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Brief Report

Mapping Breeding Birds in a Karstic Sinkhole with a Comparison between Different Sampling Methods

Corrado Battisti [1,*], Pierangelo Crucitti [2], Giuseppe Dodaro [3], Marco Giardini [4] and Francesca Marini [1]

[1] 'Torre Flavia' LTER (Long Term Ecological Research) Station, Protected Areas Service, Città Metropolitana di Roma Capitale, Via Ribotta, 41, 00144 Rome, Italy; f.marini@cittametropolitanaroma.it
[2] Società Romana di Scienze Naturali, Via Fratelli Maristi 43, 00137 Roma, Italy; info@srsn.it
[3] Fondazione per lo Sviluppo Sostenibile, Via Garigliano 61a, 00198 Roma, Italy; dodaro@susdef.it
[4] Istituto d'Istruzione Superiore Via Roma 298, Via Elsa Morante, Guidonia Montecelio, 00012 Roma, Italy; marcogiardini.sar@gmail.com
* Correspondence: c.battisti@cittametropolitanaroma.it; Tel.: +39-06-6766-3321

Abstract: Karstic sinkholes are peculiar structures hosting specific biological communities. Birds are still little studied in this regard. This note reports, for the first time, original data relating to the density of breeding species occurring within a sinkhole in central Italy obtained with a fine-grained and time-expensive sampling technique (mapping method). The results were compared with data sampled with the point counts method carried out in the same phenological period. We recorded 22 breeding species, all typical of meso-thermophilous forests and ecotonal habitats of hilly central Italy. Among them, two species (*Turdus merula* and *Troglodytes troglodytes*), typical of shady, undergrowth habitats, were recorded in the deepest part of the sinkhole (−70 m from the top). No significant differences emerged between the relative frequencies of the species obtained with the two methods, except for *Luscinia megarhynchos* (overestimated with the mapping method) and *Aegithalos caudatus* (underestimated). At the community level, the comparison of the two methods revealed similar values in univariate diversity metrics, Whittaker plots did not show a significant difference (ANCOVA test), and ordinary least squares regression between the frequencies showed a highly significant correlation. Therefore, in these peculiar habitats, data obtained from the two methods are comparable: since the point counts method needs lower sampling effort, it appeared to be more effective when compared to the mapping method to study these peculiar habitats.

Keywords: species richness; diversity; Whittaker plots; point counts; mapping method; Italy

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1. Introduction

Karst sinkholes are peculiar structures characterized by sudden changes in altitude, temperature, and lighting: these environmental gradients can influence some ecosystem components [1–3]. Among these components, birds are still little studied in these peculiar ecosystems [4,5]. This note reports data on the community structure of breeding birds in a deep karstic sinkhole located in central Italy (Pozzo del Merro; Latium), actively studied in the past decades regarding geology and vegetation [6].

Here, we report, for the first time, original data relating to the density of breeding (territorial) species within the sinkhole recorded with a mapping method: a census technique useful to obtain data at species and community level (species richness, diversity, and evenness). Furthermore, the results obtained were compared with the data obtained with the point counts method carried out in the same phenological period [4]. Although the mapping method obtains fine-grained data [7], it is time-consuming compared to the more rapid method of point counts [8]. Similar to other comparisons between sampling techniques for avian species [9], we wanted to verify whether there are differences between the two approaches both in terms of density (at species level) and regarding univariate metrics of diversity (at community level). Therefore, we defined two aims in this study: (i) a quantitative arrangement of breeding birds using a fine-grained field sampling approach (mapping method); and (ii) a comparison with another sampling technique (point count method), carried out in the same site and seasonal period. Finally, some considerations are reported on the species that occupied the deepest part of the sinkhole.

2. Materials and Methods

2.1. Study Area

The "Pozzo del Merro" sinkhole 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 [10]), and is within the "Macchia di Gattaceca e Macchia del Barco" nature reserve (997 hectares; Cornicolani Mountains). It is a funnel-shaped cenote (cave-collapse sinkholes), with a diameter of about 200 m at ground level (7 ha), narrowing to 25 m at the lake water surface 80 m below (Figure 1). Its flooded part extends at least 392 m below the water table [6]; therefore, this cenote is one of the world's deepest sinkholes [11–14].

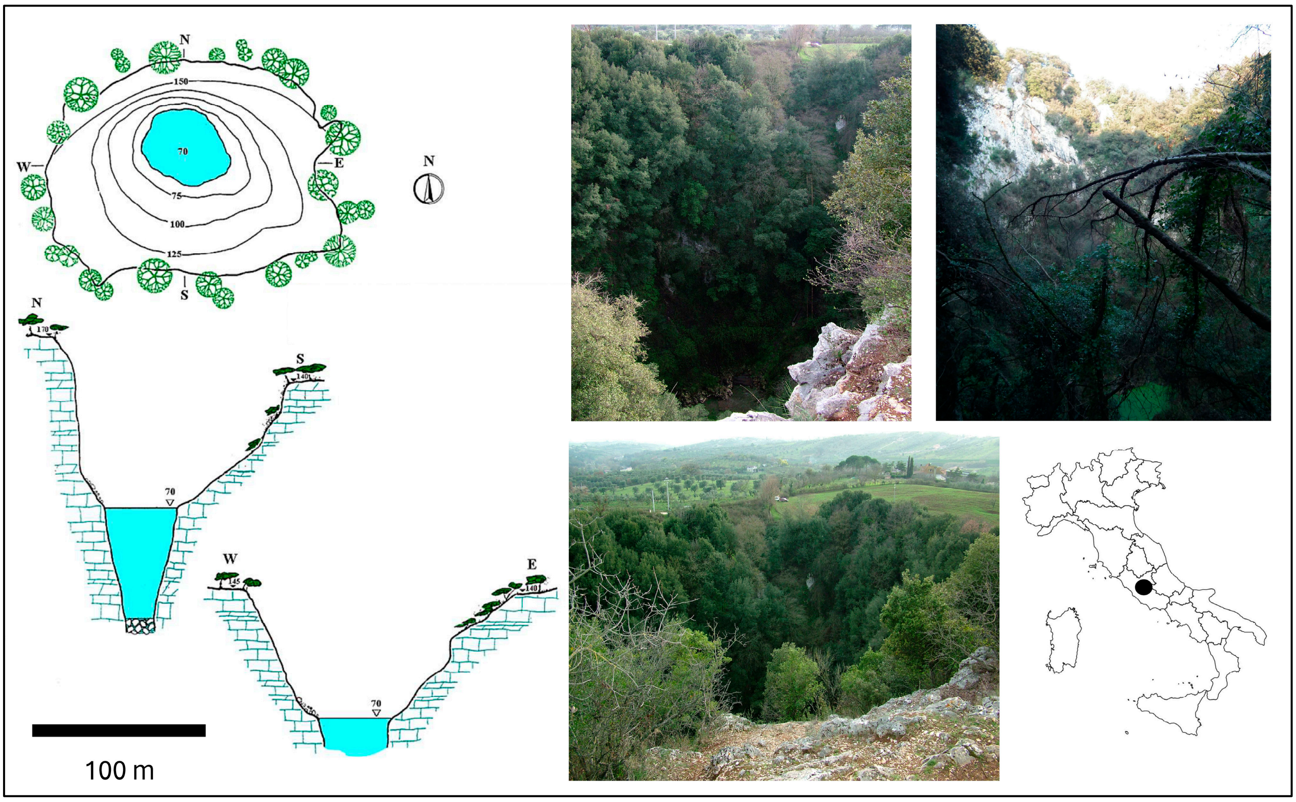

Figure 1. Study area. The 'Pozzo del Merro' sinkhole (Latium, central Italy). Top left: zenith (orthogonal) projection. Lower left: north–south and west–east sections. On the right, the sinkhole from different points of view and a map of Italy with the location of the study area (Photo: M. Giardini).

Thermal inversion occurs along the slopes of sinkholes. The internal slopes of sinkholes are covered by vegetation, showing differentiation on the upper side, and being more arid, where the vegetation is represented by Mediterranean evergreen thermo-xerophilic and sclerophylic species on calcareous soils (e.g., *Quercus ilex*, *Phyllirea latifolia*) with the presence of Balcanic deciduous species (e.g., *Cercis siliquastrum*, *Styrax officinalis*); on the bottom side, the vegetation dominated by meso-igrophilous species (with *Corylus avellana*, *Acer obtusatum*, *Ficus carica*, and *Sambucus nigra*). This plant diversity is linked to the high variability in slope inclination, soil, and exposed bare rock of the sinkhole, with different levels of light and organic matter availability. In the shady environments at the bottom of the sinkhole, there are soils which are moist, mainly carbonatic, and rich in humus. In the bottom, around the deep lake, hygrophilous vegetation is present, with *Carex pendula*, *Veronica beccabunga*, and *Scrophularia auriculata* species [4]. For a more detailed floristic description, see [4,14].

The sinkhole surface in the zenith (orthogonal) projection is approximately 3 hectares, while the overall surface considering the truncated cone is 7.9 hectares.

2.2. Bird Survey

Data on bird abundance were collected using the territory mapping method [7,8], in order to obtain quantitative data on the occurrence, density, and relative frequency of the breeding bird species. Differently from other methods widely used for assessing population parameters in birds (e.g., the line transect method and point counts), the mapping method obtains a more accurate estimate of density (as territorial breeding pairs) for single species, e.g., at the level of single male territories [8]. Inside the study period, at least three records of evident territorial behavior were considered sufficient to document the existence of a breeding pair inside the fragment. We assigned a territory for each breeding pairs; we assigned values of 0.5 to the territories partially included in the study area [7,8].

All the field sampling was carried out in the morning (7:00–11:00 a.m.) for eight replicated sessions from 22 March to 21 June 2016. During each sampling session, the observers walked at a constant speed (1.0–1.5 km/h) along a previously established route, in order to cover the whole area, and recorded each bird individual of any species seen or heard, locating them on a field-map (1:1000).

Data on bird abundance were collected by the fixed radium point count method [7,8]. 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 sessions in the same seasonal period (2016). Each session lasted 15 min. During each session, the observers recorded each bird individual of any species seen or heard within a radius of 25 m, thus obtaining a comparable value of point detection. To avoid pseudo-replication, we located points to a distance >50 m. The distance between sampling points located in contiguous belts was always higher than 70 m to reduce the chance of double-counting (pseudo-replication; [7]). Each individual record obtained from each observer has been reported on a 1:1000 map (details in [4]).

The total fieldwork consisted of about 20 h (=1200 min) of sampling effort. Samples were taken under favorable environmental conditions, avoiding extreme rain and strong wind to reduce sampling biases [8].

2.3. Data Analysis

From the field data, the number (*n*) of records for each species and the number of breeding territories were obtained. Two densities were then calculated: (i) on the surface of the sinkhole in zenith projection (3 hectares); and (ii) by calculating the area of the truncated cone represented by the sinkhole (7.9 hectares).

The following species- and community-level indices were obtained from the data: (i) the total number of detected species (S, non-normalized richness); (ii) normalized species richness (Margalef index), as $Dm = (S - 1)/\ln N$, where S is the number of species and N is the total number of recorded individuals [15,16] (this index expresses a value of richness normalized to the sampling dataset); (iii) the species density (in pairs/ha), obtaining an estimate based on the rigid application of the mapping method (one territory = at least three territorial records, with at least one contemporary with another territory [8], following prior examples [15,16]); (iv) the Shannon–Wiener diversity indices as $H' = -\Sigma\ fr_{MA} \ln fr_{MA}$, where fr_{MA} is the relative species frequency (i.e., n records of the i-th species/n. total records [16], with $fr_{MA} > 0.05$, considered as dominant species); and (v) the evenness index (e), as $J = H'/H'max$, where $H'max = \ln S$ [17–19].

To spatially explicit structural differences among assemblages, we constructed a rank/abundance plot (or Whittaker plot [20]). In this analysis, species are plotted in sequence from the most to least detected along the x-axis, and the number of detections is displayed, as relative frequency, in $\log_{10}$ format along the y-axis. To facilitate comparisons the in number of detections, data of abundance were transformed into relative frequencies. Whittaker plots highlight differences in evenness amongst assemblages: steep plots indicate assemblages with higher dominance (low evenness), and in contrast, shallower slopes imply a lower dominance (high evenness [16,21]).

The data obtained were then compared with those obtained for the same area with another sampling method (sampling point counts [8]) carried out in the same period (22 March to 21 June 2016) but involving different research efforts (1200 min for the mapping method vs. 480 with the point counts method [4]).

For comparisons of species frequencies obtained with the two methods, the χ^2 test was used (only for species showing >5 records in at least one method). We performed a non-parametric Spearman rank correlation test between the species frequencies obtained from both the methods (mapping method vs. point counts). We compared the co-variance among frequencies in Whittaker plots using a paired χ^2 test between species frequencies [22]. PAST 4.1 software [23] with alpha level at 0.05 was used. For taxonomic nomenclature, we refer to the extant literature [24].

3. Results

With the mapping method, 268 individuals belonging to 22 breeding territorial bird species were contacted (total: 25.5 territorial pairs; density: 8.5 pairs/ha considering the orthogonal size, i.e., total area of the cone projection on a flat surface; 3.23 pairs/ha, considering the cone trunk size, i.e., the sum of size areas of the sinkhole slopes).

Another 22 species were recorded outside the standard detection time or in high flight and were not considered in the quantitative analysis (see the Supplementary Materials). Among territorial breeding species, seven were dominant (showing fr_{MA} > 5%; Table 1).

Table 1. Breeding bird species recorded using the mapping method ('Pozzo del Merro' sinkhole, central Italy). The number of breeding pairs (pairs) and density (as pairs/hectare; p/ha), considering the orthogonal size (3 ha) and the cone trunk section size (7.9 ha), relative frequency (Fr_{MA}), and number of records (*n*), have been reported. The dominant species are indicated in bold (Fr_{MA} > 0.05). See the Methods for details. Nd: undetermined number of records.

		Density			
Species	**Pairs**	**p/ha (3 ha)**	**p/ha (7.9 ha)**	**Fr_{MA}**	***N***
Troglodytes troglodytes	**4**	**1.333**	**0.506**	**0.157**	**37**
Sylvia atricapilla	**3.5**	**1.167**	**0.443**	**0.137**	**29**
Turdus merula	**2.5**	**0.833**	**0.316**	**0.098**	**39**
Luscinia megarhynchos	**2**	**0.667**	**0.253**	**0.078**	**23**
Parus major	**1.5**	**0.500**	**0.190**	**0.059**	**21**
Cyanistes caeruleus	**1.5**	**0.500**	**0.190**	**0.059**	**18**
Columba palumbus	**1.5**	**0.500**	**0.190**	**0.059**	**17**
Picus viridis	0.5	0.167	0.063	0.020	11
Garrulus glandarius	0.5	0.167	0.063	0.020	10
Erithacus rubecula	1	0.333	0.127	0.039	9
Curruca melanocephala	1	0.333	0.127	0.039	8
Passer domesticus	0.5	0.167	0.063	0.020	7
Aegithalos caudatus	1	0.333	0.127	0.039	6
Oriolus oriolus	0.5	0.167	0.063	0.020	5
Cuculus canorus	0.5	0.167	0.063	0.020	4
Hippolais polyglotta	0.5	0.167	0.063	0.020	4
Phylloscopus collybita	0.5	0.167	0.063	0.020	4
Serinus serinus	0.5	0.167	0.063	0.020	4
Streptopelia decaocto	0.5	0.167	0.063	0.020	3
Sylvia cantillans	0.5	0.167	0.063	0.020	3
Corvus cornix	0.5	0.167	0.063	0.020	3
Falco peregrinus	0.5	0.167	0.063	0.020	Nd
Total	25.5	8.5	3.228	1	265

No significant differences emerged between the frequencies of the species obtained with the two methods, with the exception of *Luscinia megarhynchos* and *Aegithalos caudatus* (Table 2). Relative species frequencies, obtained from both the methods, were highly and directly correlated (r = 0.759; $p < 0.001$; Spearman rank correlation test). Diversity indices have been reported in Table 3.

Table 2. Breeding bird species (and their capitalized abbreviations) in the 'Pozzo del Merro' sinkhole (central Italy). Comparison between the two sampling methods (mapping and point counts). Relative frequencies (fr_{MA} and fr_{PC}, respectively) and the number of records (*n*) have been reported. * = $p < 0.05$.

		Mapping		Point Counts			
	Species	fr_{MA}	*n*	fr_{PC}	*n*	χ^2	*p*
TRTR	*Troglodytes troglodytes*	0.157	37	0.149	27	0.08	0.778
SYAT	*Sylvia atricapilla*	0.137	29	0.105	19	0.02	0.881
TUME	*Turdus merula*	0.098	39	0.149	27	0.003	0.953
LUME	*Luscinia megarhynchos*	0.078	23	0.028	5	6.4	0.011 *
PAMA	*Parus major*	0.059	21	0.055	10	0.958	0.328
CYCA	*Cyanistes caeruleus*	0.059	18	0.055	10	0.294	0.588
COPA	*Columba palumbus*	0.059	17	0.055	10	0.15	0.699
PIVI	*Picus viridis*	0.020	11	0.011	2	3.526	0.06
GAGL	*Garrulus glandarius*	0.020	10	0.033	6	0.065	0.798
ERRU	*Erithacus rubecula*	0.039	9	0.055	10	1.195	0.274
CUME	*Curruca melanocephala*	0.039	8	0.061	11	2.467	0.117
PADO	*Passer domesticus*	0.020	7	0.028	5	0.006	0.938
AECA	*Aegithalos caudatus*	0.039	6	0.066	12	5.292	0.021 *
OROR	*Oriolus oriolus*	0.020	5	0.011	2		
MEAP	*Merops apiaster*			0.022	4		
CUCA	*Cuculus canorus*	0.020	4				
HIPO	*Hippolais polyglotta*	0.020	4	0.011	2		
PHCO	*Phylloscopus collybita*	0.020	4	0.006	1		
SESE	*Serinus serinus*	0.020	4	0.011	2		
STDE	*Streptopelia decaocto*	0.020	3	0.006	1		
SYCA	*Sylvia cantillans*	0.020	3	0.006	1		
MOAL	*Motacilla alba*			0.006	1		
COCO	*Corvus cornix*	0.020	3	0.011	2		
REIG	*Regulus ignicapilla*			0.006	1		
FAPE	*Falco peregrinus*			0.022	4		
FATI	*Falco tinnunculus*	0.020	3	0.006	1		
	Total		268		176		

Table 3. Univariate metrics of diversity: comparison between the two sampling methods (mapping and point counts): H′: Shannon–Wiener diversity index; J: evenness; Dm: Margalef normalized richness index; S: number of species (not normalized).

Univariate Metric	**Mapping Method (Original Data)**	**Point Counts [4]**
H′	2.797	2.7
J	0.905	0.84
Dm	3.764	4.64
S	22	25

Comparison of the frequencies with the Whittaker plots showed a comparable trend between methods without significant differences in frequencies ($p > 0.01$; χ^2 test; Figure 2).

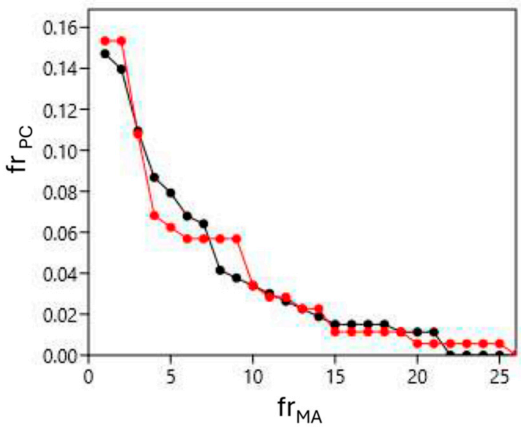

Figure 2. Whittaker plot comparing the relative frequencies of breeding bird species sampled with the two methods (fr_{MA}, black: mapping method; fr_{PC}, red: point counts), ranked in decreasing order.

4. Discussion

All the species recorded are typical of communities inhabiting meso-thermophilous forests and ecotonal environments of central Italy [25]. Similarly to what has already been found with point counts [4], two dominant species (blackbird, *Turdus merula*, and wren, *Troglodytes troglodytes*) have been recorded in the deepest part of the sinkhole (−70 m from the top of the sinkhole). Both species are typical of shady environments with dense undergrowth [26–28].

No significant differences emerged between the relative frequencies of the species obtained with the two methods, except for nightingale (*Luscinia megarhynchos*, higher in the mapping method) and long-tailed tit (*Aegithalos caudatus*, lower in the mapping method). In the first case, the specific characteristics in territorial singing [29] may have led to obtaining a larger number of records with the mapping method (which involves a long sampling time), without interruptions (unlike the point counts method, which has a fixed recording time). In the second case, although the long-tailed tit is very elusive with a barely detectable song [30], it may be easily detected due to its gregarious behavior during the breeding period: this fact may explain the overestimation during point counts sampling, which was conducted on a shorter time span. However, the effect of chance cannot be excluded, and more data will be necessary to explore the differences in detection between these two methods at single-species level.

Our data highlight the ornithological interest of karstic sinkholes. In this regard, it has been highlighted as many of these ecotopes need conservation [31]. At the community level, the comparison of the two methods revealed comparable values in univariate diversity metrics. The Whittaker plots did not show significant differences between paired frequencies, and the correlations between the specific frequencies obtained from the two methods were highly significant. Therefore, on a methodological level, the point counts method is confirmed as an effective mapping method. In fact, taking into account that the point counts method required less than half the time needed to conduct a mapping method (480 min vs. 1200), no significant differences emerged at community level. Therefore, it is advisable in field studies carried out in these extreme and peculiar habitats when time and resources are limited.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16060326/s1, Table S1: Other species recorded in the Pozzo del Merro sink-hole (outside the standard time devoted to the mapping method).

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manuscript. All authors reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: Data are available on request.

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