Cave spider trophic dynamics, network structure and competition: cave zone, taxonomy and bat presence drive hypogean interactions

Yuval Zaltz[1,2], Jordan P. Cuff[3], Efrat Gavish-Regev[2,4]

[1] The Department of Ecology, Evolution and Behaviour, The Institute of Life Sciences, The Hebrew University of Jerusalem (HUJ), Faculty of Sciences, Edmond J. Safra Campus, Givat Ram, Jerusalem 9190401, Israel.

[2] The National Natural History Collections, HUJ, Edmond J. Safra Campus, Givat Ram, Jerusalem 9190401, Israel.

[3] School of Natural and Environmental Sciences, Newcastle University, Newcastle-upon-Tyne, UK.

[4] The Department of Entomology, The Institute of Environmental Sciences, HUJ, The Robert H. Smith Faculty of Agriculture Food and Environment, Rehovot 7610001, Israel.

[+]Corresponding author: Efrat Gavish-Regev: efrat.gavish-regev@mail.huji.ac.il and Yuval Zaltz: yuval.zaltz@mail.huji.ac.il

Abstract

Cave ecosystems are shaped by unique environmental constraints, influencing species distributions and trophic interactions. Spiders are often the dominant predators in caves, but their diet is restricted by local parameters that likely intensify competition and other intraguild interactions, including the confinement of energy flows to detrital and chemoautotrophic pathways. The hypogean environment also filters biodiversity with the progression from cave entrances to complete darkness, further restricting predators' trophic niches, possibly driving dietary specialization and forming truncated, incomplete food webs. Hypogean ecological networks, however, remain scarcely studied, limiting our understanding of the trophic dynamics of these systems.

Here, we explore the dietary interactions of cave-dwelling spiders across 13 caves in the Levant through the integration of dietary metabarcoding, network analyses and multivariate modelling. Spider diets varied between spider families, spider cave affinities (troglophile vs. troglobite), cave ecological zones (entrance, twilight, dark), and caves with and without bats. Spider diets did not simply reflect prey availability in each cave, with some abundant prey absent from spider guts, while rare prey in caves were detected in many spider’s guts. The presence of bat colonies in the caves acted as a primary ecological driver, relating to distinct prey assemblages. We identified complex intraguild spider interactions in caves, with a notable reduction in potential exploitation competition at cave dark zones, suggesting that niche partitioning facilitates coexistence of spiders in these energy-limited underground environments. This study enhances our understanding of spiders as key predators of subterranean habitats and the unique structure of cave food webs.

Keywords: dietary metabarcoding, exploitation competition, high-throughput sequencing, hypergraph, molecular dietary analysis, trophic networks

Introduction

Understanding how energy availability shapes trophic networks and limits biodiversity remains a central quest in community ecology. While most ecosystems on Earth are driven by photo-autotrophic primary production, subterranean realms, as well as deep oceans and other unique environments like glacier forelands (Gravesen et al., 2024) lack primary photo-autotrophic production. Subterranean realms heavily depend on the input of organic matter transported from the surface. In turn, energy flows into caves are confined primarily to detrital and chemoautotrophic pathways, relying on microbial decomposers and invertebrate detritivores to process this input (Chen & Wise, 1999; Kováč, 2018; Por, 2007; Smith et al., 2012; Urák et al., 2025). The hypogean environment also filters biodiversity, especially with the gradual change in abiotic conditions (light, temperature and humidity) from the cave entrance, through twilight, to complete darkness at the deep ecological zones (Cuff et al., 2021). This filtering of predator and prey diversity alters prey abundances (Manenti et al., 2015; Tobin et al., 2013), which may constrain predators' trophic niches, increasing dietary specialization in some cave-dwelling species within distinct guilds (Mammola & Isaia, 2017). The extreme conditions of the hypogean environment may lead to simplified trophic networks, with shorter path lengths between resources and consumers relative to surface networks, resulting in truncated, incomplete food webs (Culver, 1982; Gibert & Deharveng, 2002; Poulson & White, 1969). As the

population dynamics of predators in caves are driven primarily by scarcity of resources, rather than by predation pressure from higher trophic levels, food webs within the hypogean environment may be characterized by bottom-up control (Chen & Wise, 1999; Mammola, 2019; Mammola & Isaia, 2017). This fundamental shift in regulatory mechanisms distinguishes hypogean ecology from epigean ecology, requiring a distinct theoretical understanding of food webs and energy flows in the dark (Mammola, 2019).

Within these truncated hypogean food webs, spiders and other arachnids are common, and frequently constitute the primary predator guild in caves due to the absence of larger vertebrate predators (Mammola, 2019; Mammola & Isaia, 2017; Reddell, 2019). Although most spiders have traditionally been categorized as generalist predators, their diet is frequently restricted to a realized niche by the local environmental constraints, their functional traits and community structure (Cuff et al., 2022; Cuff, Windsor, et al., 2023; Gajski et al., 2024). Therefore, bottom-up control in hypogean environments (Parimuchová et al., 2021) can also result from trophic specialization (stenophagy) toward specific abundant prey taxa (Cardoso, 2012; Cardoso et al., 2011; Pekár & Toft, 2015). In this context, community dynamics are frequently driven by intense competition and other predator-predator interactions (Michalko et al., 2019; Nentwig, 1986; Polis et al., 1989; Wise, 1995) and not directly by resource scarcity. In order to study and understand these processes, hypogean ecological networks must, however, first be reconstructed from observed or detected interactions.

The most fundamental and traditional approach to constructing ecological networks is through direct observation, where interactions are recorded *in situ* (Schoener, 1989). This method often fails to capture cryptic predation events, nocturnal activity, or

interactions involving small body sizes and otherwise elusive invertebrates (Andow & Paula, 2024; Cuff et al., 2022). This limitation is exacerbated in the hypogean environment, making molecular gut content analysis a useful tool for elucidating these interactions (Parimuchová et al., 2021; Symondson, 2002). Methods like dietary DNA metabarcoding identify ingested material from its remaining DNA, extracted directly from consumer guts, faeces or regurgitates (Alberdi et al., 2019; Pompanon et al., 2012). This approach is particularly effective given the long detectability half-life of prey DNA in arachnid digestive tracts (Macías-Hernández et al., 2018; Marker et al., 2026; Simone et al., 2022), providing highly taxonomically resolved detections from trace amounts of prey DNA, for the identification of otherwise unidentifiable predation events (Cuff, Kitson, et al., 2023). Such molecular approaches are particularly critical for deciphering trophic interactions within subterranean habitats, where they remain largely uncharacterized. Parimuchová et al. (2021) studied the trophic interactions of five predators in the Ardovská cave ecosystem in Slovakia using molecular gut content analysis, revealing intraguild predation and prey preferences as key drivers of the interactions (Parimuchová et al., 2021). A broader understanding of hypogean ecology requires wider scientific study of the biotic and abiotic drivers of interactions and wider ecological network complexity, including both intra- and inter-guild interactions, within these subterranean ecosystems.

Here, we studied the ecological interactions of spider communities in 13 Levantine caves using manual surveys and gut content metabarcoding. These caves are characterized by the dominance of predatory spiders that frequently prey on each other (i.e., intraguild predation) and other arthropods. We aimed to elucidate the key drivers of trophic interactions across these understudied food webs by reconstructing trophic

networks and assessing prey selectivity and competition across these networks. Specifically, we asked how the ecological zones of caves, prey availability, bat guano as a source of allochthonous nutrients, and predator taxonomy, hunting guild and cave affinity (i.e., troglobite vs. troglophile) affect trophic interactions. We hypothesized that trophic interactions, prey selectivity and competition among cave-dwelling spiders will be driven by both cave (ecological zone, bat presence), and spider characteristics (cave affinity, hunting guild, taxonomy). Elucidating the drivers of hypogean interactions and trophic network structure is key to understanding how biodiversity is structured and maintained in isolated, resource-poor environments.

Methods

Study region

The Levant region, situated at the junction of Europe, Asia, and Africa, encompasses a rich diversity of subterranean formations carved in basalt, sandstone, limestone, marl, chalk, and salt (Frumkin, 1994; Frumkin & Fischhendler, 2005; Frumkin & Naor, 2019; Sivan et al., 2016). These Levantine caves host specialized spider assemblages whose composition is shaped by local abiotic factors including precipitation, elevation and latitude, and biotic inputs such as bat guano deposition (Cuff et al., 2021; Frumkin, 2017; Gavish-Regev et al., 2021; Por et al., 2001).

Field sampling and specimen collection

Spiders were sampled from 13 caves in the Levant to study their diet composition and trophic interactions across cave zones, cave types and between spider groups. A total of 115 spider specimens were hand-collected from 13 caves across five regions: Golan, Galilee, Karmel, Samaria, and Judea, during two field surveys in 2021 and 2022 (Figure 1). For each specimen, taxonomic and sampling information (species, collection date, cave name and type, ecological zone of the cave and geographical region) were recorded.

The first survey was conducted in August and September 2021 ($n_{spiders} = 53$) in Yir'on, Sharakh, Bet Jan, Sefunim, Bet A'rif, Tinshemet and Te'omim caves. Sampling followed a standardized quadrat methodology: in each twilight and dark ecological zone of each cave, arthropods were recorded within 0.5 X 0.5 m plots representing different micro-habitats (e.g., cave floor center, floor near wall, on the wall). Each plot was visually scanned for five minutes by two experts. Spiders from these surveys were collected live and killed by freezing at -80 °C, where they were stored before the current analysis. Both the detailed methodology and the prey data resulting from these specific surveys were published by Ganem et al. (2026).

The second survey was conducted in May and July 2022 ($n_{spiders} = 62$) in Yir'on, Irav, Namer, Sharakh, Beit-Jan, Seter, Neta, and Capara caves. This sampling was performed via opportunistic hand collection across all three ecological zones: entrance, twilight and dark (Aharon et. al, in preparation). These specimens were initially preserved in 75% ethanol at room temperature, and then dried, and stored at -80 °C prior to DNA extraction.

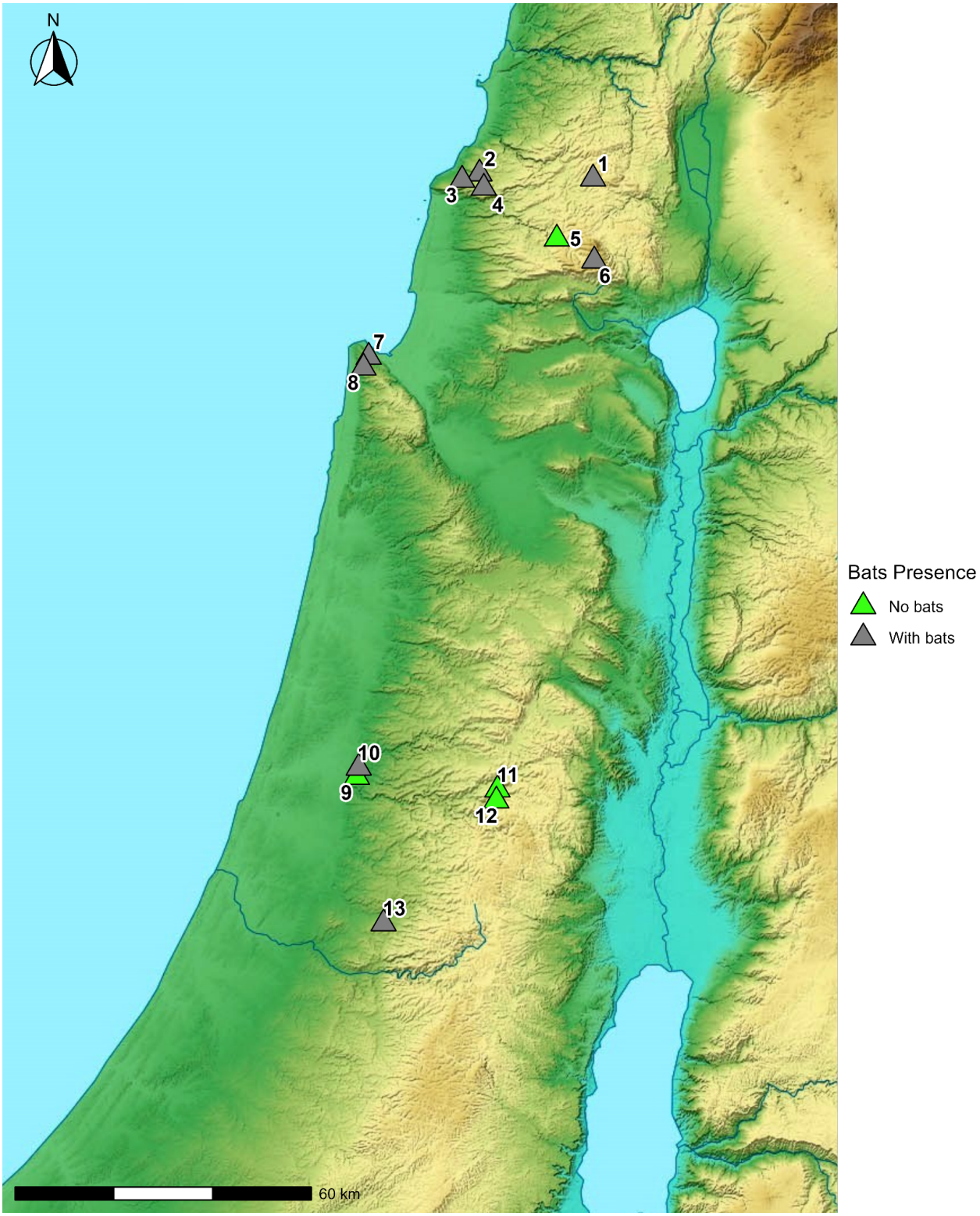


Figure 1: Map of the study area showing the locations of the sampled caves. The map displays all sites surveyed during 2021 and 2022. The sites: (1) Yiron, (2) Irav, (3) Namer, (4) Sharakh, (5) Beit-Jan Shfanim, (6) Seter, (7) Ornit, (8) Sefunim, (9) Bet Arif, (10)

Tinshemet, (11) Neta, (12) Capara, and (13) Teomim. Grey and green points denote caves with and without bats, respectively.

Table 1: The caves surveyed and their associated metadata.

Locality Number	Locality Name	Latitude	Longitude	Bat presence
1	Yiron	33.081	35.47323	With bats
2	Irav	33.07999	35.22852	With bats
3	Namer	33.07862	35.19104	With bats
4	Sharakh	33.07401	35.2378	With bats
5	Beit-Jan Shfanim	32.96153	35.39515	No bats
6	Seter	32.94179	35.47587	With bats
7	Ornit	32.75663	34.98974	With bats
8	Sefunim	32.73702	34.97843	With bats
9	Bet Arif	32.0026	34.9642	No bats
10	Tinshemet	31.9994	34.9681	With bats
11	Neta	31.95965	35.26722	No bats
12	Capara	31.95938	35.2649	No bats
13	Teomim	31.7262	35.0217	With bats

Molecular analysis

Sample processing and DNA extraction were performed at The Robert H. Smith Faculty of Agriculture Food and Environment, The Hebrew University of Jerusalem, following a protocol established for this study (Zaltz et al., 2023). Experimental controls were used at each stage of the molecular analysis, including extraction negatives, PCR negatives, PCR blanks and positive controls. All of these controls (except for the blank controls) were pooled for sequencing and the resultant data were used to define minimum sequence copy thresholds to filter false positives from the final dataset, as per Drake et al., (2022) A Qiagen DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) was used for DNA

extraction, following manufacturer protocols. A negative control was included for each batch of extractions, which comprised all of the extraction reagents with no input DNA. Prior to extraction, spider abdomens were carefully separated from the prosoma with bleach-sterilised tools to maximize the proportion of prey DNA relative to predator DNA (Macías-Hernández et al., 2018). DNA libraries were prepared for high-throughput Illumina sequencing using a two-step PCR approach. In the first PCR, a 229 bp fragment of the COI gene was amplified using the primers LCO1490 (Vrijenhoek, 1994) and 230R (Gibson et al., 2015) with 8 base pair tags according to Taberlet et al. (2018), variable length heterogeneity spacers and Nextera-compatible overhangs on the 5' end of each primer. Primer tags were organised so that each well of a 96-well plate had a unique combination of forward and reverse tags for attribution of sequencing data to each sample during bioinformatics. The primer pair used has previously been identified to have high coverage and low bias for terrestrial invertebrates (Elbrecht et al., 2019). Reactions of 25 µL contained 12.5 µL Qiagen PCR Multiplex kit, 0.2 µmol of each primer, and 1 µL of template DNA, with the remaining volume made up with molecular biology grade water. Reactions comprised 15 min at 95 °C, followed by 35 cycles of: 95 °C for 30 s, 52 °C for 90 s, and 72 °C for 90 s; followed by a final extension at 72 °C for 10 min. All PCR plates contained extraction and PCR negative controls (n = 12, water or extraction negative elution in lieu of DNA, with a negative control in each row and column), blank controls (n = 2, additional negative controls not included for sequencing) and positive controls (n = 2, a mixture of previously extracted non-target vertebrate DNA, e.g., horse). Successful amplification was confirmed via gel electrophoresis on a 2% agarose gel with SYBRSafe gel stain. PCR products were purified with a 1X ratio left-side bead clean with solid phase

reversible immobilisation (SPRI) beads at 0.1X bead concentration to normalise the concentration of amplicons recovered from each sample. All experimental controls except for blank controls were included in this normalisation and pooling, irrespective of a lack of amplification for negative controls, to ensure representation of any low prevalence false positives in subsequent data filtering. Equal volumes of these normalised PCR products were pooled per plate (n = 3 plates) ahead of a Nextera index PCR carried out to attach dual combinatorial indices and Illumina sequencing adapters. This second PCR followed the reagent and cycle conditions above, but for 12 cycles. The pooled libraries were then purified once again using a 1X ratio of SPRI beads. The final libraries were quantified and sequenced on an Illumina NextSeq 2000 platform using P1 Reagents (600 cycles) to generate paired-end reads at The Center for Genomic Technologies at The Hebrew University of Jerusalem.

Bioinformatics

Adapter and primer sequences were removed, and reads demultiplexed and assigned to samples, using cutadapt (Martin, 2011) with a maximum error rate of 15% and no insertions or deletions permitted in tag sequences. Sequences were then filtered, trimmed, denoised and assigned to amplicon sequence variants (ASVs) using DADA2 (Callahan et al., 2016). Taxonomic identities were assigned to ASVs via BLASTn with a custom database of animal COI sequence data extracted from both GenBank and BOLD. The taxonomic resolution of assignments was adjusted based on percent identity, with non-spider taxa being assigned to family level for percent identities over 90%, order for 80-89% and phylum otherwise. More resolved taxonomic levels were excluded due to the

prevalence of false identifications, likely arising due to the highly endemic and poorly described fauna of these caves. For spider detections, in order to ascribe detections to the focal predator of each sample, genus-level assignments were also permitted for a percent identity of over 95%.

Samples with fewer than 1,000 reads following taxon assignment were removed (n = 4). Some samples were repeated due to some poor performance in the first PCR, but these repeats were not ultimately used to maintain consistent PCR conditions and in light of high frequencies of false positive reads that required highly stringent minimum sequence copy thresholds which removed most detections (Drake et al., 2022). Order-level assignments of Araneae and sequences identified as belonging to the same family or genus as the focal predator were removed from their respective samples following all other data processing steps given the likelihood of these reads arising from the focal predator (Cuff, Kitson, et al., 2023). Minimum sequence copy thresholds were used to limit the prevalence of false positives in the final data, which were based on detections in the negative and blank controls, and detection of positive control taxa in samples. Where reads for a given taxon were found in negative or blank controls, any read counts equal to or less than those in the controls for that taxon were removed from samples, and a percentage sample read depth threshold was defined based on the maximum percentage sample read depth attributed to detection of positive control taxa (e.g., horse) or likely false positives (e.g., marine taxa), as per Drake et al., (2022). All detections were represented as binary presence/absence data given the inconsistently reliably quantitative data associated with metabarcoding (Deagle et al., 2019; Lamb et al., 2019).

Statistical analysis

All analyses were conducted using R v4.5.2 (R Core Team, 2025). Reproducible data processing was carried out with 'tidyverse' (Wickham et al., 2019) and data visualisation used 'ggplot2' (Wickham, 2016). The trophic interactions detected were compared between cave ecological zones (i.e., entrance, twilight, dark), spider families, cave affinities (i.e., troglobite vs. troglophile), hunting guilds (i.e., active vs. web-building) and the presence of bats or not within caves using multivariate generalized linear models (MGLMs) via the 'manyglm' command of 'mvabund' (Wang et al., 2012) with a binomial error family. Overall and univariate (i.e., prey-specific) effects were extracted by likelihood ratio test with Monte Carlo resampling. Coarse dietary differences were visualised by non-metric multidimensional scaling (NMDS) with Jaccard dissimilarity and 999 tries in 'vegan' (Oksanen et al., 2025). Prey availability based on the quadrat survey conducted in 2021 was compared between cave zones and caves with and without bats via MGLM as for the dietary data, but with a 'Poisson' error family. Data were similarly visualised by NMDS with Bray-Curtis dissimilarity. Dietary data were analysed against prey availability using null network models via the 'generate_null_net' command of 'econullnetr' (Vaughan et al., 2018) with 999 simulations.

To assess how competition between spiders may be driven by biotic (e.g., prey availability) and abiotic (e.g., light intensity) gradients within caves, we quantified potential exploitation competition between each co-occurring spider species pair based on their second order interactions (i.e., prey sharing). Second order spider-prey-spider interactions were noted when spiders shared the same prey in the same cave zone and cave, providing a proxy for competition for resources, as per Tercel et al. (2026). These

second order interactions were used to construct hypergraphs (networks in which links join more than two nodes, in this case linking predators and their spatially explicitly shared prey). Zonal meta-hypergraphs (hypergraphs constructed for each cave zone with data pooled across caves) were constructed and visualised to summarise the competitive hypergraphs using 'igraph' (Csardi & Nepusz, 2006), 'ggnetwork' (Briatte, 2021) and 'scatterpie' (Yu, 2021). The frequency of the competitive interactions was considered the number of shared interactions (i.e., if one spider taxon, which can represent multiple individuals, consumed a specific prey taxon three times, and another spider consumed that same prey four times, the frequency was considered three). The richness of the competitive interactions was considered the number of prey species featured in competitive interactions between each spider pair. Both competitive interaction frequency and richness were normalised relative to overall interaction frequency and richness (i.e., proportional values were calculated) and these normalised values were compared between cave zones using beta regression with 'betareg' (Cribari-Neto & Zeileis, 2010).

Exemplary local trophic network

To highlight how broader patterns translate to local scales, we selected Sharakh Cave for constructing a focal exemplary trophic network. Sharakh Cave represents the largest number of spiders and spider-prey interactions in our dataset. We visualised this network using 'igraph' (Csardi & Nepusz, 2006) and 'ggraph' (Pedersen, 2024) with a Fruchterman-Reingold force-directed layout to optimise node distribution (Fruchterman & Reingold, 1991). To ensure network parsimony and avoid taxonomic redundancy, lower

resolution nodes were removed (i.e., family level or coarser identifications were removed if constituent genera or species were simultaneously present).

Results

Data summary

A total of 24,554,470 reads were generated during sequencing, of which 20,252,533 were attributed to samples and controls used in this study. Following bioinformatic processing, 12,564,090 reads were retained across 142 spider samples (including repeat samples which were subsequently excluded) and controls, with 6,222,493 reads attributed to the spider samples, resulting in 36,389 ∓ 81,610 reads per sample, of which an average of 24.0% ∓ 39.0% (0-100%; Figure S1) were attributed to the focal spider of each sample, a commonly reported problem in predator-prey dietary analyses (Cuff et al., 2023). Following application of minimum sequence copy thresholds, a final 86 spider samples remained from 13 caves, with 264 interactions detected with 70 prey taxa. During prey surveys across the caves, 3515 individuals across 31 taxa were identified, resulting in 251 ∓ 155 individuals per cave zone surveyed (Ganem et al., 2026).

Dietary comparison between spider groups

Spider diets significantly related to cave zone (MGLM: $Dev_{3,82}$ = 371.2, p < 0.001; Figure 2), spider family (MGLM: $Dev_{6,76}$ = 252.5, p < 0.001; Figure 2), bat presence (MGLM: $Dev_{1,75}$ = 204.8, p < 0.001; Figure 2) and spider cave affinity (MGLM: $Dev_{1,74}$ = 61.0, p < 0.001; Figure 2), but not spider hunting guild (MGLM: $Dev_{1,74}$ = 0.0, p = 0.411). Specifically, the consumption of nine Diptera families, Phoridae (MGLM: $Dev_{3,82}$ = 18.6, p = 0.004), Psychodidae (MGLM: $Dev_{3,82}$ = 22.7, p = 0.002), Sciaridae (MGLM: $Dev_{3,82}$ = 16.9, p = 0.006), Fanniidae (MGLM: $Dev_{3,82}$ = 15.4, p = 0.018), Nycteribiidae (MGLM: $Dev_{3,82}$ = 15.4, p = 0.017), Ceratopogonidae (MGLM: $Dev_{3,82}$ = 14.6, p = 0.024), Chloropidae (MGLM: $Dev_{3,82}$ = 14.6, p = 0.024), Drosophilidae (MGLM: $Dev_{3,82}$ = 17.7, p = 0.006) and Heleomyzidae (MGLM: $Dev_{3,82}$ = 17.7, p = 0.006), significantly differed between zones. The consumption of three Diptera families, Psychodidae (MGLM: $Dev_{1,75}$ = 13.4, p = 0.009), Culicidae (MGLM: $Dev_{1,75}$ = 11.1, p = 0.027) and Dixidae (MGLM: $Dev_{1,75}$ = 19.0, p = 0.002), significantly differed between caves with and without bats. The consumption of individuals of the funnel-web spider genus *Tegenaria*, as a prey by spiders, significantly differed between spider cave affinities (i.e., troglophiles vs. troglobite spiders; MGLM: $Dev_{1,74}$ = 19.4, p = 0.001).

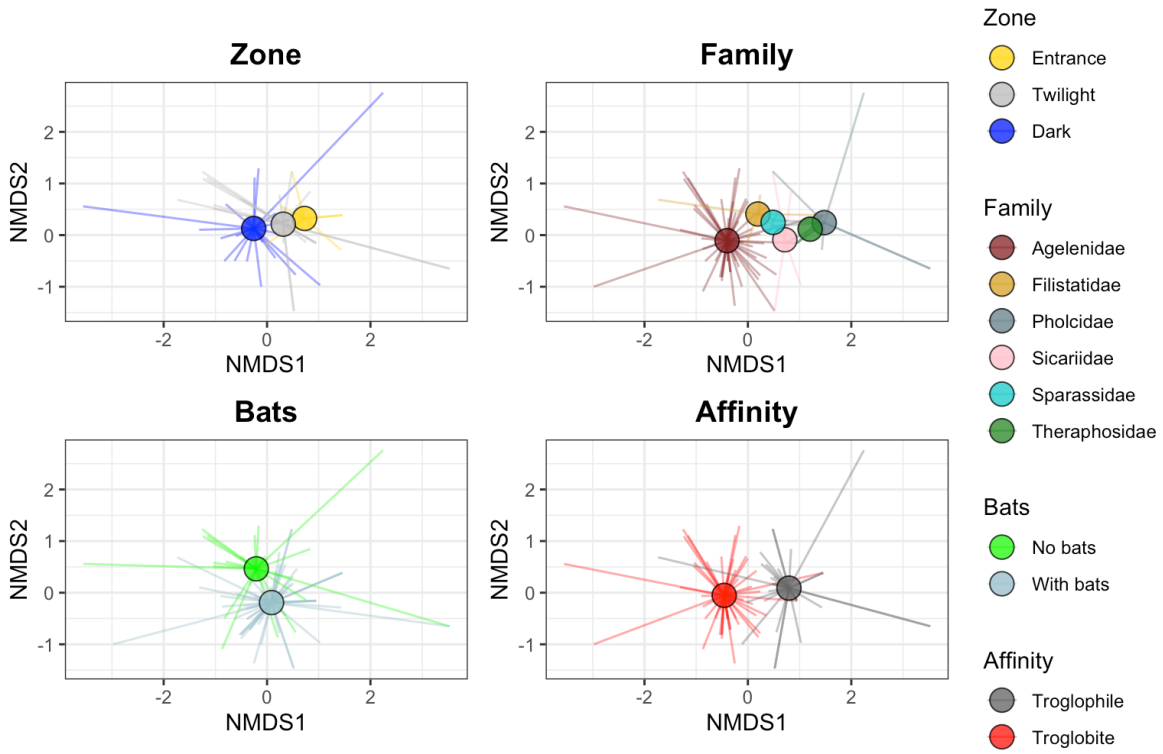


Figure 2: Plot of spider diets across cave zones, caves with and without bats, spider families and spider cave affinities, showing differences between individual (open terminal end of lines) and group average diets (large nodes, mean coordinates of the samples for each group). Stress = 0.075.

Prey availability as a driver of interactions

Prey communities significantly related to cave zone (MGLM: $Dev_{1,11} = 57.00$, $p < 0.001$; Figure 3), bat presence (MGLM: $Dev_{1,12} = 36.96$, $p = 0.019$; Figure 3) and the interaction between zone and bat presence (MGLM: $Dev_{1,10} = 16.85$, $p = 0.039$). Specifically, the abundance of Araneae (MGLM: $Dev_{1,11} = 12.10$, $p = 0.004$) and Blattodea (MGLM: $Dev_{1,11} = 8.46$, $p = 0.027$) significantly differed between cave zones.

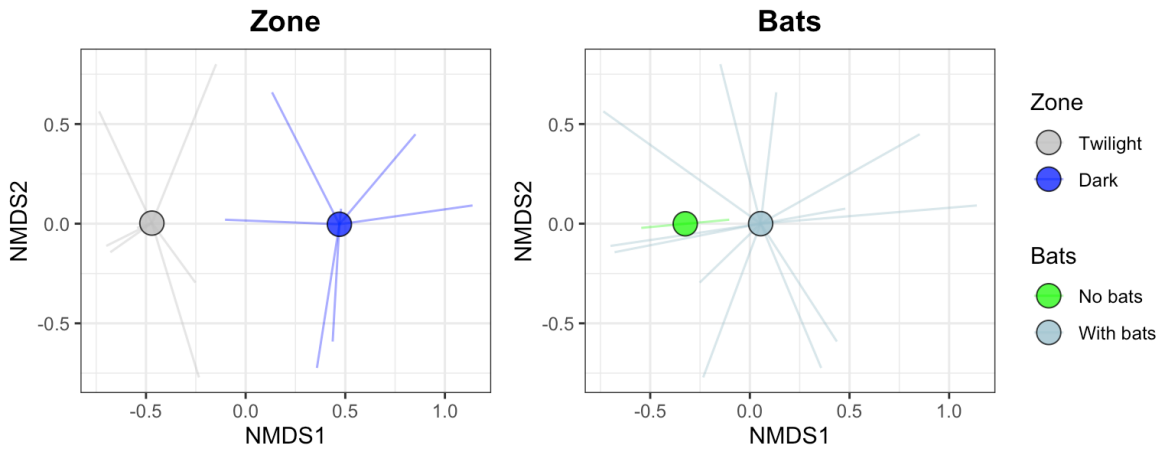


Figure 3: Plot of prey communities across cave zones, and caves with and without bats, showing differences between individual (open terminal end of lines) and group average communities (large nodes, mean coordinates of the samples for each group). Stress = 0.164.

The potential prey selectivity of spiders significantly deviated from null network models based on prey availability data. Specifically, spiders in dark zones consumed nine prey taxa more frequently than expected and two prey taxa less frequently than expected (Table 2; Figure 4). Spiders in twilight zones consumed four prey taxa more frequently than expected but no prey taxa less frequently than expected (Table 2; Figure 4). Spiders of the family Agelenidae consumed nine prey taxa more frequently than expected and two prey taxa less frequently than expected (Table S1; Figure S2). Spiders of the family Filistatidae consumed two prey taxa more frequently than expected and one prey taxon less frequently than expected (Table S1; Figure S2). Spiders of the family Pholcidae consumed one prey taxon more frequently than expected and no prey taxa less frequently than expected (Table S1; Figure S2). Spiders of the family Sicariidae consumed two prey taxa more frequently than expected and no prey taxa less frequently than expected (Table S1; Figure S2). Spiders of the family Theraphosidae consumed one prey taxon more frequently than expected and no prey taxa less frequently than expected (Table S1;

Figure S2). Spiders in caves with bats consumed five prey taxa more frequently than expected and two prey taxa less frequently than expected (Table S1; Figure S3). Spiders in caves without bats consumed two prey taxa more frequently than expected and no prey taxa less frequently than expected (Table S1; Figure S3). Troglobiont spiders consumed nine prey taxa more frequently than expected and three prey taxa less frequently than expected (Table S1; Figure S4). Troglophile spiders consumed four prey taxa more frequently than expected and one prey taxon less frequently than expected (Table S1; Figure S4).

Table 2: Significant null network model outputs indicating predation at higher or lower frequencies than expected across cave zones for each prey taxon. SES = standardised effect size. Other predator groups are represented in Table S1. Entrance zones are not represented due to a lack of prey availability data. Where SES is 'NA', the number of prey available for that taxon was 0, meaning SES could not be calculated.

Spider group	Prey taxon	Observed frequency of interactions	Null expectation of interaction frequency	Deviation from expected frequency	SES
Dark zone	Araneae	4	8.97	Less frequent	-2.96
Dark zone	Blattodea	1	0	More frequent	NA
Dark zone	Diptera	11	6.25	More frequent	3.35
Dark zone	Ephemeroptera	2	0	More frequent	NA
Dark zone	Hemiptera	1	0	More frequent	NA
Dark zone	Isopoda	0	5.31	Less frequent	-3.52
Dark zone	Mesostigmata	1	0	More frequent	NA
Dark zone	Orthoptera	1	0.01	More frequent	11.90
Dark zone	Plecoptera	1	0	More frequent	NA
Dark zone	Scorpiones	1	0	More frequent	NA
Dark zone	Trichoptera	1	0	More frequent	NA
Twilight zone	Ephemeroptera	1	0	More frequent	NA
Twilight zone	Hymenoptera	3	0.58	More frequent	3.37
Twilight zone	Orthoptera	5	0	More frequent	NA
Twilight zone	Trichoptera	1	0	More frequent	NA

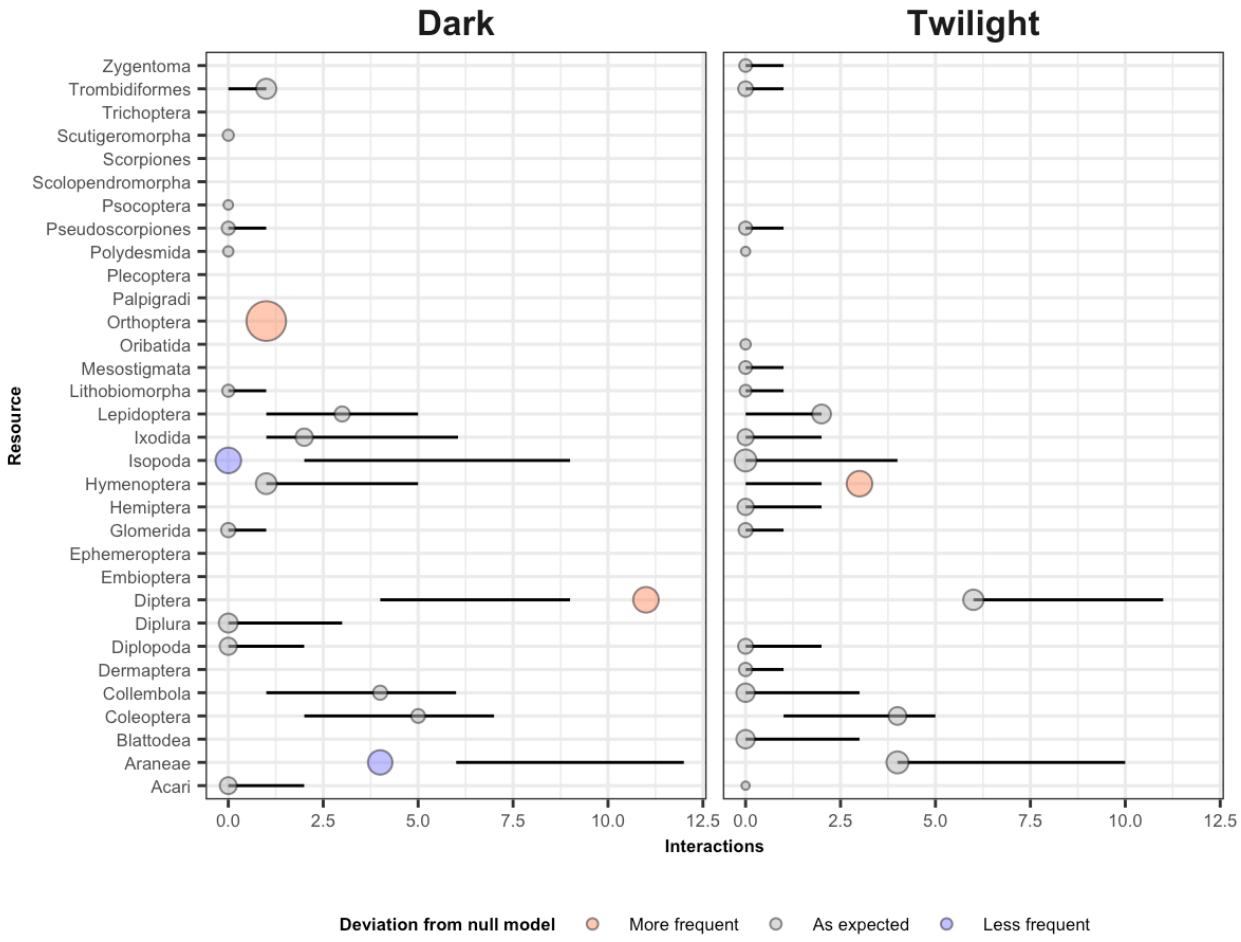


Figure 4: The frequency of predation of each prey taxon (points) deviates from the frequencies expected by null network model simulations (horizontal lines representing 95% confidence intervals of 1000 simulations) for spiders in dark and twilight zones. Several interactions occurred significantly more (orange) or less (purple) frequently than expected, whereas most aligned with null expectations (grey). Point size denotes standardised effect sizes of the deviations of observed interaction frequencies from null models. Absent points are those for which data were not available. Entrance zones are not represented due to a lack of prey availability data.

Cave trophic networks analysis and food webs

Competitive interactions (i.e., prey shared by two or more spiders in the same cave and zone) accounted for 55.4% of spider trophic interactions. The prey central to these competitive interactions represented 58.6% of the total prey richness. The proportional

richness of competitive interactions did not, however, significantly differ between zones, nor did their proportional frequency (Figure 5).

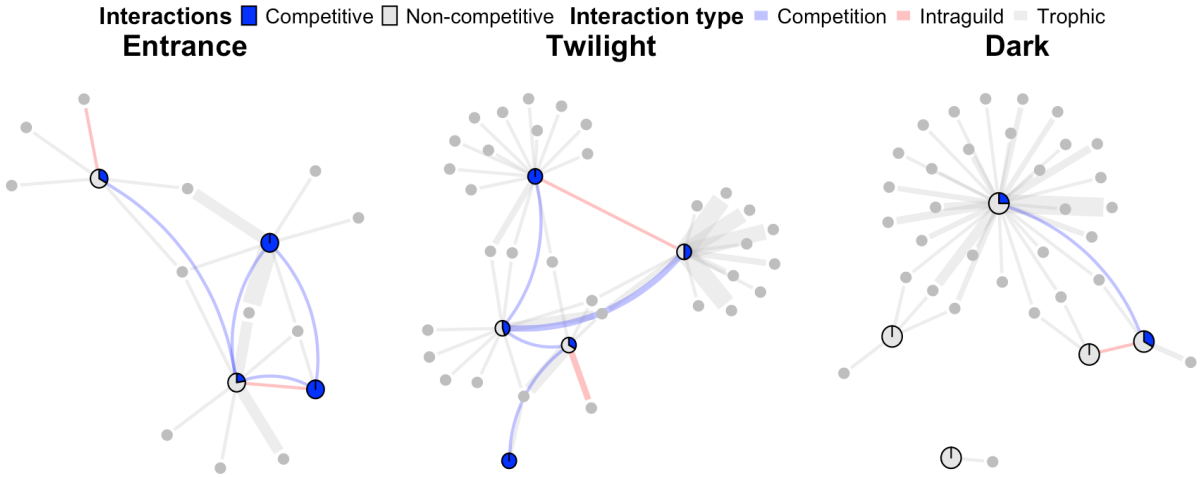


Figure 5: Networks of direct spider-prey (trophic; straight grey links) and spider-spider (intraguild; straight red links), and indirect second order spider-spider (competition; curved blue links) interactions. Pie charts represent spider nodes, with the ratio of blue to grey denoting the ratio of competitive (i.e., shared within a specific cave zone) to non-competitive (i.e., exclusive) interactions, respectively.

Spotlighting Sharakh cave trophic network

The trophic network of Sharakh Cave exemplifies a complex food web of interactions dominated by several spider species (Figure 6). In the dark zone, *Tegenaria trogalil* Aharon & Gavish-Regev, 2023 (five individuals included in the molecular gut content analysis), a troglobitic funnel-web spider, consumed the richest diet, comprising 16 arthropod taxa (Ephemeroptera, Psychodidae, Hybotidae, Plecoptera, Curculionidae, Entomobryomorpha, Hemiptera, Limnephilidae, Hymenoptera, Trombidiformes, Symphypleona, Sciaridae, Ascidae, Diptera, Noctuidae, and Tettigoniidae). Other prominent spiders exhibit taxonomically narrow diets. The space-web weaver

Hoplopholcus cecconii Kulczyński, 1908 (five individuals included in the molecular gut content analysis), and the sensing-web spider *Filistata insidiatrix* (Forskål, 1775) (four individuals included in the molecular gut content analysis), both found in the entrance and twilight zones in this cave, fed on only two insect taxa (Tettigoniidae and Formicidae), and five insect taxa (Mycetophilidae, Anthomyiidae, Lepidoptera, Noctuidae, and Tettigoniidae), respectively. One individual of the ambush-hunter spider *Loxosceles rufescens* (Dufour, 1820), found exclusively in the dark zone in this cave, consumed three insect taxa (Crambidae, Geometridae, Lepidoptera) and the spider *F. insidiatrix* (intraguild predation). Our results also demonstrate intraguild predation between *F. insidiatrix* and *H. cecconii* across cave zones.

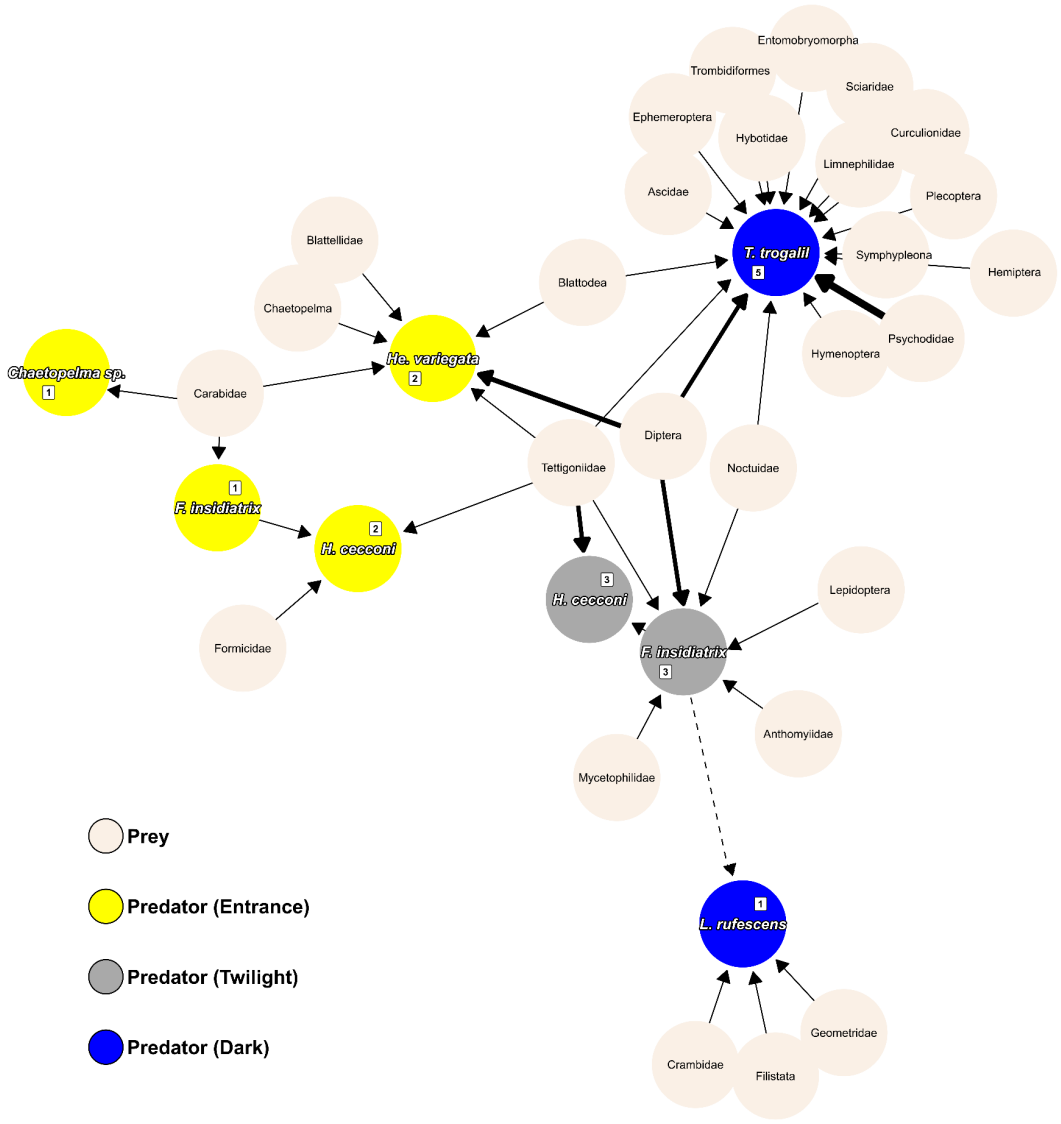


Figure 6: Spider-prey network of Sharakh Cave representing predation events detected by DNA metabarcoding, with edge thickness proportional to the number of predation events detected. Dashed lines represent trophic interactions occurring across different zones of the cave.

Discussion

This study elucidates the structure of spider-dominated trophic networks and competitive interactions across 13 Levantine caves. Our findings reveal that cave spider diets and

their interactions do not simply reflect prey availability nor broad generalist strategies, but rather represent a restricted realized niche driven by a combination of cave zone, bat presence, spider family, spider cave affinity, prey availability and competition.

Spiders are typically considered generalist predators, and we expected their diet to closely reflect the relative abundance of available prey as was previously shown in epigeic habitats (Cuff et al., 2024; Deagle et al., 2019; Michalko et al., 2019; Nyffeler, 1999; Wise, 1995). However, we found cave spiders to exhibit distinct patterns of potential selectivity for specific prey taxa relative to their local availability, and these deviations varied across cave zones. While spiders maintained diverse and rich diets within the caves studied, some spider species may be more specialized, potentially avoiding specific prey (e.g., Agelenidae spiders consuming fewer isopods than expected). Prey avoidance (Nentwig, 1986) and distinct trophic specialization (Pekár & Toft, 2015) have been documented in various spider families in epigean habitats, including the Zodariidae and Dysderidae, but evidence of avoidance and distinct trophic specialization within the subterranean realm has been lacking. Whilst we did not explicitly investigate prey specialization, we did detect higher than expected consumption of Diptera by Agelenidae, Lepidoptera by Filistatidae, and Hymenoptera by Sicariidae, but lower than expected consumption of Isopoda and Araneae by Agelenidae and Araneae by Filistatidae, suggesting taxonomically distinct prey preferences and avoidances.

It is thought that spider dietary variation is shaped by multiple interacting factors rather than a single driver (Cuff et al., 2022). In caves, local environmental conditions, such as the specific cave zone and the presence of bat colonies, dictate the structure and composition of the prey community (Ferreira & Martins, 1999; Mammola, 2019).

Simultaneously, the inherent traits of the predator, including its taxonomy and cave adaptation (troglophiles vs. troglobites), determine its foraging strategies and realized niche (Cardoso et al., 2011; Mammola & Isaia, 2017). Indeed, our results demonstrate that spider diet composition is significantly affected by both cave characteristics (cave zone and bat presence) and spider traits (family and cave affinity).

In resource-limited habitats, predators are often forced to exploit similar prey, resulting in higher dietary overlap and exploitation competition (Costa-Pereira et al., 2019; Tercel et al., 2026). For example, in epigean ecosystems, declines in prey abundance have been experimentally shown to intensify interspecific competition and dietary overlap among co-occurring spider species (Spiller, 1984). Declines in overall prey availability may lead to increased potential competition among predators (Schoener, 1974). Earlier studies suggest that prey availability, considering both abundance and species richness, is lower in caves compared to epigean habitats, and also lower in the dark zone compared to the twilight zone of caves (Mammola, 2019; Poulson & White, 1969). Yet, our network analysis, based on prey richness, revealed no significant difference in competitive interactions between spiders in the three cave ecological zones we tested (entrance, twilight and dark zones). This similar level of potential competition across zones implies that other processes like niche partitioning facilitate co-existence in these energy-limited underground environments, a mechanism consistent with broader ecological theories regarding how species avoid competitive exclusion (Polis, 1984; Schoener, 1974). Alongside niche partitioning, intraguild predation is highly common and taxonomically widespread across spider communities, as many generalist spiders

regularly prey on other spider species (Pekár & Toft, 2015), a known driver of population dynamics and community structure (Polis et al., 1989; Spiller, 1984).

Although subterranean ecosystems are often characterized by truncated food webs (Culver, 1982; Gibert & Deharveng, 2002), the trophic network of Sharakh Cave demonstrates that these networks can still maintain high interaction complexity. This complexity is driven by dense interactions among the dominant predators, specifically representatives of the spider families Agelenidae, Pholcidae, Filistatidae, and Sicariidae. These families utilize diverse foraging modes, yet they all function as generalist predators. Agelenidae and Pholcidae are considered broad generalists (Nentwig, 1983; Pekár & Toft, 2015). Similarly, predation experiments indicate that crevice weavers (Filistatidae) function as generalist predators. Ambush hunters (Sicariidae) also exhibit generalist tendencies, acting as opportunistic predators that consume a wide variety of available ground-dwelling arthropods (Magalhaes et al., 2017; Souza-Silva & Ferreira, 2014). In the dark zone of Sharakh Cave, the funnel-web spider *T. trogalil* (Agelenidae), a troglobitic spider, consumed the richest diet, comprising 16 arthropod taxa. Other prominent spiders exhibited taxonomically narrower diets relative to *T. trogalil*. The space-web weaver *H. cecconii* (Pholcidae), found across the entrance and twilight zones, fed on only two insect taxa alongside intraguild predation on the sensing-web spider *F. insidiatrix* (Filistatidae), that was detected feeding on five insect taxa. One individual of the ambush-hunter spider *L. rufescens* from the dark zone consumed two insect taxa and spiders from the species *F. insidiatrix* (intraguild predation). Our results also demonstrate intraguild predation between *F. insidiatrix* and *H. cecconii* across cave zones.

By uncovering mechanisms such as niche partitioning and intraguild predation, we reveal how these subterranean food webs persist, even under potentially severe energy constraints due to resource limitation. These insights contribute directly to our broader understanding of cave ecosystems, spider community functioning and the processes driving hypogean biodiversity, with potential implications for conservation and biodiversity management (Mammola et al., 2019). Ultimately, these findings provide a unique expansion of our understanding of subterranean predator-prey dynamics, while also demonstrating the potential for molecular gut content analysis to untangle subterranean trophic interactions and ecological network structure.

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Author contributions

Yuval Zaltz: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; visualisation; writing - original draft; writing - review and editing. Jordan P. Cuff: Conceptualization; data curation; formal analysis; investigation; methodology; visualisation; writing - review and editing. Efrat Gavish - Regev: Conceptualization; data curation; funding acquisition; investigation; methodology; project administration; supervision; writing - original draft; writing - review and editing.

Data availability

All data and analysis scripts are openly available via Zenodo: https://doi.org/10.5281/zenodo.23548449

Conflict of interest

The authors declare no competing financial or personal conflicts of interest.

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Supplementary Information

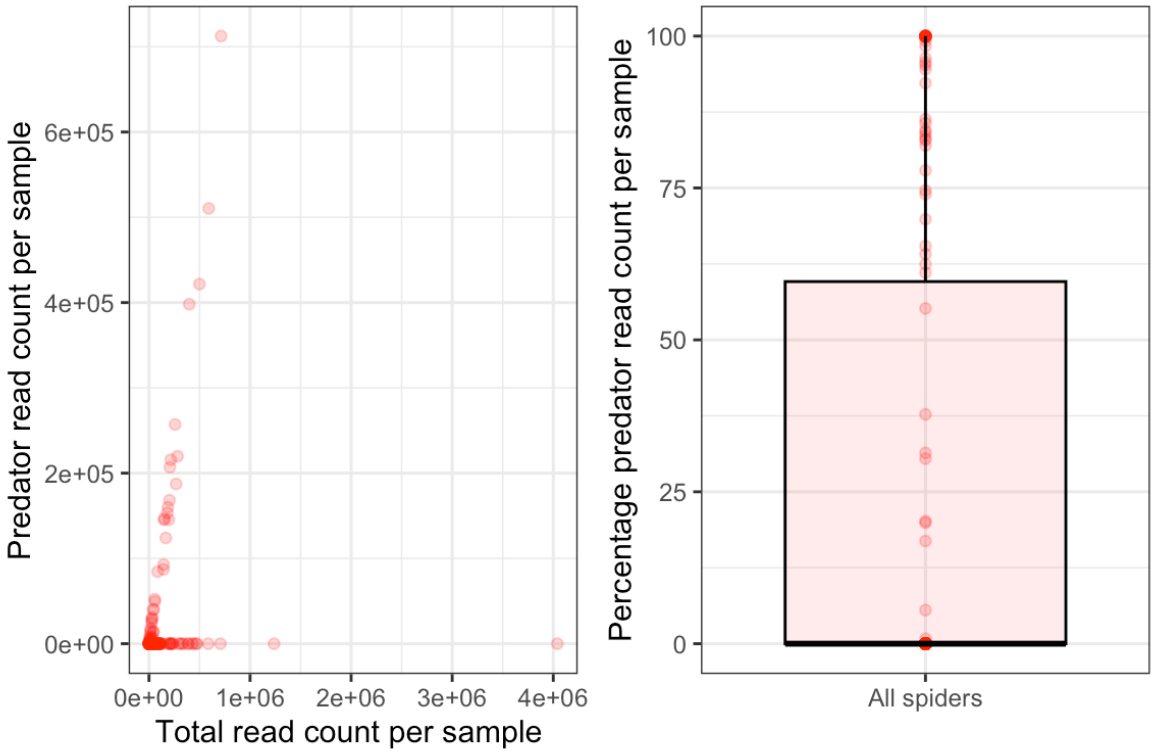


Figure S1: The proportion of metabarcoding data assigned to the focal predator of each sample. Of the 36,389 ∓ 81,610 reads assigned per sample, a mean of 8.62% ∓ 14.1% (0-100%) were attributed to the focal spider itself.

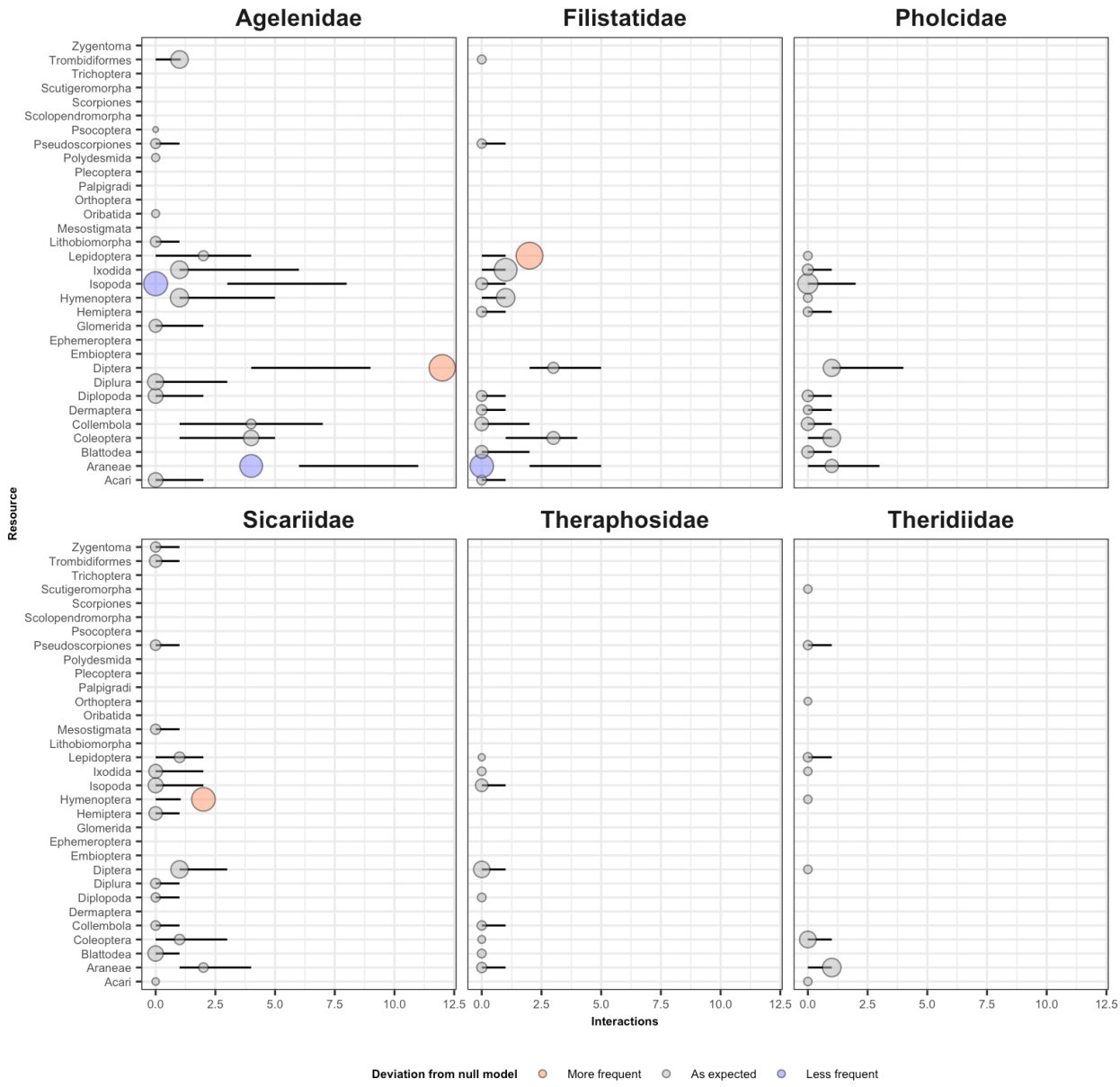


Figure S2: The frequency of predation of each prey taxon (points) deviates from the frequencies expected by null network model simulations (horizontal lines representing 95% confidence intervals of 1000 simulations) for spiders of different families. Several interactions occurred significantly more (orange) or less (purple) frequently than expected, whereas most aligned with null expectations (grey). Point size denotes standardised effect sizes of the deviations of observed interaction frequencies from null models. Absent points are those for which data were not available.

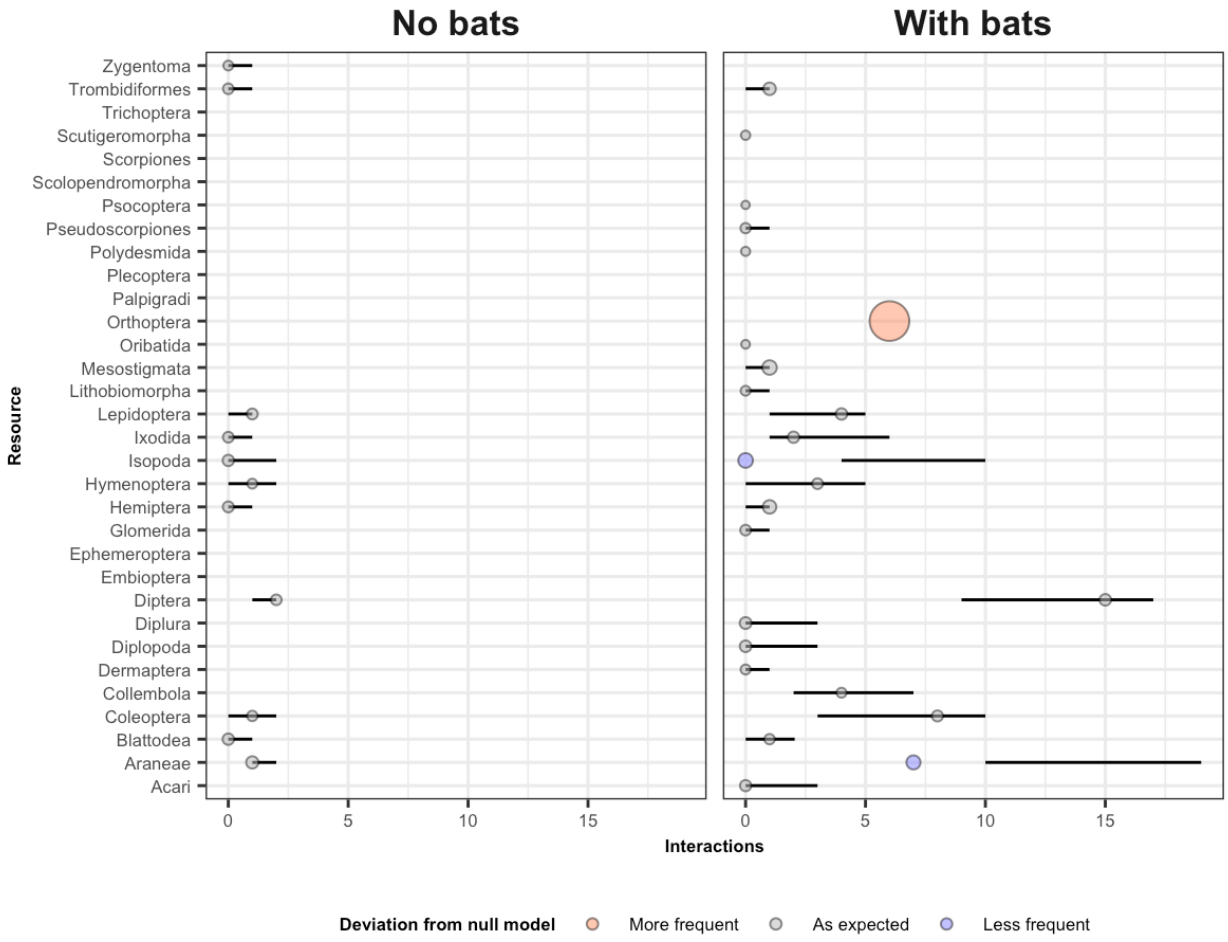


Figure S3: The frequency of predation of each prey taxon (points) deviates from the frequencies expected by null network model simulations (horizontal lines representing 95% confidence intervals of 1000 simulations) for spiders in caves with and without bats. Several interactions occurred significantly more (orange) or less (purple) frequently than expected, whereas most aligned with null expectations (grey). Point size denotes standardised effect sizes of the deviations of observed interaction frequencies from null models. Absent points are those for which data were not available.

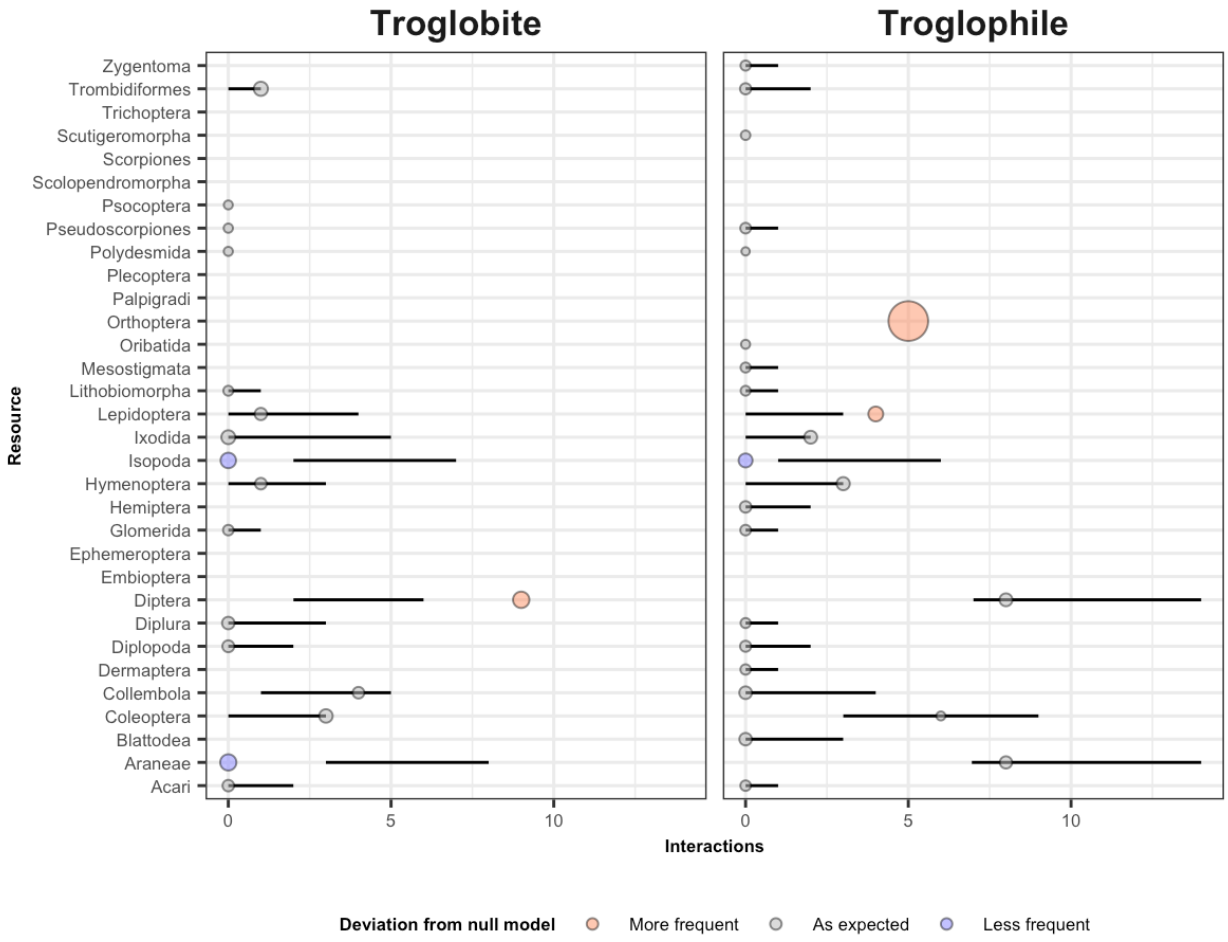


Figure S4: The frequency of predation of each prey taxon (points) deviates from the frequencies expected by null network model simulations (horizontal lines representing 95% confidence intervals of 1000 simulations) for troglobite and troglophile spiders. Several interactions occurred significantly more (orange) or less (purple) frequently than expected, whereas most aligned with null expectations (grey). Point size denotes standardised effect sizes of the deviations of observed interaction frequencies from null models. Absent points are those for which data were not available.

Table S1: Significant null network model outputs indicating predation at higher or lower frequencies than expected across spider groups for each prey taxon. SES = standardised effect size. Where SES is 'NA', the number of prey available for that taxon was 0, meaning SES could not be calculated.

Spider group	Prey taxon	Observed frequency of interactions	Null expectation of interaction frequency	Deviation from expected frequency	SES
Dark zone	Araneae	4	8.97	Less frequent	-2.96
Dark zone	Blattodea	1	0.00	More frequent	NA
Dark zone	Diptera	11	6.25	More frequent	3.35
Dark zone	Ephemeroptera	2	0.00	More frequent	NA
Dark zone	Hemiptera	1	0.00	More frequent	NA

Dark zone	Isopoda	0	5.31	Less frequent	-3.52
Dark zone	Mesostigmata	1	0.00	More frequent	NA
Dark zone	Orthoptera	1	0.01	More frequent	11.90
Dark zone	Plecoptera	1	0.00	More frequent	NA
Dark zone	Scorpiones	1	0.00	More frequent	NA
Dark zone	Trichoptera	1	0.00	More frequent	NA
Twilight zone	Ephemeroptera	1	0.00	More frequent	NA
Twilight zone	Hymenoptera	3	0.58	More frequent	3.37
Twilight zone	Orthoptera	5	0.00	More frequent	NA
Twilight zone	Trichoptera	1	0.00	More frequent	NA
Agelenidae	Araneae	4	8.59	Less frequent	-3.24
Agelenidae	Blattodea	1	0.00	More frequent	NA
Agelenidae	Diptera	12	6.32	More frequent	4.64
Agelenidae	Ephemeroptera	2	0.00	More frequent	NA
Agelenidae	Hemiptera	1	0.00	More frequent	NA
Agelenidae	Isopoda	0	5.34	Less frequent	-3.76
Agelenidae	Mesostigmata	1	0.00	More frequent	NA
Agelenidae	Orthoptera	1	0.00	More frequent	NA
Agelenidae	Plecoptera	1	0.00	More frequent	NA
Agelenidae	Scorpiones	1	0.00	More frequent	NA
Agelenidae	Trichoptera	1	0.00	More frequent	NA
Filistatidae	Araneae	0	3.43	Less frequent	-3.38
Filistatidae	Lepidoptera	2	0.18	More frequent	4.70
Filistatidae	Orthoptera	1	0.00	More frequent	NA
Pholcidae	Orthoptera	3	0.00	More frequent	NA
Sicariidae	Ephemeroptera	1	0.00	More frequent	NA
Sicariidae	Trichoptera	1	0.00	More frequent	NA
Theraphosidae	Orthoptera	1	0.00	More frequent	NA
No bats	Ephemeroptera	1	0.00	More frequent	NA
No bats	Trichoptera	1	0.00	More frequent	NA
With bats	Araneae	7	14.38	Less frequent	-3.40
With bats	Ephemeroptera	2	0.00	More frequent	NA
With bats	Isopoda	0	6.89	Less frequent	-4.13
With bats	Orthoptera	6	0.01	More frequent	77.54
With bats	Plecoptera	1	0.00	More frequent	NA
With bats	Scorpiones	1	0.00	More frequent	NA
With bats	Trichoptera	1	0.00	More frequent	NA
Troglobite	Araneae	0	5.97	Less frequent	-4.65
Troglobite	Blattodea	1	0.00	More frequent	NA
Troglobite	Diptera	9	4.00	More frequent	5.02
Troglobite	Ephemeroptera	2	0.00	More frequent	NA
Troglobite	Hemiptera	1	0.00	More frequent	NA
Troglobite	Isopoda	0	4.34	Less frequent	-3.44
Troglobite	Ixodida	0	2.98	Less frequent	-2.32
Troglobite	Mesostigmata	1	0.00	More frequent	NA
Troglobite	Orthoptera	1	0.00	More frequent	NA
Troglobite	Plecoptera	1	0.00	More frequent	NA
Troglobite	Scorpiones	1	0.00	More frequent	NA
Troglobite	Trichoptera	1	0.00	More frequent	NA
Troglophile	Ephemeroptera	1	0.00	More frequent	NA
Troglophile	Isopoda	0	2.95	Less frequent	-2.35
Troglophile	Lepidoptera	4	1.31	More frequent	3.08
Troglophile	Orthoptera	5	0.00	More frequent	79.07
Troglophile	Trichoptera	1	0.00	More frequent	NA