

1Distributinal dynamics of a specialized subterranean community

2oppose the classical understanding of the preferred subterranean

3habitats

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5Peter Kozel^{1,2,3,a}, Tanja Pipan^{2,3}, Stefano Mammola^{4,5}, David C. Culver⁶ and Tone Novak¹

7¹Department of Biology, Faculty of Natural Sciences and Mathematics, University of

8Maribor, Koroška cesta 160, 2000 Maribor, Slovenia; peter.kozel@um.si; ORCID:

90000-0002-1559-6375, ^aAuthor for correspondence.

10²Karst Research Institute ZRC SAZU, Titov trg 2, 6230 Postojna, Slovenia

11³UNESCO Chair on Karst Education, University of Nova Gorica, Glavni trg 8,

125271 Vipava, Slovenia

13⁴Department of Life Sciences and Systems Biology, University of Torino, Via

14Accademia Albertina 13, 10123 Torino, Italy

15⁵LIBRe – Laboratory for Integrative Biodiversity Research, Finnish Museum of Natural History,

16University of Helsinki, Helsinki, Finland

17⁶Department of Environmental Science, American University, 4400 Massachusetts

18Avenue, NW Washington, DC 20016, United States

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20**Abstract.** The most specialized organisms to living in the subterranean environment,

21i.e., troglobionts, reside prevalently in the deepest cave zone and shallow subterranean

22habitats and complete their whole life cycles therein. Since troglobionts in most caves

23depend on surface organic matter resources, we hypothesized that they could also select

24the surface-most cave sections, so far the other environmental conditions there are

25favorable. Over one year, we analyzed the annual distributional dynamics of a
26subterranean community consisting of 17 troglobionts species, in monthly intervals in
27relation with multiple environmental parameters. Cumulative standardized annual
28species richness and diversity clearly indicated the existence of two ecotones within the
29cave: between soil and shallow subterranean habitats, inhabited by soil and shallow
30troglobionts, and between the transition and inner cave zones, where shallow and deep
31troglobionts overlap their spatial niches. The mean standardized annual species richness
32and diversity showed inverse courses, but both contributed to a better insight in the
33dynamics of subterranean fauna. Regression analyses revealed that temperatures
34between 7–10°C, high ground substrate moisture, large cross-sections size and high
35ground substrate pH were the most important ecological drivers governing the spatio-
36temporal dynamics of troglobionts. Overall, this study show general trends in the annual
37distributional dynamics of troglobionts in shallow caves and reveal that the distribution
38patterns of troglobionts within subterranean habitats may be more complex than
39commonly assumed.

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41*Additional key words:* cave community, ecological classification, ecotone, pitfall
42trapping, seasonality, spatio-temporal dynamics

43

44Beneath the Earth's surface, various kinds of natural and artificial cavities occur, from
45large cave chambers, to millimeter-sized interspaces between sand grains (Ford &
46Williams 2007; Palmer 2007; Culver & Pipan 2009a, b, 2014). Subterranean habitats
47share some common environmental features, which differ distinctively from those found
48on the surface. Subterranean environment is generally characterized by perpetual

49darkness and reduced variability of abiotic parameters (Culver & Pipan 2009b; Hüppop
502012). However, seasonal variation in temperature, relative air humidity and some other
51parameters still occurs, which reflect a delayed response to changing weather on the
52surface (Tobin et al. 2013). Surface climate mostly affects the cave sections closest to
53the entrance, while deeper inside the cave, environmental conditions are much more
54stable and the surface effects temporally lag behind (Cigna 2002; Badino 2010; Tobin et
55al. 2013; Šebela & Turk 2014; Mammola et al. 2015). Another essential difference
56compared to the surface environment is the absence of primary producers in most
57subterranean habitats (Simon et al. 2007; Engel 2012). Consequently, the subterranean
58fauna mostly depends on organic matter input from surface habitats (Gers 1998;
59Poulson & Lavoie 2000), and is generally poorly diversified (Gibert & Deharveng
602002). In spite of this, numerous investigations have disclosed a much higher
61biodiversity and a higher degree of endemism among subterranean species than
62predicted previously (e.g., Peck 1998; Culver & Sket 2000; Elliott 2000; Christman et
63al. 2005; Pipan & Culver 2007a, b; Zagamajster et al. 2008; Reboleira et al. 2011;
64Deharveng et al. 2012).

65 This diversified subterranean fauna includes temporary and obligate residents of the
66subterranean habitats (Sket 2008; Culver & Pipan 2009a, 2014; Trajano & Carvalho
672017). Schiner (1854) and Racoviță (1907) grounded the ecological classification of the
68subterranean fauna. Despite since then many classifications have been proposed
69(reviewed in Sket 2008; Trajano & Carvalho 2017), this remains one of the most
70frequently referred classification. Schiner and Racoviță classified subterranean fauna
71into three ecological groups: (1) Troglonexenes, which do not complete their life cycle in
72subterranean habitats. Accordingly, they should be the most abundant in the entrance

73cave section. (2) Trogloniles complete their life cycles either in the subterranean or in
74the surface environments, forming populations in both habitats (Sket 2008; Mammola
752018). Some populations do, while others do not complete their life cycle underground.
76(3) Specialized taxa, troglobionts, complete the whole life cycle in the subterranean
77environment (Culver & Pipan 2009a; Romero 2009; Assmann et al. 2010; Novak et al.
782014). These species are referred to occupy the inner, most stable, cave zone.

79 The spatial distribution of these three ecological groups within subterranean habitats
80is a consequence of variability in environmental parameters and species response to this
81variability mostly on account of seasonal changes in the regional and local climate
82(Novak et al. 2004; Tobin et al. 2013; Lunghi et al. 2017). Classically, it has been
83observed that there is a gradient of subterranean specialization of the biological
84community from the entrance zone toward the deepest sectors (Mammola 2018).
85Accordingly, the community in the vicinity of the surface is usually dominated by
86troglonenes and trogloniles, whereas troglobionts became progressively more abundant
87at greater depths (Schiner 1854; Racoviță 1907; Poulson & White 1969; Culver &
88Poulson, 1970; Howarth 1983; Tobin et al. 2013). However, recent studies disclosed that
89distributions of taxa in subterranean habitats do not always comply with this general
90pattern (e.g., Novak et al. 2012, Pellegrini & Ferreira 2016). Surface organisms are
91typically observed even at great depths in caves, for example surface dipterans have
92been observed at -2140 m in the Krubera-Voronja Cave, Western Caucasus (Sendra &
93Reboleira 2012). At the same time, by studying 54 cave communities in Slovenia,
94Novak et al. (2012) showed that troglobionts may consist of two spatially segregated
95groups of species: the shallow and the deep troglobionts, the first being adapted to the
96shallow subterranean habitats and the second to deep subterranean habitats. This, in

97general, supports the Racoviță (1907) opinion that many subterranean species normally
98live in fissures and appear in caves by hazard.

99 So far, subterranean habitats have been investigated for their environmental
100conditions in relation to their associated fauna (e.g., Pipan 2005; Culver & Pipan 2010;
101Tobin et al. 2013; Lunghi et al. 2014, 2017; Prous et al. 2015; Mammola et al. 2015,
1022017; Bento et al. 2016). Distributional dynamics of subterranean fauna, especially
103troglonbionts, have rarely been studied. Juberthie (1969) observed daily migrations of the
104troglonbiotic beetle *Aphaenops cerberus* (Coleoptera: Carabidae) between a spacious
105cave and adjacent fissures, driven by changing air movements within the cave; the
106beetles promptly reacted to changes in microclimatic conditions and appeared in the
107cave in a calm only. The same was observed in *Leptodirus hochenwartii* (Coleoptera:
108Leiodidae) and *Anophthalmus hirtus* (Coleoptera: Carabidae) (Novak et al. 1980).
109Crouau-Roy et al. (1992) studied the dynamics of different populations of *Speonomus*
110*hydrophilus* (Coleoptera: Leiodidae) in a cave and in adjacent network of superficial
111interspaces in-between clastic substrate—the so-called *milieu souterrain superficiel*
112(MSS)—and observed conspicuous fluctuation in population size and vertical migration
113over the year. This was triggered by seasonal variation of environmental parameters,
114especially temperature and relative air humidity. Significant vertical migration between
115MSS and caves owing to seasonal fluctuations in temperature has also been recorded in
116other subterranean species (Chapman 1985; Polak 2012; Mammola et al. 2016). Beside
117variation in climatic conditions, trophic availability was found to be an important factor
118affecting the spatial distribution of several spider species inhabiting caves (e.g., Novak
119et al. 2010; Manenti et al. 2015; Mammola & Isaia 2014, 2016; Lunghi 2018).
120Furthermore, the spatial distribution of particular subterranean species is also affected

121by the structural complexity of cave walls (e.g., Bourne 1976, 1977; Mammola & Isaia
1222014, 2016).

123 While evident patterns in distributions of single troglotibiotic species in caves are
124reported, great gaps remains in knowledge about distributional dynamics of the whole
125troglotibiotic community. Despite extensive research in recent decades, subterranean
126organisms remain among the least understood groups in the world (Soares & Niemiller
1272013). Under this perspective, the study of distributional dynamics of troglotibionts may
128help us to achieve a better understanding of their preferred subterranean habitats and
129microhabitats. In the present research, we studied the annual dynamics of troglotibiont
130community in a cave in Slovenia, with the aim of exploring whether troglotibionts could
131occur in the surface-most cave sections, rather than exclusively in the deepest cave
132sectors, and to find out which environmental parameters would influence such
133distribution. We hypothesized that: (1) Variation in the environmental conditions along
134the cave length primarily drive spatial dynamics of troglotibionts. (2) Seasonal variation
135in the environmental parameters determine parallel seasonal changes in troglotibiotic
136community along the cave, eventually permitting the colonization of surface-most
137sector during favorable periods.

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139**Methods**

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141**Study site**

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143 We carried out the study in the cave Zguba jama (Cadastre number 6290; E
14414°12'49.57", N 45°47'47.50"; 561 m a.s.l.; Cave Register Karst Research Institute

145ZRC SAZU and Speleological Association of Slovenia) near Postojna, Slovenia (Fig.
1461). This relatively small, horizontal cave is 122 m long, with a 1.5 m wide by 1 m high
147entrance. This study site was chosen owing to its simple morphology, linear shape and
148nearly constant cross-section (2 x 2 m average cross-section, with a few largest sites
149where the ceiling reaches a 5-m-height), allowing us to sample the species and
150measuring the considered environmental parameters in a more standardized way. The
151cave is in close vicinity of the Postojna cave (Šebela 1994), one of the world's hotspots
152of subterranean fauna (Culver & Sket 2000; Zagmajster et al. 2008; Sket 2012; Culver
153& Pipan 2013). It hosts a rich and diverse community, consisting of more than 100
154species of which at least 17 can be classified as troglobionts (Kozel et al. 2017).

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156**Sampling design**

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158 To study the distributional dynamics of troglobionts, we defined 31, on average 4 m
159long, sampling sections in the cave (Fig. 2). The cave was surveyed twice a month
160within about 48 hours over one year, from March 2012 to February 2013. The
161environmental parameters, which may vary considerably in subterranean microhabitats
162across one year (Cigna 2002, Badino 2010), were measured monthly at the first monthly
163survey in each sampling site. We took monthly records of the air temperature (T_{air}) and
164relative air humidity (RH) at the ground and below the ceiling using a handheld
165aspiration psychrometer (Ahorn FN A846, Germany) fastened on a 2 m long stick to
166diminish disturbance by the researcher. A sonde thermometer (Checktemp 1, Hanna,
167Germany) was used to measure the ground temperature (T_{gr}) at a depth of 2 cm. The
168substrate samples (ca. 100 g) were collected at ca. 0.25 m² area around the pitfall traps.

169The substrate moisture content (MC) was determined after drying samples at 105°C in
170the laboratory heating oven until reaching the stable weight. Conversely, more stable
171parameters over the year were measured only once, during the first survey. The pH of
172the ground substrate (pH_{gr}) was measured with a pH-meter (Hanna Instruments HI221
173Calibration Check, Microprocessor pH Meter, Woonsocket, RI, USA), and the carbonate
174content (CC) in the substrate by means of Scheibler's calcimetry. Organic material
175content (OM) was determined after the ignition of dry substrate samples at 650°C in a
176muffle furnace until reaching a constant weight. The distance from the entrance (DisE;
177polygonal distance) and from the surface (DisS; vertical distance) of the center of the
178sampling sections were calculated upon the ground cave plan and terrain profile above
179the cave. The passage cross-section size (Size) and the sampling section area (Area),
180i.e., wall and ceiling surface area, were, simplified, considered as being an open
181cylinder. The average mean height and width of each sampling section were used to
182calculate the radius of the circle. Chimneys and concavities were additionally taken into
183account.

184 The fauna was recorded by visual inspection and by pitfall trapping. We applied
185simultaneously two sampling methods, since it is documented that complementary
186sampling approaches maximize the detection and completeness of diversity of cave-
187dwelling arthropods (Kozel et al. 2017; Wynne et al. 2018, 2019).

188 For visual inspection, we devoted 0.5 minute/m²/observer. Only individuals that we
189could not identify to the species level in the cave were taken in the laboratory for
190identification. In order to investigate simultaneously the fauna occurring on the ground
191and on walls (Kozel et al. 2017), we used two parallel sets of pitfall traps: the ground set
192and the upper set—close below the ceiling—along the cave (Fig. 2). Pitfall traps were

193baited with decomposing beef meat and apple juice with a lacing of cherry and
194maraschino essence, and a few drops of detergent to reduce the surface tension.
195Sampling of fauna was carried out within two visits a month. At the first visit, we
196visually inspected the cave for the fauna and set the traps. At the second visit, within
197about 48 hours, we picked up the traps and took them into laboratory for identification.
198The voucher specimens preserved in 70% ethanol are deposited at the Faculty of
199Natural Sciences and Mathematics, University of Maribor. For details on the traps see
200Kozel et al. (2017). Counts deriving from visual inspection and pitfall trapping were
201merged in the analysis (Wynne et al. 2019).

202

203**Data analysis**

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205 To graphically visualize the overall environmental conditions along the cave,
206microclimatic and physical parameters were mapped using ArcGIS Desktop 10.3.1 (Esri
2072015). We created a vector layer of the cave, projecting it “on the fly” over the original
208raster scheme of the cave. The point station data were interpolated by Inverse Distance
209Weighted (IDW) tool, using a power of 2 as exponent of distance and a cell size of 2 for
210all resulting grid maps.

211 The sampling sections on each sampling date were used as basic sample units. Mean
212values of T_{air} and RH from the ground and below-ceiling measurements, and the
213distances from the entrance and from the surface were used in the statistical analyses.
214Data on fauna recorded by visual inspection and pitfall trapping were pooled for the
215analyses.

216 In order to investigate the annual dynamics of troglobionts with respect to the
217 environmental parameters, we applied generalized linear mixed models (GLMMs). The
218 mixed procedure allowed us to deal with replicated prospections of the sampling
219 section, by specifying the sampling section as a random factor. We modeled abundance
220 and presence-absence data of troglobionts separately. In the abundance approach, we
221 used counts of individuals deriving from pitfall and visual inspections as the response
222 variable. To adjust the abundance for the different sizes of the sampling sections, we
223 included the sampling section area (Area) as an offset in the model.

224 Given the low richness of species per sampling section (ranging from 0 to 4
225 species, with most values being either 0 or 1), we expressed richness as presence or
226 absence of troglobionts within the sampling plot (i.e., Bernoulli distribution 0–1). This
227 model allowed us to uncover fundamental conditions governing the presence of
228 troglobionts in the cave, thus complementing the abundance approach.

229 The following independent variables were addressed as potential predictors of the
230 dependent variables: distance from the entrance (DisE), distance from the surface
231 (DisS), Season (Sea; consisting of four levels: “Spring”, “Summer”, “Autumn”,
232 “Winter”), ground temperature (Tgr), air temperature (Tair), relative air humidity (RH),
233 substrate moisture content (MC), ground substrate pH (pHgr), organic material content
234 (OM), carbonate content (CC), and passage cross-section size (Size). All variables but
235 season were continuous.

236 Prior to model fitting, a systematic data exploration was carried out following the
237 protocol proposed by Zuur et al. (2010). Including outliers or highly correlated variables
238 increase type I and II statistical errors and may potentially result in incorrect ecological
239 conclusions (Zuur et al. 2009, 2010). Accordingly, Cleveland dotplots were used to

240 assess the presence of outliers in response and explanatory variables and multi-
241 collinearity among continuous variables was tested using Pearson correlation
242 coefficients and variance inflation factors (VIF), setting $r \geq \pm 0.7$ and $VIF > 3$ as cut-off
243 values (Zuur et al. 2013). Collinearity between continuous variables and the categorical
244 variable Season was assessed graphically through boxplots. Possible non-linear effects
245 of the continuous variables included in the models were investigated with generalised
246 additive models (GAM), using the *gam* R package (Hastie 2016). Coplots were further
247 constructed to check for potential interactions between Season and the continuous
248 covariates.

249 The abundance of troglobionts was modeled choosing a negative binomial
250 distribution family, the distribution applied for over-dispersed count data (Zuur et al.
251 2009). The presence-absence of troglobionts was modeled through Binomial GLMM
252 using a complementary log-log link (clog-log), owing to unbalanced zeros (absences)
253 and ones (presences) in the dataset (Zuur et al. 2009). All models were fitted through the
254 *glmer* command from the *lme4* R package (Bates et al. 2016).

255 After fitting the initial models, including all non-collinear covariates of interest, we
256 conducted model selection (Johnson & Omland 2004). A backward elimination
257 procedure was chosen, whereby variables were progressively deleted according to AICc
258 values (Burnham & Anderson, 2002). AICc values were calculated in the *MuMIn* R
259 package (Bartón 2018). The backward elimination procedure was reiterated manually
260 until a minimum adequate model consisting exclusively of statistically significant
261 variables was obtained. Model validation was carried out on the final models in
262 accordance with Zuur et al. (2009, 2013). Factor significance was checked via
263 likelihood ratio test.

264 Species richness and diversity of troglobionts for each sampling section and each
265 month were calculated. Species diversity was calculated through the Shannon-Weaver
266 index (H') for each sampling section and each month using the *vegan* R package
267 (Oksanen et al. 2016). Given the unequal sampling section sizes, both variables were
268 standardized by dividing the corresponding value by the sampling section area.
269 Spearman rank correlation coefficient (r_s) between species richness and diversity was
270 calculated.

271 Variation in species richness and diversity along the cave was evaluated by fitting a
272 GAM into the data. All statistical analyses were carried out in the R environment (R
273 Development Core Team, 2018).

274

275 **Results**

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277 **Environmental parameters**

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279 The annual characteristics of the environmental parameters along the cave are
280 presented in Fig. 3. In terms of variation of climatic conditions, we distinguished three
281 cave zones: the entrance (sampling sections 1–8), the transition (sampling sections 9–
282 13) and the inner zone (sampling sections 14–31). The entrance zone showed the
283 greatest variation in annual temperature (T_{air} , T_{gr}) and humidity (RH), which was
284 considerably influenced by the external climatic conditions (Fig. 3A–C). In this
285 illuminated zone, air temperature varied annually from -3.6 to 15.1°C , ground
286 temperature from -0.4 to 14.4°C and relative humidity from 80.1 to 100%. The
287 transition zone was characterized by intermediate variations of these parameters with

288respect to the entrance and the inner zones, including twilight in the beginning and total
289darkness in the inner part of the transition zone. Air temperature varied from 6.6 to
2908.5°C, ground temperature from 7.2 to 8.5°C and relative humidity was close to
291saturation (variation between 98.5°C and 100%). The inner, completely dark zone was
292relatively stable, with only limited variations in air (7.9–9.1°C) and ground temperature
293(8.0–9.1°C). Relative humidity remained constant at saturation. Annual mean air and
294ground temperature and relative humidity gradually increased, and variation in these
295parameters were progressively attenuated, along with increasing distance from the
296entrance.

297 The substrate moisture content (Fig. 3D) varied from 7.92 to 50.26% and carbonate
298content (Fig. 3E) from 0 to 53.35%. Both parameters showed no pattern with respect to
299distance from the entrance. The organic material varied from 2.36 to 59.79% along the
300cave. The greatest share (Fig. 3F) was recorded in the entrance zone, and the lowest in
301the middle of the cave. Disregarding the sampling section 1, with a large amount of
302organic matter in the floor substrate, relatively low variation of organic material among
303other sampling sections was found. The pH (Fig. 3G) varied from 7.82 to 8.38 and was
304relatively stable along the cave.

305

306Annual and monthly species richness and diversity of troglobionts

307

308 Altogether, we recorded 1,546 individuals belonging to the following 17 troglobiont
309species: *Absolonia gigantea*, *Androniscus* sp., *Arrhopalites postumicus*,
310*Bathyscimorphus byssinus*, *Bathysciotes khevenhuelleri*, *Brachydesmus subterraneus*,
311cf. *Linopodes* sp., *Oncopodura cavernarum*, *Leptodirus hochenwartii*, *Neelus* sp.,

312 *Neobisium spelaeum*, *Onychiuroides postumicus*, *Plusiocampa* sp., *Stalita taenaria*,
313 *Titanethes albus*, *Troglopedetes pallidus*, *Zospeum spelaeum spelaeum*.

314 Cumulative standardized annual species richness and diversity of troglobionts
315 showed a very high correlation ($r_s=0.87$, $p<0.001$). Both were lowest in the entrance and
316 transition zones and greatest in the inner zone. However, two peaks appeared in the
317 sampling section 1 in the contact zone of surface and subterranean environments, and in
318 the sampling section 15 in the contact of the transition zone and the inner cave zone
319 (Fig. 5A).

320 Mean standardized annual species richness and diversity of troglobionts showed
321 moderate correlation ($r_s=0.44$, $p<0.013$). Comparing cumulative and standardized
322 curves, they showed inverse courses. Both curves showed the lowest values at the very
323 entrance, in the contact between the transition and inner zones, and at the very end of
324 the cave, with two inconspicuous peaks in the entrance and inner zones (Fig. 5B). High
325 correlation ($r_s=0.76$, $p=0.004$) was found between monthly species richness and
326 diversity of troglobionts. There were three distinctive peaks in species richness (May–
327 June 2012, August–September 2012, December 2012) and diversity (June 2012,
328 September 2012, January 2013) (Fig. 6). The lowest species richness was recorded in
329 July 2012 and the lowest diversity in March 2012. Both showed a trend of slight
330 increasing from March 2012 until January 2013.

331

332 **Environmental parameters influencing the abundance of troglobionts**

333

334 Data exploration revealed the presence of one outlying value in the dependent
335 variable (abundance), which was removed from the dataset. The variables organic

336material content, carbonate content and relative air humidity contained outliers that
337could not be stabilized even after the transformation. Since excluding these numerous
338outliers would significantly reduce the dimension of the dataset, and since data
339exploration revealed that these three variables were not strongly correlated with our
340dependent variable, we removed these three variables from the analysis. The variable
341passage cross-section size was log-transformed to homogenize its distribution. Air
342temperature, being collinear with ground substrate temperature ($r>0.7$), and distance
343from the entrance, being collinear with distance from the surface ($r<0.7$), were dropped
344from the analysis. Coplots revealed a possible interaction between distance from the
345entrance and the seasons. Finally, a non-linear effect of the ground temperature was
346detected during data exploration, and accounted for by introducing squared ground
347temperature as a term in the regression structure. Accordingly, we included, as variables
348in the initial model, the interaction between distance from the entrance and Season, log-
349transformed passage cross-section size, substrate moisture content, ground substrate
350temperature and its square, and ground substrate pH.

351 According to model selection based on AICc values (Table 1), the most appropriate
352model that explained the abundance of troglobionts had the following structure:

353

354 $y \sim \text{DisE} * \text{Season} + \text{Tgr} + \text{Tgr}^2 + \text{MC} + \text{offset}(\text{Area}) + 1 | \text{Sampling site}.$

355

356 The combined effect of these variables on the abundance of troglobionts is illustrated
357in Fig. 7. The abundance of troglobionts was significantly greater near the cave entrance
358and decreased with increasing distance from the entrance in Spring
359(Season_Spring*DisE Estimated $\beta \pm \text{SE}$: -0.0163 ± 0.0052 , $p=0.002$) and Summer

360(Season_Summer*DisE Estimated $\beta \pm SE$: -0.0183 ± 0.0047 , $p < 0.001$) relative to
361Autumn. Instead, the abundance of troglobionts was lower and not significantly
362different in Winter (Season_Winter*DisE Estimated $\beta \pm SE$: -0.0082 ± 0.0052 , $p = 0.120$).
363The abundance of troglobionts also increased with increasing ground substrate
364temperature (Tgr Estimated $\beta \pm SE$: 1.1850 ± 0.3840 , $p = 0.002$) and decreased with
365squared ground substrate temperature (Tgr² Estimated $\beta \pm SE$: -0.0839 ± 0.0279 ,
366 $p = 0.003$). The combination of the latter two effects resulted in a parabolic trend, with
367the greatest abundance of troglobionts at temperatures comprised between 7 and 10°C.
368Moreover, we found an increase in the abundance of troglobionts with increasing values
369of substrate moisture content (MC Estimated $\beta \pm SE$: 0.0307 ± 0.0114 , $p = 0.007$).

370

371 **Environmental parameters influencing the presence of troglobionts**

372

373 During data exploration, no outliers were detected. The same procedure as described
374in detail in the previous analysis, and the same variables, except Tgr², were included in
375the initial model. According to model selection (Table 1), based on AICc values, the
376most appropriate model explaining the probability of the presence of troglobionts, had
377the following structure:

378

379 $y \sim \text{DisE} * \text{Season} + \log \text{Size} + \text{pHgr} + 1 | \text{Sampling site.}$

380

381 The combined effect of these variables on the probability of the presence of
382troglobionts is illustrated in Fig. 8. The probability of the presence of troglobionts
383decreased significantly with increasing distance from the entrance in Spring

384(Season_Spring*DisE Estimated $\beta \pm SE$: -0.0156 ± 0.0057 , $p=0.006$) and Summer
385(Season_Summer*DisE Estimated $\beta \pm SE$: -0.0146 ± 0.0056 , $p=0.010$) with respect to
386Autumn. On the other hand, the presence of troglobionts was lower in Winter, but not
387significantly different (Season_Winter*DisE Estimated $\beta \pm SE$: -0.0057 ± 0.0055 ,
388 $p=0.299$). We also found an increase in the probability of the presence of troglobionts
389with increasing log-transformed passage cross-section size (logSize Estimated $\beta \pm SE$:
390 0.5380 ± 0.2178 , $p=0.013$) and ground substrate pH (pHgr Estimated $\beta \pm SE$:
391 1.7096 ± 0.8268 , $p=0.039$).

392

393**Discussion**

394

395 Subterranean habitats are suitable for studying species interactions, ecological
396niches, and temporal and spatial patterns in terrestrial subterranean communities.
397However, subterranean communities as a whole are only rarely considered in relation
398with environmental conditions (e.g., Di Russo et al. 1997; Pipan et al. 2011; Sendra &
399Reboleira 2012; Tobin et al. 2013; Bento et al. 2016; Mammola et al. 2017; Mammola
400& Isaia 2018). Consequently, community responses to environmental conditions are still
401poorly understood.

402 High correlations between cumulative standardized species richness and diversity of
403troglobionts on annual and monthly scales suggest that either of these measures provide
404equivalent insight into the dynamics of the troglobiotic community. In the entrance cave
405zone, the gradual decrease of both species richness and Shannon-Weaver diversity of
406troglobionts, with the highest values at the very entrance, suggests that there is an
407ecotone between soil and shallow karst habitats, where soil and shallow subterranean

408 troglobionts temporarily occur. A similar situation also occurs at the boundary between
409 the transition and the inner cave zones. This suggests that there is another ecotone
410 where shallow troglobionts, which regularly inhabit shallow subterranean habitats, meet
411 deep troglobionts, regularly occurring in the inner cave zone. Thus, in the entrance cave
412 zone, troglonexes, troglaphiles and, temporary, shallow troglobionts regularly occur,
413 while in the inner cave zone deep troglobionts, and, temporary, troglaphiles and
414 troglonexes occur. Culver and Pipan (2014) argued that deep soil habitats host a range
415 of troglomorphic species, which have been largely neglected in current speleobiological
416 studies. Until very recently, troglobionts have been discussed as a unique, convergent
417 ecological and morphological group. However, divergence governed by competition and
418 other factors also takes place in the subterranean habitat (Pipan & Culver 2012). The
419 result of this study support previously published evidence (Novak et al. 2012, 2014),
420 suggesting that there is a clear separation of troglobiotic species inhabiting shallow and
421 deep subterranean habitats. The entrance cave zone may thus be seen as an ecotone
422 between the surface and subterranean environments (Prous et al. 2004, 2015), the
423 boundaries of which are poorly defined (Mammola et al. 2017) and which is
424 characterized by relatively high fluctuation of meteorological conditions (Pipan et al.
425 2011). Compared to inner cave sectors, this ecotone has received only limited study (but
426 see, e.g., Prous et al. 2004, 2015; Hobbs 2012; Culver & Pipan 2014; Yao et al. 2016;
427 Lunghi et al. 2017). Consequently, it has remained uncovered whether deep edaphic and
428 cryptic ground species with troglomorphic appearance should be distinguished from
429 shallow troglobionts or not. However, further research on the life cycles, physiology and
430 population structure of troglobionts inhabiting these two zones are required. The mean
431 standardized species richness and diversity of troglobionts on annual scale, on the other

432hand, reflect other community characteristics, referring the environmental conditions
433within the cave. Over the year, most favorable environmental conditions for shallow
434troglonbionts appeared at the end of the entrance zone, while such conditions for deep
435troglonbionts were in the middle of the inner zone. Consequently, both approaches
436contribute to better understanding the dynamics of troglonbiont communities.

437 Seasonality manifests in species distributions through altering the presence and the
438abundance of certain species in a given place at a stated time (Murray et al. 2013;
439Lunghi et al. 2017). Seasonality affects many biotic functions, such as growth, feeding,
440and reproduction (Araújo et al. 2010; Hjernquist et al. 2012). In our study, troglonbionts
441showed a conspicuous spatial distribution pattern that varied seasonally. In spring and
442summer, greater abundance and higher probability of presence were found near the
443entrance; these declined with increasing distance from the entrance. The same
444distributional pattern also applies in the case of distance from the surface, being
445collinear with the distance from the entrance. Although in Zguba jama, air and ground
446temperature and humidity, passage cross-section size and pH significantly influenced
447the troglonbionts, this may not be the general rule in other cave types, e.g., in ice caves
448(cf. Raschmanová et al. 2018). A similar spatial pattern has been observed in the
449troglonbiont beetle *Sphodropsis ghilianii* (Mammola et al. 2015). Our results show that
450greater abundance of troglonbionts occurs at ground temperatures between 7 and 10 °C
451and high ground substrate moisture, which may represent optimal microclimatic
452conditions. The optimal temperatures well fit the average annual temperature in
453Postojna (Postojna Meteorological Station). Such conditions were present near the
454entrance in spring and summer, which may in turn explain the greater abundance of
455troglonbionts in these seasons. Our findings are in accordance with the generally

456accepted understanding that troglobionts are adapted to narrow ranges of temperature
457and humidity (Barr & Kuehnelt 1971; Howarth 1980). They are more susceptible to
458desiccation, owing to their thinner exoskeleton, as compared to their surface
459counterparts (Howarth 1980). Furthermore, the higher availability of prey and organic
460matter deposits in the vicinity of the entrance (Tobin et al. 2013; Mammola & Isaia
4612016) likely constitute additional essential factors determining the highest abundance of
462troglobionts in the entrance zone in spring and summer. In autumn and winter, lower
463abundance and probability of the presence of troglobionts near the entrance refer to
464unfavorable conditions. Most likely, these triggered the migration of troglobionts from
465the cave into the adjacent fissure network and partly towards climatically more stable
466inner cave zone. Prompt migrations, as a response to changes in microclimatic
467conditions, have been reported in different subterranean beetles (e.g., *Aphaenops*
468*cerberus* – Juberthie 1969; *Drimeotus* – Racoviță 1983; *Speonomus hydrophilus* –
469Crouau-Roy et al. 1992; *Parapropus sericeus* and *Prospelaeobates brelihi* – Polak
4702012; *Sphodropsis ghiliani* – Mammola et al. 2015). Troglobionts found near the
471entrance in winter are presumably adapted to more severe and variable environmental
472conditions. They probably possess moderate freezing tolerance, being able to withstand
473short exposure to sub-zero temperatures (e.g., Lencioni et al. 2010; Novak et al. 2014), a
474general feature of shallow (Novak et al. 2014) and some deep troglobionts
475(Raschmanová et al. 2018). In autumn and winter, slightly greater species richness and
476diversity were recorded. This might suggest that the most suitable microclimatic
477conditions for troglobionts occurred in these periods. On the other hand, low abundance,
478along with higher species richness resulted in greater diversity of troglobionts in these
479seasons.

480 Larger passage cross-section size corresponded to higher probability of the presence
481of troglobionts probably because these cave sections provide more microhabitat types
482and a calmer atmosphere. This apparently contradicts findings in some troglolobiotic
483beetles, which show strong preference for narrow spaces but are highly sensitive to air
484currents in large passages (e.g., *Aphaenops* – Juberthie 1969; Juberthie & Bouillon
4851983; *Speonomus* – Delay 1978; *Leptodirus*, *Anophthalmus* – Kuštor & Novak 1980a,
486b). In relatively small caves, such as Zguba jama, the Bernoulli principle seems to be the
487most important agent affecting the air current velocity leaving no calm portions in
488narrow passages, but allowing some calm microhabitats in more spacious cave parts.

489 Ground substrate pH was found to be another factor importantly affecting the
490presence of troglobionts; the higher the substrate pH, the greater the probability of their
491presence. A study on bacterial community in a cave indicated that higher substrate pH
492proposed a greater diversity of bacteria (Yun et al. 2016). As already reported by Vandel
493(1965), obligate argilivory, i.e., feeding on clay, is common in troglolobiotic taxa, such as
494beetles of the family Leiodidae (Moldovan 2012) and the harvestman *Ischyropsalis*
495*hadzii* (own unpublished data). Leahy and Colwell (1990) found that a decrease in pH
496from 8.5 to 7.4 significantly increased the decomposition rates of organic matter by
497microbes, which might consequently result in lower food availability for troglobionts.
498Organic matter did not affect the distribution of troglobionts, since, except the humus
499accumulation at the entrance, there were no further accumulations of organic matter, and
500its contents in the floor substrate was relatively low and stable along the cave.

501 It is worth noting that abundance and richness data deriving from baited pitfall-
502trapping must be carefully considered since these results might differ in comparison to
503other methods (Poulson & Culver, 1968; Juberthie, 1969; Kuštor & Novak, 1980a, b;

504Weinstein & Slaney, 1995; Culver & Pipan, 2009; Kozel et al. 2017; Wynne et al.,
5052019). However, in previous studies (Novak et al. 2012; Kozel et al. 2017) we showed
506that a monthly two-day setting of the traps being 3–4 m distant from each other provides
507enough data for many statistical analyses, along with any detectable influence on the
508depletion of the fauna.

509 Our finding that most troglobiotic species preferred the inner, climatically the most
510stable, cave zone, is in accordance with general trends that species richness and
511diversity of troglobionts are typically greatest deeper inside caves (Schiner 1854;
512Racoviță 1907; Poulson & White 1969; Howarth 1983; Tobin et al. 2013). However,
513this is in contrast to Novak et al. (2012), who found that most troglobionts are
514distributed within the upper 10 m of subsurface strata. This difference in the findings
515might be the consequence of either comparing only one (our study) vs. a large number
516of caves (Novak et al. 2012) or due to different sampling efforts (monthly vs. seasonal
517sampling).

518 In conclusion, in speleological terms, Zguba jama is a relatively short cave, shallow
519beneath the surface. The mean annual air temperature roughly corresponds to the mean
520annual air temperature outside. The other environmental characteristics, such as relative
521humidity, substrate moisture content, organic matter content and pH, are typical of caves
522in the region (own unpublished data). Although partitioning caves into the entrance,
523intermediate and inner zones is conceptually clear, in caves some practical guidelines
524are needed. In moderate climates, the entrance cave zone is illuminated, the air and
525ground temperatures may fall below 0°C and the relative humidity is nearly all the time
526below 100 %. The intermediate zone is a twilight or completely dark zone, where the
527temperatures never fall below 0°C, while the relative humidity may be lower than 100

528% at least in one season. The inner zone is completely dark and thermally (temperature
529variation usually less than 1°C; Cigna 2002) and humidity stable (saturated or
530subsaturated). In most caves the three zones can only be identified after checking the
531illumination, and measuring annual variation of air and ground temperature and relative
532humidity at least once each season. Two ecotones: the entrance and the inner one can be
533distinguished in the cave, the first one sharing the edaphic and shallow troglobiont
534faunas, and the second one the shallow troglobiont and deep troglobiont faunas.
535Cumulative and standardized data on species richness and diversity reveal different
536population properties; consequently, both approaches should be applied in future studies
537to get a better insight in the dynamics of subterranean fauna. In this study we identified
538the most important environmental parameters affecting spatio-temporal dynamics of
539troglobionts in the cave. Hosting a relatively rich fauna (Kozel et al. 2017) and being
540easily modeled due to its horizontal development and simple shape, this cave well
541serves as an amenable model to study the annual distributional dynamics of troglobionts
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543

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551

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790**Fig. 1.** Geographical location of the study cave (source: LIDAR DEM, Cave Register
7912018, TTN5).

792

793**Fig. 2.** The ground plan of Zguba jama showing sampling sections (red lines) and
794pitfall-trap sites (blue squares) with a pair of the ground and the upper trap. Modified
795from Šebela (1994) and Kozel et al. (2017).

796

797**Fig. 3. (A–D)** Annual mean and range of monthly measured environmental parameters;
798**(E–G)** parameters measured only once along the cave.

799

800**Fig. 4. (A)** Cumulative standardized annual species richness and the Shannon-Weaver
801index (H') of troglobionts per sampling section. Regression lines were obtained by
802fitting a GAM into the data (annual species richness: $df=3$, $F=2.91$, $p=0.053$; Shannon-
803Weaver index: $df=3$, $F=5.73$, $p=0.004$). **(B)** Mean \pm SD standardized annual species
804richness and Shannon-Weaver index (H') of troglobionts per sampling section.
805Regression lines were obtained by fitting a GAM into the data (annual species richness:
806 $df=3$, $F=8.39$, $p<0.001$; Shannon-Weaver index: $df=3$, $F=2.63$, $p=0.071$). For better
807visualization, confidence intervals are not shown.

808

809**Fig. 5.** Monthly species richness and values of the Shannon-Weaver index (H') of
810troglobionts in Zguba jama.

811

812**Fig. 6.** Predicted values of the effects of distance from the entrance in interaction with
813the Season and **(A)** ground substrate temperature, and **(B)** substrate moisture content on
814the abundance of troglobionts obtained from the Generalized Linear Mixed Model
815(GLMM). In each panel, to generate the prediction, the unplotted variable is set at its
816mean value.

817

818**Fig. 7.** Predicted values of the effects of distance from the entrance in interaction with
819the Season and **(A)** log-transformed passage cross-section size, and **(B)** ground substrate
820pH on the probability of the presence of troglobionts obtained from the Generalized
821Linear Mixed Model (GLMM). In each panel, to generate the prediction, the unplotted
822variable is set at its mean value.

823

824**Table 1.** Model selection according to the corrected Akaike Information Criterion
825(AICc) and the Aikake weight. Models are ordered from the most to the least
826appropriate. Df, degrees of freedom; w_i (AICc), Akaike weight.

Model structure	Df	AICc	w_i (AICc)
Troglobionts abundance models			
$y \sim \text{DisE} * \text{Season} + \text{Tgr} + \text{Tgr}^2 + \text{MC} + \text{offset}(\text{Area}) + 1 \text{Sampling section}$	13	1633.11	0.64
$y \sim \text{DisE} * \text{Season} + \text{Tgr} + \text{Tgr}^2 + \text{MC} + \text{logSize} + \text{offset}(\text{Area}) + 1 \text{Sampling section}$	14	1635.20	0.22
$y \sim \text{DisE} * \text{Season} + \text{Tgr} + \text{Tgr}^2 + \text{MC} + \text{pH} + \text{logSize} + \text{offset}(\text{Area}) + 1 \text{Sampling section}$	15	1636.17	0.14
Troglobionts presence-absence models			
$y \sim \text{DisE} * \text{Season} + \text{logSize} + \text{pH} + 1 \text{Sampling section}$	11	420.41	0.56
$y \sim \text{DisE} * \text{Season} + \text{logSize} + \text{pH} + \text{MC} + 1 \text{Sampling section}$	12	421.72	0.27
$y \sim \text{DisE} * \text{Season} + \text{logSize} + \text{pH} + \text{MC} + \text{Tgr} + 1 \text{Sampling section}$	13	422.47	0.13

827