative humidity values during the day (white boxes) and at night (grey boxes) in the four seasons. Significant differences are highlighted by asterisks (Signif. codes: \*\*\*  $p < 0.001$ , \*\*  $p < 0.01$ ).



**Figure 4.** Interaction plot showing the effect of the interaction between seasonality and the day–night cycle on the abundance of troglonemes.

ity of *M. menardi* was higher at night (night mean activity  $\pm$  sd =  $2.56 \pm 5.60$ ) than during the day (day mean activity  $\pm$  sd =  $0.83 \pm 2.41$ ), and this difference was statistically significant ( $p = 0.02$ ). No significant differences in seasonal activity were detected, therefore the variable season was dropped during model selection.



**Figure 5.** Predicted values (filled lines) and 95% confidence intervals (dotted lines) of the effect of distance from the main entrance in interaction with the sampling season on the abundance of trogliphiles derived from the generalized linear mixed model (GLMM). Day and night trends are shown.

## Discussion

Whilst the forefront on analysis of the temporal patterns in cave communities has relied on the seasonal timescale (e.g., Tobin et al. 2013, Pellegrini and Ferreira 2016, Lunghi et al. 2017, Mammola et al. 2017), this study aimed at incorporating a day/night timescale in the analysis of abundance and activity patterns of subterranean invertebrates. We observed significant day–night variations in the abiotic conditions of the twilight zone in term of temperature, humidity and illuminance and, in parallel, variations in the composition of the community and the abundance of certain species. These variations differed across seasons, with the most significant discrepancies between day and night in winter and spring.

It is self-evident that the most easily detectable day–night variation in the environmental conditions of the twilight zone pertain the illuminance. Most species inhabiting the twilight zone should theoretically perceive and respond to variations in light availability within the typical circadian cycle of 24 hours – although some erratic patterns of activity have been documented (Koilaraj et al. 2000, Hoenen 2005, Pasquali and Sbordoni 2014). Variations in the composition of the community between day and night were indeed observed. Aside from the obvious differential daily availability of sunlight, at least in the vicinity of the entrance there were also variation in the climatic conditions, especially in respect to higher moisture content at night. A reduced relative humidity in particular, is a well-known limiting factor for the presence of the subterranean fauna (Howarth 1980, 1983, Sharratt et al. 2000). Pronounced sensitivity to saturation deficit was experimentally demonstrated in subterranean beetles (Boyer-Lefèvre 1971), crickets (Yoder et al. 2011) and spiders (Howarth 1980, Hadley et al. 1981, Mammola and Isaia 2017). The fact that subterranean species are preferentially associated with humid microhabitats (Howarth 1980, 1983), indirectly implies that, at night, the twilight zone should represent a more suitable habitat for the subterranean fauna due to the higher levels of relative humidity – at least in winter and spring.

In conjunction with these disparate diurnal–nocturnal and seasonal conditions, we observed variation in the abundance and composition of the animal community. The parietal community was primarily composed by troglophile predators, some troglloxenes plus a variety of accidental species (Table 1). It is worth noting that prior to this study, only two species were recorded in the study site, namely the spiders *Pimoida graphitica* (Mammola et al. 2016b) and *Troglohyphantes lucifer* (Isaia et al. 2017). Across the four seasons, the abundance of troglloxenes was in general higher at night. There were also variations in the presence of troglloxenes in the study area, with greater nocturnal abundances during summertime and in autumn. It has been demonstrated that predators inhabiting the twilight zone have access to a range of potential prey, which mostly includes accidental organisms that move into the subterranean system from the external environment (Smithers 2005b). The temporal differences in the abundance of troglloxenes and accidental elements in the twilight zone (mostly dipterans), in turn, determines a differential availability of potential prey items for the resident predators. During summertime and autumn, when the availability of these potential prey items was higher at night, troglloxenes predators (*Dolichopoda azami*, *Meta menardi*, *Pimoida graphitica*) displaced themselves toward the entrance of the mineshaft, most likely in order to exploit this increased availability of trophic resources. Conversely, during winter, the predicted abundance of troglloxenes was higher deeper inside the mine.

We also noticed that some of the taxa used the mine either during the day or at night. For instance, the nocturnal moth [*Triphosa* cf. *dubitata* (Lepidoptera: Geometridae)] likely uses the mine as a diurnal shelter, whereas myriapods were exclusively documented at night. In contrast to moths, the latter case may not reflect a true biological pattern, but can be explained in light of a differential detectability of the species between day and night, i.e. myriapods are mostly active at night and preferably occur in sheltered and hardly accessible microhabitats during the day. Finally, a small part of the community used the mine for overwintering, e.g. the herald moth *Scoliopteryx libatrix* (Linnaeus, 1758) (Lepidoptera: Noctuidae).

### Activity patterns

The high nocturnal activity that we observed in *Meta menardi* can be explained considering the parallel higher nocturnal availability of potential prey items. Indeed, a relation between the presence of *Meta* spiders and the availability of prey was previously documented (Mammola and Isaia 2014, Manenti et al. 2015). The other species considered in this study, instead, were mostly inactive. This is not surprising in the case of *Pimoida graphitica*, which is regarded as a sit-and-wait hunter (Mammola et al. 2016a, 2016b). Conversely, our results do not meet our expectation for the cave-dwelling cricket *Dolichopoda azami*. In particular, given that the recorded activity pattern was extremely low, and abundance of individuals was similar during day and night, our data seemingly did not confirm the hypothesis that *Dolichopoda* crickets leaves the cave environment at night to forage outside (see, e.g., Di Russo et al. 1991, 1994, Carchini et al. 1994, Lana 2001), at least in this specific site.

No significant variations in the activity patterns were detected in respect to the other environmental predictors considered in this study. However, given that these results were obtained in uncontrolled environment, the picture obtained of activity patterns remains preliminary. Detailed experiments performed in laboratory conditions would be useful, specifically aimed to establish rhythms of activity during controlled light/dark cycles and to evaluate the circadian clock of troglophile species inhabiting the twilight zone.

## Concluding remarks

Although based on a single subterranean community, these results of this study can be used as a jumping-off point to introduce new ideas about our perception of subterranean ecosystems. We acknowledge that it may seem counterintuitive to use caves as model systems where to investigate biological cycles related to light availability. In fact “[...] *the subterranean ecological theater is, by definition, dark*” (Culver 2016, p. 67). Yet, in the twilight zone, variations in the availability of sunlight can be detected within the regular daily light–dark cycle. Our data evidence that, in parallel, there are daily variations in some microclimatic and trophic conditions, conditioning the animal community resident in this transitional area.

For many years, deep cave habitats have been the central models for studying the ecology and evolution of subterranean life (Culver and Pipan 2015). In recent years, however, the twilight zone is receiving increasingly attention as a model system in ecology and evolution. These transitional habitats represent ecotones characterized by clear environmental gradients (Prous et al. 2004), which can be used as ideal models for to study of transition in species assemblages (Sharipova and Abdullin 2007, Moseley 2009b, Prous et al. 2015, Mammola et al. 2017). Furthermore, non-strictly cave species inhabiting these transitional habitats offer intriguing opportunities for shedding light on the process of adaptation to subterranean environments (Yao et al. 2016) and for the study of classic ecological topics, such as competition dynamics, the niche theory and the predator-prey interactions (Novak et al. 2010b, Luštrik et al. 2011, Mammola and Isaia 2014, Mammola et al. 2016a). In this frame, our data emphasize the fact that the study of cave twilight zone communities should preferably incorporate a temporal perspective, as already suggested by other authors (e.g. Culver and Sket 2002, Bichuette et al. 2017, Lunghi et al. 2017).

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