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International Journal of Speleology

Official Journal of Union Internationale de Spéléologie



Seasonal dynamics and micro-climatic preference of two Alpine endemic hypogean beetles

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Abstract: Hypogean beetles generally live in stable environments, characterized by constant temperature and high relative humidity. Changes in the underground microclimatic conditions generally induce local migrations of the beetles through the hypogean environment in search of suitable microhabitats. We studied the seasonal dynamics and the micro-climatic preference of two Alpine endemic hypogean beetles - *Sphodropsis ghiliani* (Coleoptera, Carabidae) and *Dellabeffaella roccae* (Coleoptera, Cholevidae) - in the hypogean complex of Pugnetto (Graian Alps, Italy). We surveyed the two species for one year, using baited pitfall traps and measuring temperature and humidity along the two main caves. We used logistic regression mixed models (GLMMs) to relate the presence of the two species to several variables, namely microclimate (seasonality, temperature, and humidity), subyacency and cave length. In addition, we tested the attractive power of the bait on the two species. The thermic optimum for *S. ghiliani* was found to be around 7°C, with an increasing probability of finding the species in the vicinity of the cave entrance during summer, autumn and spring. The species migrates inside the cave in winter, in response to the drop in the mean daily temperature and in the relative humidity occurring in the outer parts of the cave. On the contrary, *D. roccae* showed a significant preference for the deeper sections of the cave, characterized by an almost constant temperature of 9°C in air saturated with water vapour. Males and females individuals of both species were found to be equally affected by the environmental variables included in the analysis. We also provided information on the life history of the two species and methodological insights about the use of the bait in the traps.

Keywords: Carabidae; Cholevidae; microclimatic preference; Western Italian Alps, cave ecology, *Sphodropsis ghiliani*; *Dellabeffaella roccae*

Received 10 February 2015; Revised 29 May 2015; Accepted 4 June 2015

Citation: Mammola S., Piano E., Giachino P.M. and Isaia M., 2015. Seasonal dynamics and micro-climatic preference of two Alpine endemic hypogean beetles. *International Journal of Speleology*, 44 (3), 239-249. Tampa, FL (USA) ISSN 0392-6672 <http://dx.doi.org/10.5038/1827-806X.44.3.3>

INTRODUCTION

The climatic conditions occurring underground have a profound influence on hypogean fauna (Culver & Pipan, 2010). As far as hypogean beetles are concerned, it is well known that they generally dwell in environments which are relatively stable from an environmental point of view, characterized by constant temperature and an atmosphere saturated with water vapor (Moldovan, 2005). Indeed several hypogean beetles are adapted to narrow ranges of temperature and relative humidity, and thus classified as stenothermic and stenohygic (Howarth, 1980). Hence, it has been observed that they are able to react quickly when significant changes in the microclimatic conditions occurs (Juberthie, 1969), migrating inside the inner parts of the cave (and through the network

of cracks) in search of other suitable micro-habitats (Novak et al., 2004; Moldovan, 2005; Giachino & Vailati, 2010). Moreover, movements during the season proved to be influenced by air temperature, resulting in spatial migrations (e.g. Casale, 1988), seasonal rhythmicity (e.g. Crouau-Roy et al., 1992) and fluctuations in the abundances of the beetles captured in the traps (e.g. Rendoš et al., 2012). In order to achieve a better understanding of the relationship between the hypogean fauna and the abiotic parameters occurring underground, these phenomena were investigated in two species of hypogean beetles inhabiting the Pugnetto hypogean complex (Graian Alps, NW-Italy). *Sphodropsis ghiliani* (Schaum, 1858) s.l. (Coleoptera, Carabidae, Sphodrina) is an endemic ground beetle of the Western Alps, distributed from the Ligurian-Maritime to the Lepontine Alps (Casale,

1988). This beetle is frequently found in caves, but also able to colonize several terrestrial shallow subterranean habitat (SSH *sensu* Culver & Pipan, 2009), such as the *Milieu Souterrain Superficiel* (*sensu* Juberthie et al., 1980; 1981 - hereinafter MSS), various type of rocky habitats and, in particular conditions, the forest leaf litter (Casale, 1988). *Dellebeffaella roccae* (Capra, 1924) (Coleoptera, Cholevidae, Leptodirinae), has been described as one of the most specialized Leptodirinae species in the Western Italian Alps (Vigna Taglianti, 1968; Vailati, 1988). This species has been exclusively found in the Pugnetto hypogean complex (Vailati, 1988), where it inhabits the innermost parts of the caves. Since the earliest biospeleological investigations of the site, both species have been reported to be particularly abundant in the Pugnetto hypogean complex (e.g., Capra, 1924; Sturani, 1942; Capra & Conci, 1951; Martinotti, 1968; Fig. 1).



Fig. 1. *Sphodropsis ghiliani* and *Dellebeffaella roccae*. Historical oil painting of Mario Sturani (Ancona, 1906 – Torino, 1978) representing individuals of *Sphodropsis ghiliani* (foreground) and *Dellebeffaella roccae* (background) dwelling in the Borna Maggiore di Pugnetto. By courtesy of the family Sturani, published in Sturani (1942).

In order to study how the environmental conditions influence the dynamics of the two beetles from a spatial and a seasonal perspective, we designed a pitfall trap study-survey in two caves of the hypogean complex of Pugnetto, hypothesizing that i) there is a significant preference of the two species for a bound and determinate range of temperature and relative humidity; ii) the two species dwell preferentially in different sections of the caves and are able to migrate inside the cave according to the microclimatic changes occurring in the cave; iii) males and females are equally affected by the environmental variables included in the analysis (i.e., the sex ratio does not change through space and time).

Moreover, because of the saprophytic feeding behavior of *D. roccae* (Capra & Conci, 1951) and the generalist predator habits of *S. ghiliani* (Morisi, 1971; Casale, 1988), we hypothesize them to respond differently to the presence of baits in the traps.

This study was also an opportunity to investigate the so far unknown ecology of *D. roccae*, and to confirm from a statistical point of view some of the ecological and phenological observation regarding *S. ghiliani* reported in the literature. In particular, Casale (1988) hypothesized a relatively high thermophily and hygrophily in the latter species and pointed out that should be preferentially found in the area close to the cave entrance (or even outside) at dusk and in the early morning during spring and summer.

MATERIAL & METHODS

Study area

The Pugnetto hypogean complex is located in the vicinity of the hamlet of Pugnetto, municipality of Mezenile, Lanzo Valley, Graian Alps, Piedmont (NW Italy). The site is surrounded by a *Luzulo-Fagetum* beech forest (Sindaco et al., 2009) and hosts several natural caves classified as “Caves not open to the public” (H 8310) and thus protected under the European Habitat Directive 43/92 (S.C.I. IT 1110048) (Fig. 2). Our study was conducted in the Borna di Pugnetto cave (cadastral number 1501 Pi/TO, entrance at N 45°16'19", E 5°02'26", altitude 820 m a.s.l.) and in the Grotta Superiore di Pugnetto cave, also known as Creusa d'le Tampe (cadastral number 1503 Pi/TO, entrance at N 45°16'12", E 5°02'33", altitude 870 m a.s.l.). For the sake of clarity, hereinafter we will refer to the first cave as “Borna” and to the second as “Creusa”. Due to the difficult of access and the small dimensions, the other minor caves of the hypogean complex were excluded from the study, namely the Tana del Lupo cave (1502 Pi/TO), and the Tana della Volpe cave (1504 Pi/TO). A fifth minor cave (“Cavernetta”) mentioned by Muratore (1946) in the original description of the hypogean complex was also excluded. The caves are developed in schists and serpentine formations of Jurassic age and originated in lens of carbonate-rich calcschist included within insoluble metamorphic rocks (gneiss, prasinite and serpentinite; Selvaggi & Sindaco, 2001). The Borna has a planimetric development of 765 m. It consists of a main gallery (300 m) directed north-south, which splits at the end into two branches (*Ramo della Madonna* and *Ramo della Fontana*). Near the main entrance there are also some labyrinthine sideways galleries (*Gallerie di Sinistra*), which were not sampled during this study. The Creusa has a planimetric development of 47 m. It consists of a single tunnel, which leads to the final chamber after a narrow passage. Due to the spatial proximity of the two caves (see Fig. 2), it is likely that they are connected via small passages, not even accessible to speleologists (Balbiano D'Aramengo, 1993). For this reason we assumed that the hypogean species inhabiting the two caves (and the associated SSHs) belong to the same population and hence we pooled

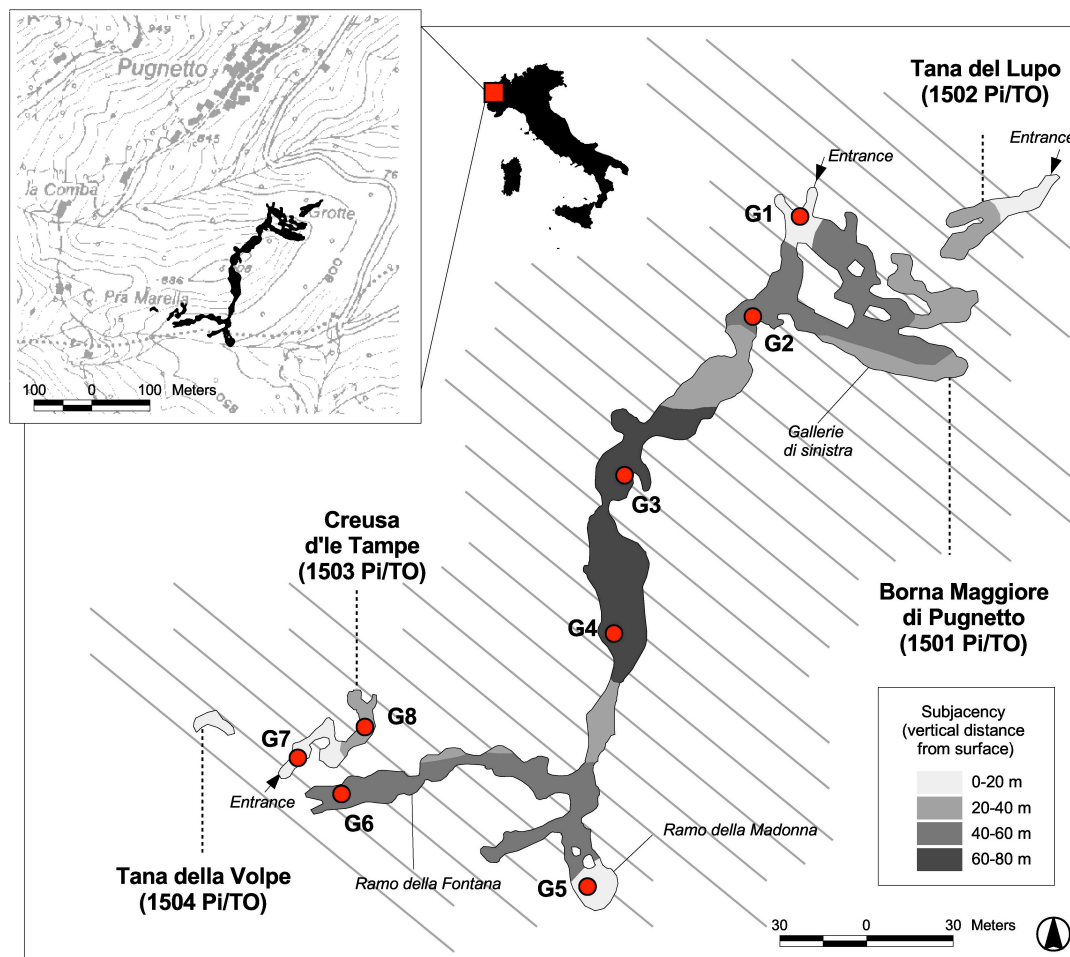


Fig. 2. Map of the study area. The shape and the topographic position of the four caves (Borna Maggiore di Pugnetto, Tana del Lupo, Creusa d'le Tampe, Tana della Volpe) was obtained from the original planimetric drawings of Muratore (1946). The position of the sampling triplets is represented by eight red dots, namely G1 (4 m from the cave entrance), G2 (30 m), G3 (90 m), G4 (150 m), G5 (230 m) and G6 (350 m) in the Borna Maggiore di Pugnetto and G7 (5 m) and G8 (25 m) in the Creusa d'le Tampe. The different sectors of the cave are coloured with different shades of grey representing the subgency (i.e., vertical distance from the surface) according to Motta & Motta (2015).

together the records collected in the two caves in the statistical analysis.

Sampling design

For our study we used twenty-four pitfall traps (plastic cups diameter 9 cm, volume 40 cl). The traps were baited with chicken meat and filled with a supersaturated preserving solution of water and NaCl. The pitfall traps were arranged in groups of three (hereinafter Triplet), at a distance of ~5 m one from the others. Six triplets (18 traps) were placed in the Borna at 4, 30, 90, 150, and 230 (Ramo della Madonna) and 350 (Ramo della Fontana) meters from the main entrance. Two triplets (6 traps) were placed in the Creusa at 5 and 25 m from the main entrance. Figure 2 shows the position of the triplets within the two caves. The distance of each pitfall triplet from the cave entrance (DST) was measured at the first survey. We derived the subgency (DEPTH) of each trap (i.e., the direct vertical distance from the surface) from the geological survey of Motta & Motta (2015). In particular, we created three categories of subgency (Fig. 2): 0-20 m (triplets G1, G5, G7, G8), 20-40 m (G2, G6), and 60-80 m (G3, G4).

We replaced the traps approximately once a month from June 2012 to June 2013. However, the access

to the Borna is forbidden from 1st November to 31th March in order to protect the roosting bats inhabiting the cave. In order to reduce disturbance, in this period we accessed the Borna only twice (December and March), resulting in ten total sampling sessions over the year.

In order to evaluate the effect of the bait on the sampling probability, we replaced the bait every two sampling sessions, thus resulting in 5 sampling sessions with fresh and 5 with exhausted baits. Trapped *Sphodropsis ghilianii* and *Dellabeffaella roccae* were sorted, identified and sexed (males, females, immatures and larvae).

The climatic factors that could hypothetically affect the distribution of the species were measured. In correspondence of each pitfall trap, we placed a Hygrochron™ temperature and humidity datalogger programmed to sample temperature (T) and relative humidity (RH) every three hours for the whole sampling period (accuracy of $\pm 0.5^{\circ}\text{C}$ and $\pm 1\%$ respectively). For each sampling period and for each pitfall trap we calculated the mean temperature (Tmean) and relative humidity (RHmean), the mean of the minimum (Tmin; RHmin) and maximum (Tmax; RHmax) and the associated range (Tmax-Tmin = Trange; RHmin-RHmax = RHrange). We also derived

the external daily mean temperature (Text) to the same periods from the nearest thermo-hygro-pluviometric weather station (Fua, Lanzo Torinese (cod. 111); N 45° 17' 23", E 7° 29' 38"; 550 m a.s.l.). The temperature values recorded by the weather station were corrected with the standard environmental lapse rate (i.e. the change of temperature with altitude for the stationary atmosphere; 6.49 K(C°)/1000 m [International Civil Aviation Organization, 1993]) in order to obtain a climatic characterization of the Pugnetto site. In all analyses relating the presence/absence of the two focal species with the explanatory parameters (see later sections), we used the pseudoreplicates as basic sample units.

Data analysis

Due to malfunctioning, eleven out of twenty-four dataloggers did not record measurements of temperature and relative humidity during the sampling period. Consequently, the removal of such missing data (NA) nearly halved the dimension of the dataset. Therefore, we decided to split the analysis in two and consider:

- 1) the temporal trends of the focal species in the four seasons by creating a four-level corresponding categorical variable (SEA) according to the astronomic calendar (Summer [Su]: 2 sampling sessions, samples from July to August 2012; Autumn [Au]: 3 sampling sessions, samples from September to November 2012; Winter [Wi]: 2 sampling sessions, samples from December 2012 to March 2013; Spring [Sp]: 3 sampling sessions, samples from April to June 2013). The creation of four categorical variables allowed us to bypass the bias created by the missing data and to use the entire set of data.
- 2) the direct response of the two species to the microclimatic parameters, using only the observations referring to valid data of temperature and relative humidity (halved dataset, i.e. with NA removed).

All the statistical analyses were performed in R (R Development Core Team, 2013).

Seasonal migrations

Data were analyzed with generalized linear mixed models (GLMMs - Zuur et al., 2009) which accounted for multiple observations from the same pitfall traps and repeated observations. For each basic sample unit, we chose counts (immature + adults) of *Sphodropsis ghilianii* (SG) and *Dellabeffaella roccae* (DR) as dependent variables. However, both variables were characterized by the presence of several outliers. To avoid log-transformation (Manning & Mullahy, 2001) or the removal of too many outliers (Zuur et al., 2010), we expressed the counts of the two species in term of presence/absence and modeled them assuming a Bernoulli distribution (0-1). Moreover, in order to test if adult males and females of the two species responded differently to the variables that we considered in this study, we computed an additional set of models using the sex ratio as dependent variable (SEX-RATIO). We expressed the sex ratio as the percentage of males

on the total counts of individuals in each pitfall trap at each sampling session [males/(males + females)]. We excluded from the analysis the traps with zero counts. Sex Ratio was modeled assuming a Binomial distribution (0-1 continuous). We carried out data exploration following Zuur et al. (2010). According to Zuur et al. (2009) the inclusion of outliers and highly correlated predictors in the regression analysis leads to misleading results. We therefore evaluated the presence of outliers in the independent variables *via* Cleveland dotplots and the collinearity among the covariates *via* pairwise Pearson's *r* correlations and boxplots.

In order to account for seasonal effects, we tested for interaction between the different covariates and the season (SEA*DST; SEA*DEPTH). To evaluate the effect of the bait on the probability of sampling the two species, we also included a categorical variable (BAIT) made up of two levels, "Fresh" and "Exhausted" (i.e. five surveys with a fresh bait and five with the exhausted one).

The GLMM mixed procedure allowed us to deal with temporal and spatial dependence, so we included the spatial factor "Triplet" (namely the identification number of each group of three traps) and the temporal factor "Session" (namely the ten replacements of each pitfall trap) as random factors. Such structure allowed us to deal with repeated observations of the same trap (given the removal sampling technique adopted in this study) and the clumped spatial distribution of the sampling triples within the cave.

The regression models were fitted via the *glmer* command in the *lme4* (Bates et al., 2013) R package. The structure of the initial models was:

$$y \sim \text{DST} + \text{DEPTH} + \text{SEA} + \text{BAIT} + (1 | \text{Triplet}) + (1 | \text{Session}) \quad (1)$$

where *y* = one of SG, DR or SEX-RATIO (Dependent variables); DST = distance from the cave entrance (Fixed effect); DEPTH = subjacency of the trap (Fixed effect); SEA = categorical variable representing the season (Fixed effect); BAIT = type of bait (fresh or exhausted) (Fixed effect). The random part of the model includes the effect of the temporal grouping variable (Session) and the spatial variable (Triplet).

Once we fitted the general model we applied model selection (Johnson & Omland, 2004), in order to identify the best model structure supported by observations. We performed a backward elimination, progressively excluding variables and potential interactions between variables according to AICc values (Zuur et al., 2009). Variables not contributing to the fit of the model (i.e. increasing the AICc value) were progressively dropped from the models thus avoiding overfitting (Howkins, 2004). Model validation was carried out following Zuur et al. (2009).

Microclimatic preferences

In order to investigate the microclimatic preferences of *Sphodropsis ghilianii* and *Dellabeffaella roccae*, we related their presence/absence to the mean temperature and the mean relative humidity of the

sampling session using binomial GLMMs (see details in the previous section). Since we found a high degree of collinearity between the two explanatory variables (Pearson $r = 0.79$), we fitted the mean temperature (2) and the mean relative humidity models (3) separately. Other variables concerning temperature and relative humidity (namely minimum, maximum and range) were not considered in the analysis given their lower correlation with the dependent variable. In order to deal with spatial and temporal dependence we used the mixed procedure as specified above.

$$y \sim T_{\text{mean}} + (1 | \text{Triplet}) + (1 | \text{Session}) \quad (2)$$

$$y \sim RH_{\text{mean}} + (1 | \text{Triplet}) + (1 | \text{Session}) \quad (3)$$

During model validation, we found a non-linear response of SG to temperature. To assess this kind of distribution, we fitted a quadratic polynomial GLM introducing the quadratic term (T_{mean}^2) as a covariate (4).

$$SG \sim T_{\text{mean}} + T_{\text{mean}}^2 + (1 | \text{Triplet}) + (1 | \text{Session}) \quad (4)$$

RESULTS

Microclimatic characterization of the caves

Relative humidity inside the two caves was almost constantly close to saturation, with values ranging from 90 to 100%. However, in the outermost portion of the cave in winter the relative humidity dropped down to 75%.

With regard to temperature, changes and max-min ranges were attenuated with increasing distance from the cave entrance and delayed compared to the values recorded outside the caves (Fig. 3). The mean annual temperature values deep inside the two caves were comparable ($T_{\text{mean}} \pm \text{SD}$: Borna = $9.0 \pm 0.4^\circ\text{C}$; Creusa = $8.9 \pm 0.8^\circ\text{C}$) and showed little variation over the year. In the outermost section of the caves the conditions were quite stable in summer, spring and autumn. Over the year, the range of temperature variation ($T_{\text{max}} - T_{\text{min}}$) in the vicinity of the cave entrance was 4.5°C for both the Borna and the Creusa, while the external range of temperature variation was around 41°C . However, the microclimate at the entrance zone

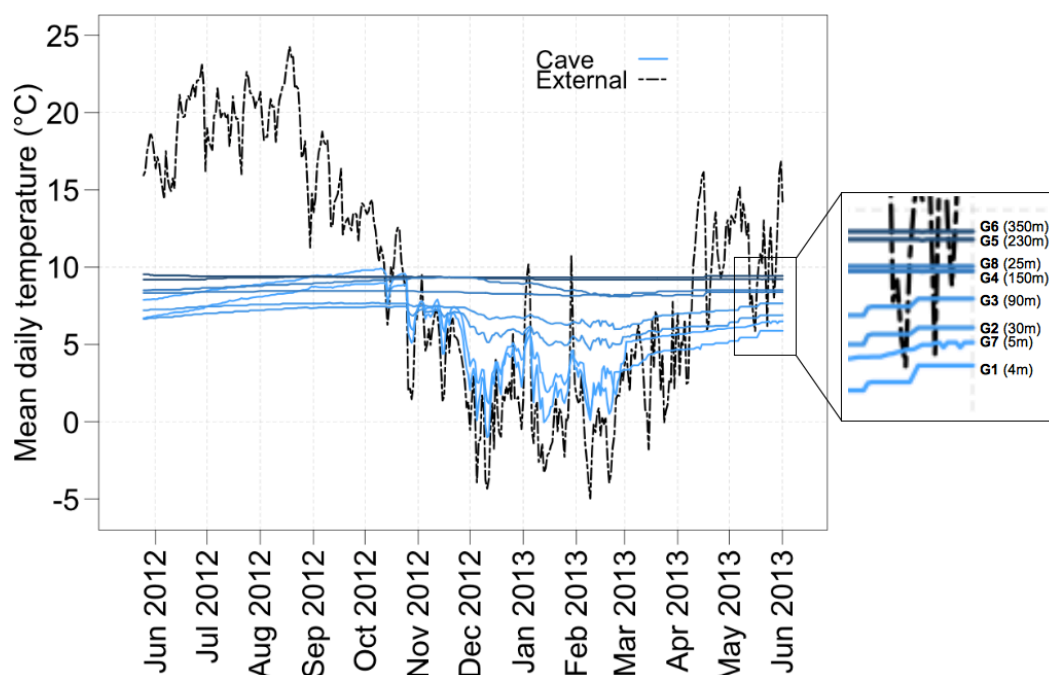


Fig. 3. Microclimate of the caves. Annual trends of external temperatures derived from the closest weather station (dotted line) and internal ones, registered by the dataloggers placed at each sampling point (filled lines). Only one datalogger *per* Triplet is shown (Triplet's codes are explained in Fig. 1; from G1 to G6 refers to the Borna cave and G7 - G8 to the Creusa cave). The shade of blues indicate the relative position of the dataloggers in the cave, from the outermost parts (lighter blues) to the innermost parts (darker blues).

drastically changed during winter, when we observed a drop in the mean temperature values (mean values always below 6°C). The coldest temperature values were recorded in December and January (T_{min} : Borna = -2.0°C and Creusa = -0.9°C).

Life history and seasonal migrations

No pitfall traps were lost during the study. Over the year, we sampled 179 individuals of *Sphodropsis ghiliani* in the Borna and 202 in the Creusa, for a total of 381 individuals. We sampled 991 individuals of *Dellabeffaella rocae*, of which 785 in the Borna and 206 in the Creusa. Abundance of adults and immature varied considerably through the year

(Fig. 4). Adult males and females of *S. ghiliani* were abundant from June to September. Abundance of adults decreased dramatically from October onwards and started to increase again in March. The annual sex ratio [males/(males + females)] was 0.49, and persisted during most of the sampling period. A remarkable exception to this trend was observed in spring (February - June) when we captured more males than females, although this discrepancy in the sex ratio was not statistically significant in respect to the baseline (SEA_Au) (SEA_Wi = Estimate $\beta \pm \text{SD}$: 0.7709 ± 1.013 , $p = 0.447$ n.s.; SEA_Su = Estimate $\beta \pm \text{SD}$: 0.6862 ± 0.744 , $p = 0.356$ n.s.; SEA_Sp = Estimate $\beta \pm \text{SD}$: 1.2155 ± 0.736 , $p = 0.090$ n.s.). Immatures were

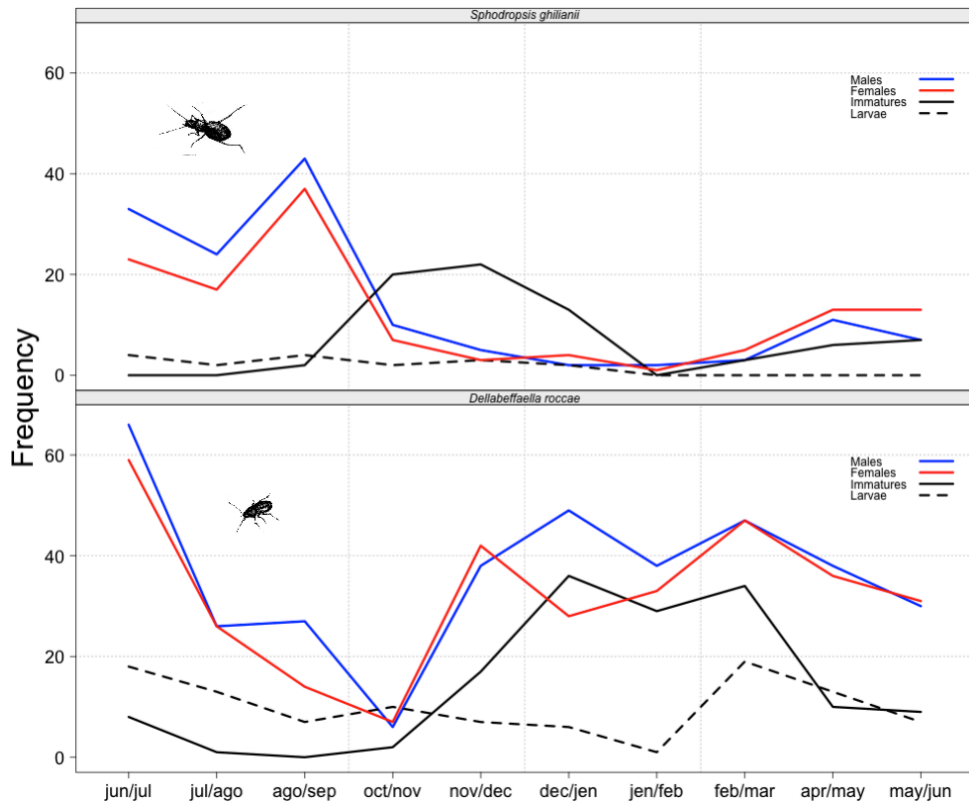


Fig. 4. Phenology. Abundance trends of adult male, female, immature individuals, and larvae of *Sphodropsis ghiliani* and *Dellabeffaella roccae* in the study area.

generally less abundant than adults, with a peak of abundance in autumn (September - November). Larvae were very scarce (17 specimens) and were only found in the Creusa.

Concerning the seasonal migration analysis, the best model (i.e., lower AICc) explaining the presence of *S. ghiliani* (SG) in the two study sites was $y \sim \text{DST} * \text{SEA} + \text{BAIT} + (1 | \text{Triplet}) + (1 | \text{Session})$. In particular, a significant interaction between the distance from the cave entrance and the season was

observed: the probability of the presence of *S. ghiliani* was found to be significantly higher at lower distances from the cave entrance in summer (SEA_Su*DE = Estimate $\beta \pm \text{SD}$: -0.0323 ± 0.0120 , $p = 0.007^{**}$) with respect to autumn (reference category) (Fig. 5). The same trend (close to significance) was observed in spring (SEA_Sp*DE = Estimate $\beta \pm \text{SD}$: -0.0196 ± 0.0108 , $p = 0.070[*]$). Instead, the probability of presence was lower and not significant in winter (SEA_Wi*DE = Estimate $\beta \pm \text{SD}$: 0.0114 ± 0.0069 , $p = 0.097$). Moreover,

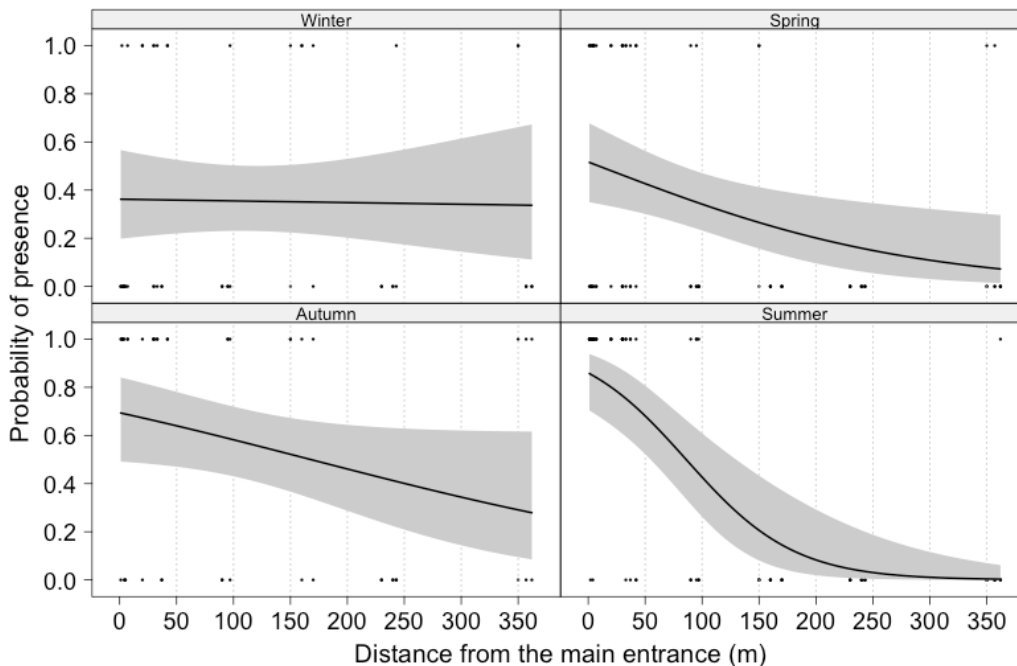


Fig. 5. Spatial dynamics. Predicted values (black lines) and 95% confidence intervals (grey surfaces) of the effect of distance from the main entrance in interaction with the season (DE*SEA) on the probability of presence of *Sphodropsis ghiliani* derived from the generalized linear mixed model (GLMM). Only fixed effects are shown. Due to the proximity of several values, various points appear superimposed.

there was no significant effect of the type of bait on the probability of presence of *S. ghilianii* (BAIT = Estimate $\beta \pm \text{SE}$: 0.5421 \pm 0.4459, $p = 0.224$ n.s.). The latter variable did not influence the sex ratio neither (Estimate $\beta \pm \text{SE}$: 0.0339 \pm 0.5163, $p = 0.947$ n.s.).

Adult males and females of *Dellabeffaella roccae* were abundant from November to June and decreased at the end of summer (minimum in September/October). The mean sex ratio was 0.43, and was constant over the year in respect to the baseline (SEA_Au) (SEA_Wi = Estimate $\beta \pm \text{SE}$: -0.5933 \pm 0.644, $p = 0.357$ n.s.; SEA_Su = Estimate $\beta \pm \text{SE}$: -0.5042 \pm 0.591, $p = 0.394$ n.s.; SEA_Sp = Estimate $\beta \pm \text{SE}$: -0.2210 \pm 0.602, $p = 0.714$ n.s.).

Immatures were mostly collected from October to May, with two peaks in October/November and February/March. Larvae occurred in low abundance through the whole year, with a peak in February/March (19 specimen) and another one in June/July (18 specimens). The most appropriate model (i.e., lower AICc) explaining the spatial and temporal presence of *D. roccae* in the two study sites was found to be $y \sim \text{DEPTH} + \text{SEA} + \text{BAIT} + (1|\text{Triplet}) + (1|\text{Session})$. The probability of presence of *D. roccae* was found to increase significantly with increasing subjacency (DEPTH 20-40 m = Estimate $\beta \pm \text{SE}$: 2.229 \pm 0.442, $p < 0.000$ ***; 60-80 m = Estimate $\beta \pm \text{SE}$: 1.715 \pm 0.390, $p < 0.000$ ***) in respect to the reference category (0-20 m). Concerning the seasonal differences, we observed that the probability of presence of *D. roccae* was significantly higher in summer (SEA_Su = Estimate $\beta \pm \text{SE}$: 1.375 \pm 0.449, $p = 0.002$ **), in spring (SEA_Sp = Estimate $\beta \pm \text{SE}$: 1.172 \pm 0.440, $p = 0.007$ ***) and in winter (SEA_Wi =

Estimate $\beta \pm \text{SE}$: 0.944 \pm 0.473, $p = 0.046$ *) in respect to the reference category (autumn). Moreover, there was no significant effect of the type of bait on the probability of presence of *D. roccae* (BAIT = Estimate $\beta \pm \text{SE}$: -0.586 \pm 0.317, $p = 0.074$ n.s.) or on the sex ratio of the captured individuals (BAIT = Estimate $\beta \pm \text{SE}$: 0.1726 \pm 0.396, $p = 0.663$ n.s.).

Microclimatic preference of *Sphodropsis ghilianii* and *Dellabeffaella roccae*

Due to malfunction of several dataloggers, the analysis concerning the thermal preference of the two species was performed with a reduced dataset. *Sphodropsis ghilianii* showed a non-linear trend (Fig. 6a) in respect to temperature, with significant effects of both temperature (Tmean = Estimate $\beta \pm \text{SE}$ 3.4798 \pm 1.0219, $p = 0.000$ ***) and squared temperature (Tmean² = Estimate β -0.2381 \pm 0.0717, $p = 0.000$ ***). In particular the probability of presence of *S. ghilianii* was found to be higher between 5 and 9°C, with the optimum around 7.3°C.

The response of *Dellabeffaella roccae* to temperature was better described by a (linear) logistic function (Fig 6c). The probability of presence of the species was found to increase with the increase of temperature (Tmean = Estimate $\beta \pm \text{SE}$ -3.1324 \pm SE 0.9097, $p < 0.000$ ***). In particular, it reached 50% at 7.6°C. There was also a positive and significant effect of the mean relative humidity for both species, with increasing probability of presence at increasing values of relative humidity (*S. ghilianii*, RH = Estimate $\beta \pm \text{SE}$ 0.1688 \pm 0.0642, $p = 0.008$ **); *D. roccae*, RH = Estimate $\beta \pm \text{SE}$ 0.2029 \pm 0.08047, $p = 0.011$ *; Fig. 6b; Fig. 6d).

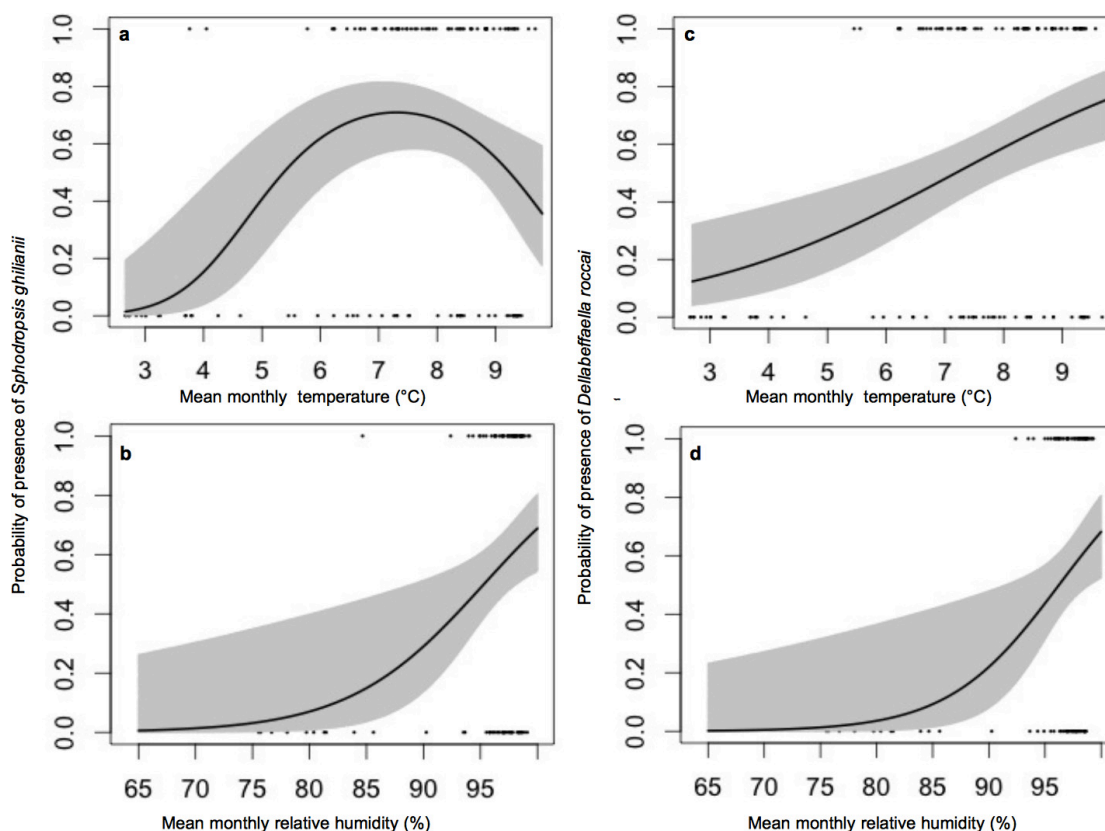


Fig. 6. Microclimatic preference. Predicted values (black line) and 95% confidence intervals (grey surface) of the effect of the mean temperature (a, c) and relative humidity (b, d) on the probability of presence of *Sphodropsis ghilianii* (left panels) and *Dellabeffaella roccae* (right panels) derived from GLMM analysis. Only fixed effects are shown. Due to the proximity of several values, various points appear superimposed.

DISCUSSION

It is generally accepted that seasonality may affect the underground climate, resulting in seasonal variations in cave temperature and relative humidity, with major effects close to the cave entrance. On the contrary, seasonal patterns are strongly attenuated deeper inside caves, where the response to the external climate is temporally lagged (Smithson, 1991; Badino, 2010; Cigna, 2012). As expected, in the Pugnetto hypogean complex we observed different microclimatic conditions in different sections of the cave. Deep inside, temperature variations were very feeble (0.2°C annual range), while the climate of the outermost sections was more variable (4.5°C annual range). Interestingly, despite the mean annual temperature was similar to the one recorded in the innermost sections, such condition did not persist in winter, when an alignment with the external conditions was observed (i.e., drop of temperature and relative humidity). According to Badino (2010) this kind of “*meteora*” is related to the convective circulation of the air within the cave, and characterize caves with openings at different altitudes. According to Motta & Motta (2015) in the Pugnetto hypogean complex, the presence of secondary openings through the hill that overcome the caves (Trüchtle Tampe), allows a convective air movement that determine the winter thermal anomaly in the vicinity of the entrance. The peculiarity of this condition seems to influence the seasonal migrations of *Sphodropsis ghilianii*. The probability of presence of the species was found to be higher at lower distance from the cave entrance (i.e., in the epigeal/hypogean ecotone *sensu* Prous et al. [2004]) in spring, summer and autumn (see Fig. 5). During these seasons, the most suitable microclimatic conditions for *S. ghilianii* were indeed close to the cave entrance area. This trend was particularly clear in summer, with higher abundance within the first 100 m of the cave. Our trends find an empirical confirmation in Casale (1988), who pointed out that *S. ghilianii* is a “spring breeder” (Brandmayr et al., 2005; see also, Fig. 4) and particularly abundant in spring and summer in the area close to the cave entrance (or even outside the cave). Moreover, the higher prey availability found in this area should play an additional important role for a generalist predator such as *S. ghilianii* (Novak et al., 2012; Tobin et al., 2013).

However, during winter, the alignment with the external conditions triggers the spatial migration of the species inside the cave (see also Weber et al., 1994, 1995). According to our results, this migration can be related to the unsuitable conditions occurring in winter in the nearby of the entrance (Fig. 3). Accordingly, the number of individuals in winter was found to be very low, as previously reported for other cave-dwelling Sphodrina beetles (Casale, 1988). Most likely, the drop of abundance that we observed is related to the migration of individuals from the cave chambers to the network of fissures connected with the cave. In this respect, Chapman (1985) observed that certain vagile specialized beetles were able to appear and disappear

rapidly in the cave, accessing the larger chamber from the adjacent labyrinth of voids.

On the base of our observations (Fig. 4), we hypothesize that reproduction takes place in early summer. From that time on, adults decrease dramatically and, in parallel, immature increase (September to November). In this respect, it is worth to mention that we did not find any significant discrepancy in the sex ratio during the year. Given the low abundance of larvae in the cave over the year, it appears likely that the reproduction and the development of larvae possibly takes place in the fissures and SSHs connected with the caves.

While *S. ghilianii* was preferentially found around the cave entrance, the probability of presence of *Dellabeffaella roccae* was found to increase in the deeper zone of the cave, characterized by higher subjacency (>20 m) and buffered from the external climatic variability. The highest abundance of adults was recorded inside the Borna, as previously reported by Capra & Conci (1951). In this sense, because of the higher planimetric development (765 m) and subjacency (up to 80 m), the Borna offers the most appropriate habitat within the Pugnetto hypogean complex. Instead, as observed for other subterranean arthropods (Giachino & Vailati, 2010; Novak et al., 2012), the presence of sporadic individuals in the vicinity of the surface may reflect migrations from the MSS environment. On the base of our working hypothesis, given the climatic stability of the internal part of the cave, no seasonal migration should occur. As a matter of fact, the pattern of spatial distribution within the cave did not change throughout the year, and no migrations along the cave were detected. On the other hand, seasonality affected abundance (Fig. 4). It appears likely that the reproduction took place in late summer. Thenceforth, the number of adults in the cave underwent a decline (September/October), possibly in relation to movements from the interstitial-like habitats (Giachino & Vailati, 2010; Novak et al., 2012). Subsequently, the development of immature individuals determined the new upswing in the abundance of individuals in the cave, with the maximum of records between December and March. Also in this species we did not find any significant discrepancy in the sex ratio during the year.

Regarding the thermal preference, *D. roccae* was preferentially collected around 8-9°C. Such values were mainly recorded in the most stable (i.e. deeper) sections of the Borna. It is indeed generally accepted that the higher diversity of specialized hypogean species occurs in the environmentally stable portions of caves (e.g., Tobin et al., 2013), as previously observed in other stenothermal cave-dwelling Cholevidae (Lencioni et al., 2010; Bernabò et al., 2011). Moreover, relative humidity seems to play an equally important role in determining the probability of presence of the species. As highlighted in Fig. 7, between 7 and 9°C the cave air was almost always saturated in terms of water vapor content (95-100%). Indeed, according to the pressure-temperature relation (Clausius-Clapeyron equation), the value of the vapour equilibrium pressure depends essentially on

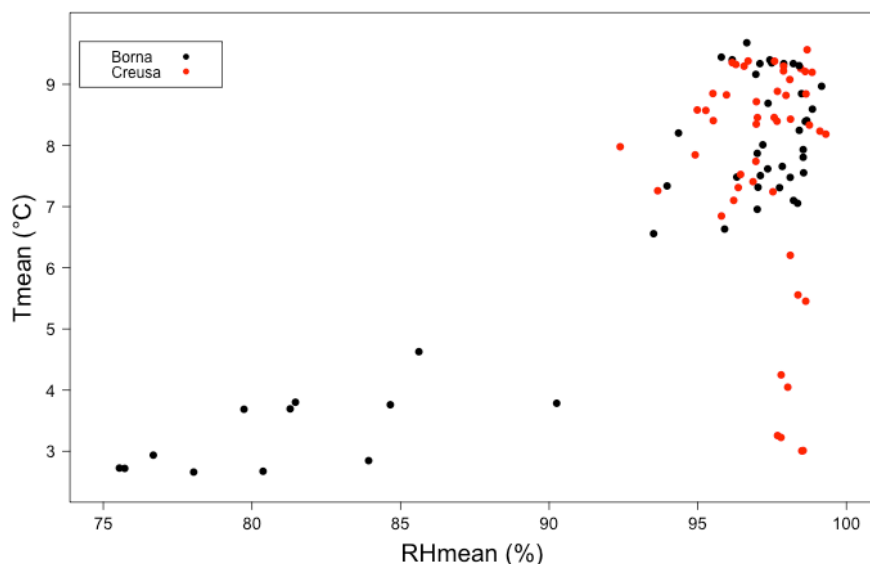


Fig. 7. Relation between temperature and relative humidity. The scatterplot shows the relation existing between the mean relative humidity (RH, x-axis) and the mean temperature (T , y-axis) at each trap within the Borna (black dots) and the Creusa (red dots) caves. Due to the proximity of several values, various points appear superimposed.

the temperature of the system (Clapeyron, 1853). Due to thinner exoskeletons, hypogean beetles usually show higher rates of desiccation compared to surface taxa (Boyer-Lefèvre, 1971; Howarth, 1980) and therefore humidity is considered to be one of the most important ecological limiting factor in the hypogean domain (Latella & Stoch, 2001). We thus hypothesize that the preference of *D. roccae* for the stable deepest portion of the Pugnetto caves is also related to the need for a saturated environment in terms of relative humidity.

Concerning the effectiveness of the bait, neither the probability of capturing the two species nor the sex ratio was found to vary with the aging of the bait - at least up to two months. It worth to specify that this analysis should not be interpreted as a denial of the effect of the bait *per se*, but as a demonstration that the effect of aging is not very influential, at least with regard of the two investigated species. Indeed, unbaited pitfall traps are considered to be almost useless in caves as the density of hypogean invertebrates is very scarce (Hunt & Millar, 2001). Concerning *D. roccae*, the attractiveness of the bait is likely related to its saprophytic feeding behavior, thus irrespectively to its aging. Regarding *S. ghilianii*, Morisi (1971) and Casale (1988) observed that decaying meat or cheese attract this species indirectly, because the bait itself attract its potential preys. Also in this case, both a fresh or an exhausted bait may attract potential preys and thus it may equally attract individuals of *S. ghilianii*.

CONCLUSIONS

In this study we characterized the microclimatic preference of *S. ghilianii* and *D. roccae* by defining their optimal conditions of temperature and relative humidity. The narrow microclimatic range that we defined logically points out *S. ghilianii* and *D. roccae* as stenoeicous subterranean species. Given their narrow ecological requirements, we expect that environmental

changes, especially in the underground climate, could dramatically affect their survival. In this sense, future risings of the outside temperatures due to global warming are expected to induce changes in the underground climatic conditions (Badino, 2004b; see also evidence in Fejér & Moldovan, 2013; Dominguez-Villar et al., 2014). However, little is known about the response to global climatic changes of the hypogean fauna (e.g. Brandmayr et al., 2013), and thus knowledge of the microclimatic requirements of the subterranean species appear to be of crucial importance.

ACKNOWLEDGEMENTS

This study was set within the CaveLab project “From microclimate to climate change: caves as laboratories for the study of the effects of temperature on ecosystems and biodiversity”, funded by University of Turin and Compagnia di San Paolo (Progetti di Ateneo 2011, Cod. ORTO11T92F). We would like to thank Dr. Paolo Debernardi (Mandria Natural Park) for allowing us to access the Borna di Pugnetto during the winter period, to Dr. Dan Chamberlain for the useful discussion about the polynomial GLMMs and to Alice Spencer for proof reading the English.

We are thankful to Prof. Achille Casale, who pre-revised the manuscript and provided important insights on the biology of subterranean beetles. A special thanks goes to Matteo Sturani, who provided the scanning of the Fig. 1, and to all friends and colleagues involved in the samplings: Mauro Paschetta, Fabio Ferrero, Enrico Lana, Elena Piano Sr., Davide Giuliano, and Martina Dalle.

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