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Diversity metrics, species turnovers and nestedness of bird assemblages in a deep karst sinkhole

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Abstract We reported a study on breeding birds occurring inside an 80 m-deep karst sinkhole, with the characterization of the assemblages recorded along its semi-vertical slopes from the upper edge until the bottom. The internal sides of the sinkhole have been vertically subdivided in four belts about 20 m high. The highest belt (at the upper edge of the cenote) showed the highest values in mean number of bird detections, mean and normalized species richness, and Shannon diversity index. The averaged values of number of detections and species richness significantly differ among belts. Species turnover (Cody's β-diversity) was maximum between the highest belts. Whittaker plots showed a marked difference among assemblages shaping from broken-stick model to geometric series, and explicited a spatial progressive stress with a disruption in evenness towards the deepest belts. Bird assemblages evidenced a nested subset structure with deeper belts containing successive subsets of the species occurring in the upper belts. We hypothesize that, at least during the daytime in breeding season, the observed non-random distribution of species along the vertical stratification is likely due to (i) the progressive simplification both of the floristic composition and vegetation structure, and (ii) the paucity of sunlight as resources from the upper edge to the inner side of the cenote.

Keywords: sinkhole; diversity metrics; Cody's β-diversity; nestedness; vegetation structure; sunlight

Introduction

Cenotes (or sinkholes) are depressed and partially exposed structures resulting from the collapse of the surface layer that exposes groundwater underneath, often caused by karst processes (Whittow 1984; Culver 2016). In their internal slope they show a specific geo-climate characterization (high humidity, low sunlight, constant temperatures, minimum wind speed, high slope) that may affect the composition and richness of local vegetation and associated fauna (Vrbek and Fiedler 2000; Schmitter-Soto et al. 2002; Polli 2005; Lewin and Woodward 2007; Özkan et al. 2010; Vilisics et al. 2011; Bátori et al. 2011; Bátori et al. 2014), both specialized (as endemic troglobites and cave-dwelling; Barr and Holsinger 1985; Hamilton-Smith 2001) or not.

Surface habitats, both aphotic karst habitats and twilight habitats, such as sinkholes, karst springs, thin soils, and rock faces, may serve as important temporary refuges for organisms avoiding extremes temperature on the surface (Culver 2016). Among vertebrates, few species are strictly restricted to these karst environments mainly amphibians and bats (Friend 2002; Romano et al. 2012), but more typically, a large number of species utilizes temporarily and opportunistically karst contexts as one of a variety of suitable habitats (Reynolds 2014). For example, although birds are widespread in surface sides of karst habitats (Rimmer et al. 2005; Acevedo and Aide 2008), they occur occasionally in caves, sinkhole ponds and cenotes (e.g. Hicks 1938; Hutchinson 1999; Klaas 2011; Gerrard 2015; Roble and Stevenson 1998; Cokendolpher and Polyak 1996; Culver et al. 2000; Schmitter-Soto et al. 2002).

Although partially exposed karst habitats are peculiar and having an important ecological role and conservation interest (Jones et al. 2003), in Mediterranean area studies carried out on the opportunistic vertebrate fauna living in these habitats are very limited in number and focused only to single species level (e.g. Romano et al. 2012).

Here, we report a study focused on breeding bird assemblages occurring along the semi-vertical slopes inside a deep sinkhole with a lake at the bottom (80 m-depth; Bono 2001), characterizing it along a gradient from the upper edge to the bottom. More particularly, our aim is to define the vertical patterns in diversity metrics (individual detection, species richness, Shannon diversity, evenness and species turnover) along the cenote from the upper edge to the bottom. Our hypothesis is that, moving towards the deeper sides of the sinkhole, the increasingly severe geo-ecological conditions affect the structure of the bird assemblages, so inducing a progressive depletion of several diversity metrics because most bird species' requirements are not met there. Moreover, we hypothesize that the species composition

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*Corresponding author. E-mail: c.battisti@cittametropolitanaroma.gov.it of assemblages characterized by low species number (i.e. likely those colonizing the deeper belts) presents a non-random pattern, i.e. the species in assemblages of the deepest belts are sub-set of highest belts (nested structure).

Materials and Methods

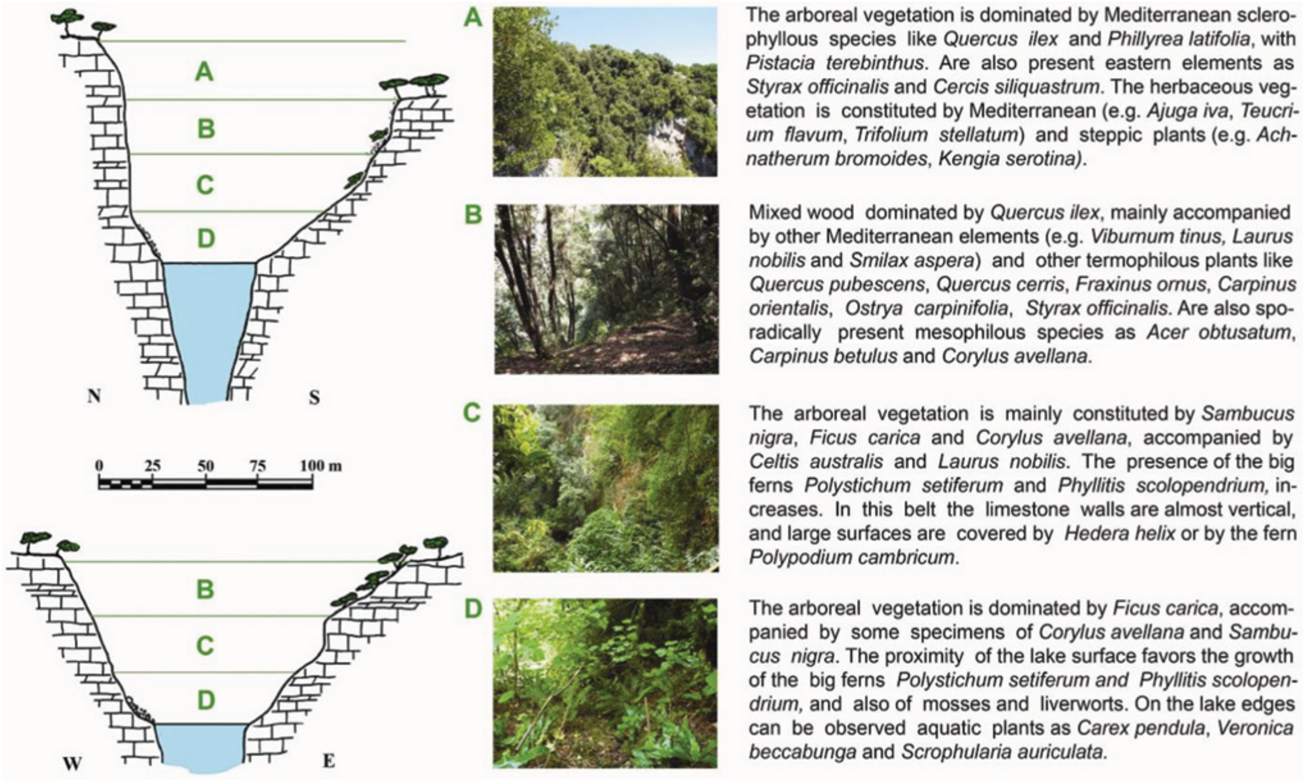
Study area

The "Pozzo del Merro" sink-hole is located at 140 m a.s.l., on the southern slopes of Cornicolani Mountains (site La Selva 42°02'21" N, 12° 40'50" E, Sant'Angelo Romano, Latium, central Italy) and is included in the "Macchia di Gattaceca e Macchia del Barco" nature reserve (Cornicolani mountains). It is a funnel-shaped cenote (cave collapse sinkholes), with a diameter of about 200 m at ground level (7 ha-wide), narrowing to 25 m at the lake water surface 80 m below (Fig. 1). Its flooded part extends at least 392 m below the water table (Giardini and Caramanna 2012): therefore this cenote is one of the world's deepest sinkhole (Bono 2001; Gary et al. 2003; Palozzi et al. 2010). The lake at the base of Pozzo del Merro host a peculiar fauna also with endemic species (Iannilli and Vigna Taglianti 2005; Romano et al. 2012).

The internal semi-vertical slopes of sinkhole are covered by a luxuriant vegetation, surprisingly rich of woody taxa of various geographic origin and different ecological needs. It vertically shows a clear differentation in four belts (for details see Figure 1), but it can be roughly divided into two parts: the upper side, more arid and well-lighted, where the vegetation is floristically richer and mostly constituted by Mediterranean evergreen sclerophyllic elements (e.g. *Quercus ilex* and *Phillyrea latifolia*), and the bottom side where the vegetation, floristically poorer, is dominated by mesoigrophilous and moderately nitrophilous species (e.g. *Ficus carica* and *Sambucus nigra*). In this chasm, the Mediterranean species largely dominate but are also present eastern termophilous taxa and some really mesophilous species. This floristic and vegetational diversity is linked to the high ecological variability of the sinkhole, due to the presence of bare rock or of a more or less developed soil, to a greater or lesser inclination of the walls, to a greater or lesser water, light and organic matter availability. For these reasons the vegetation changes from top to bottom of the sinkhole, showing also a thermal inversion, a typical phenomenon of the karst sinkholes. This phenomenon is evident if we observe the abundance of termophilous evergreen sclerophillic taxa, like *Quercus ilex*, in the upper part of the cavity, and the presence of mesophilous arboreal taxa, e.g. *Acer obtusatum*, further down. A further confirmation is represented to the presence, in the deeper part of the sinkhole, of *Cardamine impatiens*, a species that prefers shady environments on cool and moist soils, mainly carbonatic and rich in humus, that usually lives in Italy between 400 and 1300 m a.s.l. For plant composition along the semi-vertical belts of sinkhole, see Fig. 1. For a more detailed floristic description, see also Giardini et al. (2001) and Giardini (2012a).

Protocol

The internal sides of Pozzo del Merro have been vertically divided in four 20 m-deep belts (total vertical range = 80 m, corresponding to the depth from the edge of cenote to the small lake on the bottom).



The arboreal vegetation is dominated by Mediterranean sclerophyllous species like *Quercus ilex* and *Phillyrea latifolia*, with *Pistacia terebinthus*. Are also present eastern elements as *Styrax officinalis* and *Cercis siliquastrum*. The herbaceous vegetation is constituted by Mediterranean (e.g. *Ajuga iva*, *Teucrium flavum*, *Trifolium stellatum*) and steppic plants (e.g. *Achnatherum bromoides*, *Kengia serotina)*.

Mixed wood dominated by *Quercus ilex*, mainly accompanied by other Mediterranean elements (e.g. *Viburnum tinus, Laurus nobilis* and *Smilax aspera*) and other termophilous plants like *Quercus pubescens*, *Quercus cerris*, *Fraxinus ornus*, *Carpinus orientalis*, *Ostrya carpinifolia*, *Styrax officinalis.* Are also sporadically present mesophilous species as *Acer obtusatum*, *Carpinus betulus* and *Corylus avellana*.

The arboreal vegetation is mainly constituted by *Sambucus nigra*, *Ficus carica* and *Corylus avellana*, accompanied by *Celtis australis* and *Laurus nobilis*. The presence of the big ferns *Polystichum setiferum* and *Phyllitis scolopendrium*, increases. In this belt the limestone walls are almost vertical, and large surfaces are covered by *Hedera helix* or by the fern *Polypodium cambricum*.

The arboreal vegetation is dominated by *Ficus carica*, accompanied by some specimens of *Corylus avellana* and *Sambucus nigra*. The proximity of the lake surface favors the growth of the big ferns *Polystichum setiferum and Phyllitis scolopendrium,* and also of mosses and liverworts. On the lake edges can be observed aquatic plants as *Carex pendula*, *Veronica beccabunga* and *Scrophularia auriculata*.

Figure 1. Plant composition in the 20 m-deep semi-vertical belts along the cenote (From A to D; on the right). On the left two sections (North–South and West–East) have been reported (Original pictures: M. Giardini).

Vegetation composition and structure. – To compare the vegetation structure between the surface edge belt and the inner side, we carried out a structural characterization of the vegetation using the Range Finder Circle Method (James and Shugart 1970, modified), locating a set of plots of 0.04 ha in size along the slopes of the cenote. Within each plot, we randomly selected the first ten trees immediately surrounding the plot centroid. We measured each tree with a diameter (>7.5 cm) at the breast height (hereafter, dbh: 1.40 m; n = 120 plants measured). From these sampling, we obtained the number and frequency of trees for a number of dbh classes (>7.5–15; >15–23; >23–38; >38–45; >45 cm). It was not possible to make a balanced structural characterization for all the belts because of the inability to obtain a representative sample of tree vegetation located along the deepest sub-vertical belts. Therefore, to test if there are significant differences in tree vegetation structure from the edge of the cenote and the inner sides, we subdivided the total sample in two main sub-samples, the first referring to the belt A (the edge of cenote, represented by a Mediterranean sclerophyllic vegetation; see study area), the second one referring to the sum of belts B, C and D (the inner sides of cenote, dominated first by Mediterranean evergreen oak woodland and then by mesoigrophilous and moderately nitrophilous vegetation; see study area).

Bird sampling. – Data on bird abundance were collected by the fixed radium-Point Count Method (Bibby and Burgess 1992; Sutherland 2006). We located four sampling points in each 20 m-deep belt using a GPS Garmin E-trex. Point counts were sampled in the morning (7:00–11:00 a.m.) for eight replicated session from 22 March to 21 June 2016. Each session lasted 15 min. During each session, the observers recorded each bird individual of any species seen or heard within a radium of 25 m, so obtaining a comparable value of point detection. Distance between sampling points located in contiguous belts was always higher than 70 m and each session has been carried out contemporarily by four researchers (CB, FM, LDR, GD) to reduce the chance of double counting (pseudo-replication; Bibby and Burgess 1992; Battisti et al. 2014). To reduce a possible bias due to the observer effect, in each replicated session each researcher randomly changed its location along the belts. Each individual record obtained from each observer has been reported on a 1:1,000 map. The total fieldwork summed up to 480 min. Samples were taken under favorable environmental conditions, avoiding extreme rain and strong wind (Bibby and Burgess 1992).

Data analysis

At assemblage level, we calculated the following parameters for each 20 m-deep belt: (i) mean number of individual bird detections (mean point detection, MPD, and standard deviation, s. d.); (ii) total number of detected species (S); (iii) mean species richness (S_{mean} and standard deviation, s. d.), as the averaged number of species detected in each sampling point; (iv) normalized species richness (Margalef index), as Dm = (S−1)/ln N, where S is the number of species and N the number of detected individuals (Magurran 2004); this index expresses a value of richness normalized to the sampling data-set; (v) Shannon diversity index, as $H' = -\Sigma \, fr_i \ln fr_i$, where fr_i is the relative species frequency (Shannon and Weaver, 1963; we considered 'dominant' a species with a $fr_i > 0.05$); (vi) evenness index (e), as $e = H'/H'max$, where $H'max = lnS$ (Lloyd and Ghelardi 1964).

To assess the turnover in species composition along habitat gradients (in our case, along paired semi-vertical belts), we calculated the Cody measure as $\beta_C = [gS + lS]/2$ (Cody 1975; Koleff et al. 2003; Magurran 2004). This index checks the lost (lS) and gained (gS) species when comparing two paired assemblages.

To spatially explicit structural differences among assemblages we performed a rank/abundance plot (or Whittaker plot; Whittaker 1965). In this analysis, species are plotted in sequence from the most to least detected along the x-axis, and their number of detections is displayed in a $\log_{10}$ format along the y-axis. To facilitate comparison in number of detections, data were transformed in relative frequency. Whittaker plots highlight differences in evenness amongst assemblages in a graphical way: steep plots indicate assemblages with high dominance and, at the opposite, shallower slopes imply a low dominance (Magurran 2004; Magurran and McGill 2011).

To compare the averaged values of MPD and S_{mean} among the four belts we used the Friedman test. To compare paired belts among them, we performed the Wilcoxon paired sample test. We tested for differences in relative frequency among tree dbh categories using a χ^2 test (Dytham 2011). To perform all the analyses, we used the SPSS 13.0 software (SPSS Inc. 2003).

To test the potential structure of our study system in nested sub-assemblages, we conducted a nestedness analysis on the distribution of avian species along the semi-vertical belts of sinkhole. We estimated three nestedness metrics with the NeD software (ecosoft/alwaysdata.net; Strona et al. 2014): (i) the matrix temperature (MT), which uses the Euclidian distances of unexpected empty or filled cells from the isocline that separates presences from absences in a perfectly nested matrix; the sum of these distances is rescaled relative to the maximum possible value for a given matrix size and fill (Rodriguéz-Gironés and Santamarìa 2006); (ii) Brualdi and Sanderson Discrepancy (BR), which is a count of the number of discrepancies (absences or presence) that must be "corrected" to produce a perfectly nested matrix (Brualdi and Sanderson 1999); (iii) nestedness measure based on overlap and decreasing fills (NODF), which is the percentage of presences in inferior rows and in right columns that are in the same position (column or row) of the presences in, respectively, upper rows and left columns with higher marginal totals for all pairs of columns and rows (Almeida-Neto et al. 2008). In addition to the absolute metrics' values, we also computed the respective standardized effect sizes as z-values based on 1000 simulated null matrices. Null matrices were constructed using the 'Equiprobable row totals, fixed column totals (EF)' algorithm, which maintains observed column totals (i.e. species richness per belt) but allows row totals (i.e. species occurrence frequencies) to vary randomly and equiprobably (Ulrich and Gotelli 2007). We selected this algorithm in order to maintain the observed belt species richness that we hypothesized as constrained by belt-specific carrying capacity.

For taxonomic nomenclature, we refer to Fracasso et al. (2009) and Brichetti and Fracasso (2015); for Italian Sparrow we refer to the nomenclature of *Passer italiae* since Hermansen et al. (2011) established definitively that this species is a stabilized hybrid.

Results

Vegetation structure

Overall, the more represented dbh size category was 7.5–15 cm (43%, n = 120; Table 1, Figure 2; Supplementary materials S1), significantly more frequent in the inner side of the cenote (belts B+C+D; frequency = 0.68, n = 41) when compared to the upper edge (belt A; frequency = 0.29, n = 79; χ^2 = 15.389, p = 0.000); differently, mature trees (category 23–38 cm) were significantly more frequent in the upper edge of the cenote (0.39 vs. 0.07; χ^2 = 12.020, p = 0.000; Figure 2).

Bird sampling

During the point count method, we obtained 181 detections belonging to 28 species (Table 1). Other 15 species have been recorded outside the standard method (Supplementary materials S2). We did not obtained a significant difference among replicated sessions for both number of detections (χ^2 = 7.223, p = 0.406) and number of species (χ^2 = 8.553, p = 0.286, df = 7; Friedman test).

The belt A (upper edge of the cenote) showed the highest values in MPD and S_{mean} (Figures 3 and 4). These last

Table 1. Species and relative frequencies detected in the four semi-vertical belts (A, B, C and D) in the Pozzo del Merro cenote. In bold, the dominant species (fr > 0.05). N: total number of individual detected; fr_i relative frequency.

	Belts			
	A	B	C	D
Species	fr_i	fr_i	fr_i	fr_i
Falco peregrinus		**0.073**		
Falco tinnunculus	0.010			
Columba palumbus	0.040	**0.109**		
Streptopelia decaocto	0.010			
Merops apiaster	0.040			
Picus viridis	0.020			
Dendrocopos major		0.018	0.043	
Motacilla alba	0.010			
Garrulus glandarius	0.020	**0.073**		
Corvus cornix	0.020			
Troglodytes troglodytes	**0.079**	**0.200**	**0.304**	**0.5**
Hippolais polyglotta	0.020			
Sylvia atricapilla	**0.099**	**0.145**	0.043	
Sylvia melanocephala	**0.099**	0.018		
Sylvia cantillans	0.010			
Phylloscopus collybita	0.010			
Regulus ignicapilla	0.010			
Luscinia megarhynchos	0.050			
Erithacus rubecula	0.050	**0.091**		
Turdus merula	**0.099**	**0.145**	**0.348**	**0.5**
Cyanistes caeruleus	0.030	**0.091**	**0.087**	
Parus major	**0.089**		0.043	
Aegithalos caudatus	**0.069**	0.036	**0.130**	
Oriouls oriolus	0.020			
Passer italiae	0.050			
Fringilla coelebs	0.010			
Serinus serinus	0.020			
Carduelis chloris	0.020			
N	101	55	23	2

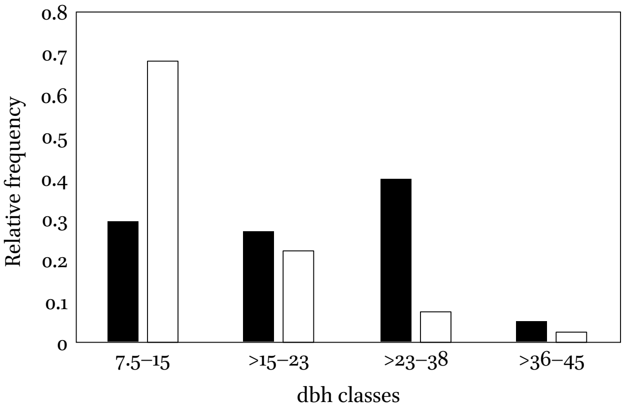


Figure 2. Relative frequencies for different dbh size classes on the belt A (the upper edge of cenote; in black) and in the belts B, C and D summed (the inner belt of cenote; in white).

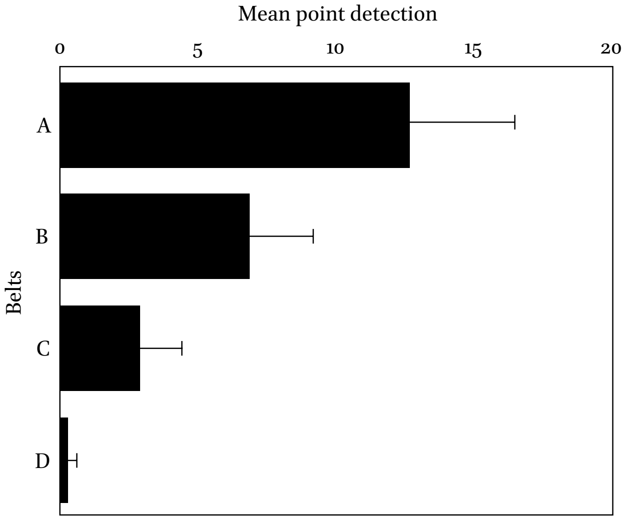


Figure 3. Mean point detection (and standard deviation) in the four 20 m-deep semi-vertical belts (from A to D; see Methods).

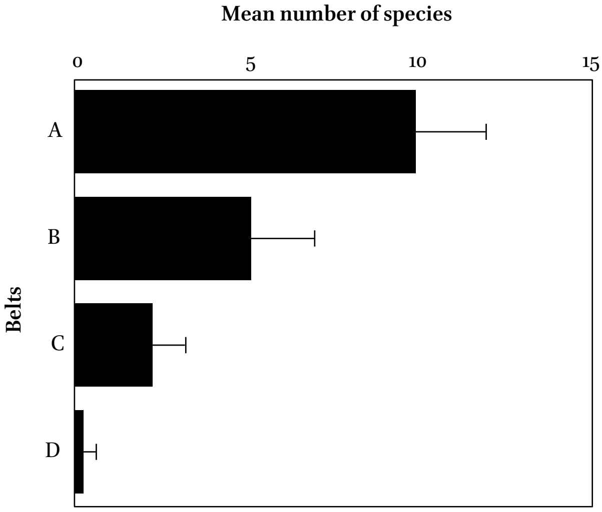


Figure 4. Mean number of species (S_{mean}; and standard deviation) in the four 20 m-deep semi-vertical belts (from A to D; see Methods).

metrics significantly decreases toward the deepest belts (respectively, $\chi^2 = 23.734$, $p = 0.000$ and $\chi^2 = 24.000$, $p = 0.000$ df = 3, Friedman test). Differences were significant also when we performed a paired comparison between adiacent belts both for MPD (A-B: $Z = -2.527$, $p = 0.012$; B-C: $Z = -2.533$, $p = 0.011$; C-D: $Z = -2.555$, $p = 0.011$) and for S_{mean} (A-B: $Z = -2.366$, $p = 0.018$; B-C: $Z = -2.524$, $p = 0.012$; C-D: $Z = -2.555$, $p = 0.012$; Wilcoxon paired test). Analogously, normalized species richness and Shannon diversity index both progressively decrease from belt A to D.

Evenness index decreases from the uppest belts (A and B) to belt C. In belt D, the maximum value of 1 is due to the detection of only two species of similar relative frequency (0.5) (Table 2). Whittaker plot showed a marked difference among assemblages, spatially expliciting the progressive change in frequency structure from the A to C belts (assemblage of belt D is only represented from two species and show a right line: Figure 5).

The Cody measure of turnover shows the highest value between the highest belts (A vs. B) and progressively decrease toward the deepest belts (Table 2). Nestedness analysis revealed that sinkhole assemblages were nested for all the used metrics (matrix size: 112, fill: 42%; BR = 2, $z = -7.783$, $p < 0.001$; temperature = 13.145, $z = -6.158$, $P<0.001$; NODF=55.909, $z=7.608$, $P<0.001$; Supplementary materials S3): i.e. the assemblages in the deepest belts are nested (i.e. represented a sub-set) in the highest ones.

Table 2. Structural parameters of breeding bird assemblages along the four 20 m-deep belts of the cenote (from A to D). N: total number of recorded occurrences, MPD: mean point detection richness (and standard deviation, s.d.); S: species richness; S_{mean}: mean species richness (and standard deviation, s.d.); H': Shannon diversity index; e: evenness; Dm: normalized species richness (Margalef index); β_C = Cody's β diversity measure.

Metrics	Belts			
	A	B	C	D
N	101	55	23	2
MPD (and s.d.)	12.63 (3.89)	6.88 (2.36)	2.88 (1.64)	0.25 (0.46)
S	26	11	7	2
S_{mean} (and s.d.)	9.88 (2.17)	5.13 (1.89)	2.25 (1.04)	0.25 (0.46)
H'	2.96	2.21	1.62	0.69
e	0.91	0.92	0.83	1
Dm	5.42	2.50	1.91	1.44
β_C		9.5		
			3	
				2.5

Discussion

At community level, we observed a rapid decrease of number of detections, species richness (absolute, averaged and normalized) and Shannon diversity index from the highest belts of cenote toward the progressively deepest belts, where only occasional individual detections have been obtained. Moreover, although the number of detections is limited, the four assemblages showed an apparent nested subset structure with deepest belts containing successive subsets of the species yet occurring in higher belts. Finally, Whittaker plots move from shallower slopes of the curves of the A assemblage (having a "broken-stick" shape) until plot tending to geometric series in C assemblage. The deepest assemblage showed a right line (only two species).

It has been highlighted that (i) sinkholes may act as refuge for many species since these areas show a lower or absent frequency of natural and anthropogenic disturbances (e.g. fires, windstorms, grazing, human frequentation and noise), and that (ii) they could function as sink for biomass

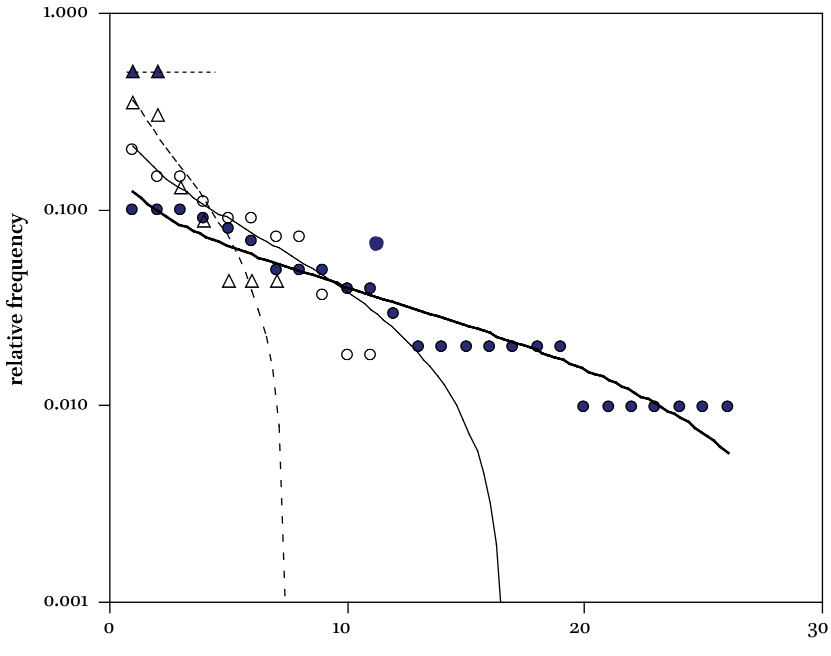


Figure 5. Rank/abundance plots (Whittaker plots) for the assemblages in the four 20-m depth semi-vertical belts of Pozzo del Merro sinkhole. Assemblage A: black circles and bold line; B: white circles and line; C: white triangles and dashed line; D: black triangles and dotted line. Y-axis is log-transformed (Magurran 2004).

accumulation (and consequently trophic resources; Keiller 2011). However moving towards the deepest belts of the cenote, (i) the reduced availability of sunlight, (ii) the floristic impoverishment (e.g., here the number of woody *taxa* decreased from over 20 to less than 5), also linked to a reduced size area and a consequent lower amount of trophic resources, and (iii) a simplification in vegetation structure (with a reduced number of spatial niches) might be all considered strong limiting factors with consequent stress in bird assemblages (Wiens 1989). All these factors together may likely induce (i) a disruption in assemblage structure (Whittaker plots; see Dornelas et al. 2011), (ii) an evident species turnover (Cody's β measure) in richest and highest belts, (iii) a progressive decrease in richness (absolute and normalized) and diversity toward the deepest belts and (iv) a nested structure of the assemblages from top to bottom.

At single species level, *Troglodytes troglodytes* and *Turdus merula* are the only two 'shadow-related' species, occasionally detected in the deepest belts. These species, everywhere dominant in all the belts, are known as sciaphilous species, linked to undergrowth conditions (Dabelsteen et al. 1993; Camprodon and Brotons 2006; Tomiaojæ and Bursell 2006; Orłowski et al. 2008). A limited set of other species (*Cyanistes caeruleus, Parus major, Aegithalos caudatus, Dendrocopos major, Sylvia atricapilla*) occurred in the belt C located below other 40–60 m from the upper edge. These are both generalist and specialized forest species however linked to interior and mesophilous habitats (e.g. Hinsley et al. 1995; Bianconi et al. 2004; Lorenzetti and Battisti 2007; Zangari et al. 2013). Contrarily, a heterogeneous set of termophilous and edge-mosaic species (e.g., *Merops apiaster, Sylvia melanocephala, S. cantillans*) but also forest canopy species linked to mature trees (e.g. *Columba palumbus, Picus viridis, Garrulus glandarius*) and patchy synanthropic landscapes (e.g. *Falco tinnunculus, Streptorpelia decaocto, Corvus cornix*; Bellamy et al. 1996; Cieslak 1985; Hinsley et al. 1995; Møller 1987; Opdam et al. 1985), occurred exclusively in the lighting edge area on the border of cenote (belt A) and in immediately surrounding belt B.

However, for single specialized species, cenotes may also act as "key structures" (sensu Tews et al. 2004), improving the landscape heterogeneity, so allowing the presence of rare birds. For example, the isolated presence of *Falco peregrinus*, nesting inside the cenote thanks to availability of vertical rocky slopes (rarely occurring in the surrounding; Giardini 2012b), is likely linked to the peculiar characteristic of this context.

Although a large number of research have been carried out in karst habitats around the world (e.g. Barr and Holsinger 1985; Hamilton-Smith 2001; Schmitter-Soto et al. 2002; Lewin and Woodward 2007), this is the first study on bird assemblages occurring in the emerging semi-vertical slopes of a deep karst sinkhole. Nevertheless, we would highlight a caveat of our study: we carried out the field research in a limited seasonal period (spring) and during day-time. However, in order to control this seasonal-related bias, we carried out the samplings in a unique phenological period (there are not significant differences in species detection and richness among replicated sessions), and controlled for other possible sources of data unreliability (in particular pseudo-replication). Moreover, as with point count method we obtained only bird detection, considered a weak proxy of true density (Freckleton et al. 2006; Toms et al. 2006), the observed depletion of MPD values along the belts could be also addressed to a reduced singing behaviour of birds due to aphotic conditions (Sorjonen 1986; Kroodsma and Byers 1991), irrespective of a true reduction in their density. In this sense, further studies should separately analyse sound and sight detections. Finally, we reported only a pattern in bird detection without an analysis of relationships between birds and environmental factors along a vertical gradient. Therefore, further studies should investigate the temporal patterns of true density in the deepest belts of the cenote to detect time regimes of permanence (duration and frequency) and turnovers of the species at both seasonal and circadian time scales and its relationships with environmental factors (e.g. vegetation, light). In this sense, our explorative data allowed us to postulate further a-posteriori hypotheses that should be tested in future studies (inductive approach; Romesburg 1981; Guthery 2007). For example, a possible prediction could be that the sinkhole, due to its local climatic features (low wind speed, constant regime in temperatures and thermal inversion in cold days; Culver 2016) might act as temporary refuge for birds in winter, hosting crowded roosts of gregarious species, differently from the breeding period when this key habitat apparently host only occasional singing individuals.

The results of this work can be used for the planning of conservation strategies of this site and other karst environments. These areas are extremely vulnerable and generally poorly resilient. Their management requires an holistic approach that takes into account all the various aspects – biotic, geological, hydrological, microclimatic – that contribute to their overall balance. Moreover, it should take in account the regime of specific threats and disturbances (Sauro 1993; Watson et al. 1997; Day and Urich 2000; Urich et al. 2001; Van Beynen and Towsend 2005; Angulo et al. 2013; Li et al. 2013a, b; Battisti et al. 2016), thus providing valuable information to manage these peculiar contexts.

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No potential conflict of interest was reported by the authors.

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Supplementary material

Supplementary material S1. Frequency distribution of trees for different diameter at breast height (dbh) classes (A, B, C, D: semi-vertical 20 m-deep belts in the cenote).

dbh classes	A		B + C + D	
7.5–15	23	0.29	28	0.68
>15–23	21	0.27	9	0.22
>23–38	31	0.39	3	0.073
>36–45	4	0.05	1	0.024
Tot (n = 120)	79		41	

Supplementary material S2. Bird species recorded in the study area outside the standardized sampling.

Athene noctua, Pernis apivorus, Accipiter nisus, Larus cachinnans, Cuculus canorus, Streptopelia turtur, Apus apus, Upupa epops, Hirundo rustica, Delichon urbicum, Saxicola torquatus, Muscicapa striata, Certhia brachydactyla, Pica pica, Emberiza cirlus.

Supplementary material S3. Binary matrix representing the birds communities occurring in the four semi-vertical belts (A, B, C, and D). A filled square indicates an observed presence of a given species in a given belt. Assemblages are arranged in the order of decreasing number of bird species occurring in them, and bird species are ranked in the order of decreasing number of occurrences in the various belts, in a way that both minimizes unexpectedness.

Species/Sites	A	B	C	D
Troglodytes troglodytes	■	■	■	■
Turdus merula	■	■	■	■
Sylvia atricapilla	■	■	■	
Parus caeruleus	■	■	■	
Aegithalos caudatus	■	■	■	
Parus major	■	■	■	
Picoides major		■	■	
Columba palumbus	■	■		
Garrulus glandarius	■	■		
Sylvia melanocephala	■	■		
Falco peregrinus		■		
Erithacus rubecula	■	■		
Hippolais polyglotta	■			
Corvus cornix	■			
Sylvia cantillans	■			
Phylloscopus collybita	■			
Regulus ignicapilla	■			
Luscinia megarhynchos	■			
Falco tinnunculus	■			
Streptopelia decaocto	■			
Merops apiaster	■			
Picus viridis	■			
Motacilla alba	■			
Oriouls oriolus	■			
Passer italiae	■			
Fringilla coelebs	■			
Serinus serinus	■			
Carduelis chloris	■			