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Diet of the exotic Madeiran wall lizard: first insights into trophic interactions in an Atlantic seabird sanctuary

[running title: Exotic lizard diet in the Azores]

Verónica Neves,¹ Dorothee Rund², Catarina J. Pinho^{3,4,5}, Raquel Vasconcelos^{3,4}, Paco Bustamante^{6,7}, Petra Quillfeldt²

¹ Ocean Sciences Institute — Okeanos, University of the Azores, Horta, Portugal

² Department of Animal Ecology & Systematics, Justus Liebig University, Giessen, Heinrich-Buff-Ring, 35390 Giessen, Germany

³ CIBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos, InBIO Laboratório Associado, Campus de Vairão, Universidade do Porto, 4485–661 Vairão, Portugal

⁴ BIOPOLIS Program in Genomics, Biodiversity and Land Planning, CIBIO, Campus de Vairão, 4485-661 Vairão, Portugal

⁵ Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre 1021/1055, 4169–007 Porto, Portugal

⁶ Littoral Environment et Sociétés (LIENSs), UMR 7266 CNRS — La Rochelle Université, 2 rue Olympe de Gouges, 1700 La Rochelle, France

⁷ Institut Universitaire de France (IUF), 1 rue Descartes 75005 Paris, France

Corresponding author: Verónica Neves (veronica.rc.neves@uac.pt)

Abstract

The Madeiran wall lizard *Teira dugesii* is a relatively new species to the Azores Archipelago, where it was accidentally introduced about 150 to 200 years ago. This lacertid quickly became naturalized and now occurs in all the nine main islands of the Azores. At Praia Islet, off Graciosa Island, the Madeiran wall lizard was recently observed preying on chicks of the threatened Monteiro's storm-petrel *Hydrobates monteiroi*. To characterize the lizards' trophic niche, we conducted a study of the diet of the Madeiran wall lizard at Praia using stable isotope analysis and next-generation sequencing. Our results indicate that the Madeiran wall lizard has a varied diet, consisting of at least 23 invertebrate taxa, 17 plant species, and occasionally birds (two species detected, including storm-petrels). Marine derived food items were present in few samples, and it is vital to monitor the trophic interactions at Praia Islet to ensure the conservation of this threatened storm-petrel.

Key Words: Azores, conservation, Macaronesia, next-generation sequencing, Praia islet, stable isotopes

Introduction

Invasive species can pose a risk to local wildlife as predators (Mack et al. 2000) and disrupt ecosystem dynamics, especially on islands, where ecosystems are more vulnerable to introductions (Butterfield et al. 1997). The Madeiran wall lizard *Teira dugesii* is the only land reptile occurring in the Azores Archipelago (Malkmus 1995; Borges et al. 2022), where it was introduced around 150–200 years ago (Godman 1870) by the shipping trade with the Madeira Islands.

Even though lizards and other reptiles are frequently the only terrestrial vertebrates on oceanic islands, there are few studies documenting interactions between reptiles and seabirds. The interactions between the endemic tuatara *Sphenodon punctatus* and the fairy prion *Pachyptila turtur* in Stephens Island, New Zealand, are perhaps the most studied case (e.g., Wall 1978; Cree et al. 1999). The study of Matias et al. (2009) at Selvagens Islands (Madeira, Portugal) is one of the first documented evidence of a lacertid lizard, the Madeiran wall lizard (in this case endemic) preying on seabirds, including storm-petrels, in Macaronesia. Recently, Pinho et al. (2018) found evidence that a common prey of an endemic and “Endangered” lizard, the giant wall gecko *Tarentola gigas*, is also an endemic and “Near Threatened” seabird, the Cabo Verde shearwater *Calonectris edwardsii*, showing how intricate these trophic links can be. Also in Macaronesia, Gil et al. (2020) found evidence of a trophic link between the Selvagens gecko *Tarentola (boettgeri) bischoffi* and Cory's shearwater *Calonectris borealis*.

Ground-breeding seabirds often nest exclusively on small islands, which are probably selected because of a lack of predators (Schreiber and Burger 2001); that is the case for the small Hydrobatidae species breeding in the Azores (Monteiro et al. 1999), such as the endemic Monteiro's storm-petrel, only known to breed in the islets off Graciosa Island (Bolton et al. 2008). Bried and Neves (2014) reported Madeiran wall lizard scavenging dead Monteiro's storm-petrel *Hydrobates monteiroi* chicks on Praia Islet from Graciosa in the Azores. More recently, the killing of Monteiro's storm-petrel chicks by those lizards was confirmed (Neves et al. 2017). This small seabird is classified as globally ‘Vulnerable’ to extinction on <https://docs.google.com/document/d/1U91WVZLNKkOzsWIsFoTMsanzThNSK5TaiBw>

[RbKcDtT4g/edit?usp=sharing](#) the IUCN Red List (BirdLife International 2018). On those grounds, the lizard predation events detected are a cause of concern and, to our knowledge, are the first documented evidence of an introduced lacertid affecting seabirds.

This study aims to investigate the diet of Madeiran wall lizards at Praia islet as a first step to effectively protecting the endemic Monteiro's storm-petrel. To assess the lizards' trophic niche, we studied their diet using Stable Isotopes (SI) in blood and next-generation sequencing in faeces. Stable isotopes analyses provide information on the trophic position and diet (Kartizinel and Pringle 2015) and next-generation sequencing identifies the prey items (Pompanon et al. 2012). Stable isotopes can provide a time-integrated depiction of consumer diet and trophic relationships (Phillips and Eldridge 2006).

Methods

Praia Islet is located about 1.2 km off Graciosa Island, one of the nine main islands of the Azores Archipelago (Fig. 1) and is a breeding site for several seabird species, including three Charadriiforms and five Procellariiforms — two species are winter breeders (Barolo Shearwater, *Puffinus lherminieri* and Band-rumped storm-petrel, *Hydrobates castro*) and all the others breed during the summer (Monteiro et al. 1999, Bolton et al. 2008). The islet is a natural reserve and Graciosa is classified by UNESCO as a Biosphere Reserve (UNESCO 2007). Fieldwork took place from May to July 2016, coinciding with the breeding season of most seabirds that breed in the islet (Cory's Shearwater, *Calonectris borealis*; Monteiro's storm-petrel, *H. monteiroi*, Bulwer's petrel, *Bulweria bulwerii*; Common tern, *Sterna hirundo*; Roseate tern, *S. dougallii* and Sooty tern, *Onychoprion fuscatus*). Lizards were caught by hand or in traps made from 5-litres plastic containers baited with pieces of fresh fruit. Samples for SI analysis were collected in two different areas, approximately 200 m apart to try to minimise the resampling of individuals across sampling areas. One area was around the support house on the west side of the islet (Fig. 1), which was defined as the area with a low density of nesting birds. The other area was on the east side of the islet around an area with 58 artificial nests of Monteiro's storm-petrel (Fig. 1). Sample analysis was divided into two periods: 31 May–8 June, corresponding to the incubation stage of Monteiro's storm-petrels and 3–16 July, corresponding to the chick-rearing stage (Bolton et al. 2008).

Caught lizards were brought to the support house, measured (snout–vent length, SVL) and weighed. Faecal samples were collected by gentle palpation of the abdomen and stored in 70% ethanol. A blood sample (5–10 μ L) was drawn with a sterile insulin syringe (0.33 x 12.7 mm, 29G) from the caudal vein (see Divers and Mader, 2005) and stored in 70% ethanol. The base of the tail was disinfected using cotton dampened in 99% ethanol. After the procedure, which took approximately 10 minutes, the lizards were released where they were captured.

To provide an isotopic baseline of the food resources, we also sampled 18 food items for SI analysis: six arthropods and two plant species as terrestrial food, as well as blood from 10 Monteiro's storm-petrel chicks as marine-derived food. Blood samples were briefly centrifuged, supernatant (i.e., plasma) was discarded, and the Eppendorf tubes were put into a drying chamber to evaporate the water content of the blood cells. The dried samples were then pulverized using a mortar and pestle to obtain a fine powder, and a subsample of 0.35–0.45 mg was weighed into tin capsules. Some samples were slightly lighter if there was not sufficient blood, but no less than 0.12 mg. Stable isotope ratio for carbon and nitrogen was determined with an Isotope Ratio Mass Spectrometer at the University of La Rochelle, LIENSs SI Facility. Carbon and nitrogen ratios were determined with a continuous-flow mass spectrometer (Delta V Advantage or Delta V Plus with a Conflo IV interface, Thermo Scientific, Bremen, Germany) coupled with an elemental analyser (Flash EA 1112 or Flash 2000, Thermo Scientific, Milan, Italy). Measurements of internal laboratory standards were conducted using acetanilide (Thermo Scientific) and peptone (Sigma-Aldrich) and indicated an experimental precision of ± 0.15 ‰ for both elements. Results are expressed in parts per thousand (‰), in the usual δ notation relative to Vienna Pee-Dee Belemnite for $\delta^{13}\text{C}$ and atmospheric N_2 for $\delta^{15}\text{N}$, following the formula: $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$, where R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$, respectively. Differences in the mean $\delta^{13}\text{C}$ in the different habitats before and after the hatching of the chicks were analysed using Wilcoxon rank sum test with continuity correction because the data were not normally distributed (R v3.3.2, R Core Team, 2016). Significance was assumed at $P < 0.05$.

Faecal samples were dried in an incubator at 50°C to remove the ethanol. The DNA was extracted using the Stool DNA Isolation Kit (Norgen Biotek Corp., Canada), following the manufacturer's instructions. In the end, two DNA elutions were obtained with a final volume of 50 µL each, but only the first elution was used for the next steps. To correctly identify the different prey types (vertebrates, invertebrates, and plants) three different DNA fragments were selected for amplification. For vertebrates, the V5-loop fragment of the mitochondrial 12S gene (supplementary Table S1) was selected. The marker selected for invertebrates was the mitochondrial 16S rRNA (supplementary Table S1) and for plants the short P6-loop of chloroplast trnL (UAA) intron (supplementary Table S1). To allow the individual identification of each sample, all primers were modified with an individual barcode as detailed in Pinho et al. (2018). Each PCR was performed following the same protocol as detailed in Pinho et al. (2018) and the product was tested on a 2% agarose gel.

Library preparation was carried out following Illumina MiSeq protocol "16S Metagenomic Sequencing Library Preparation" (Illumina 2013) according to the method described in Pinho et al. (2018). OBITools (<https://git.metabarcoding.org/obitools/obitools>) was used for general sequence processing according to the methods described in Pinho et al. (2018). Results were compared with Azores databases (Borges et al. 2022) and the Encyclopedia of Life database (<http://eol.org>). We removed a few haplotypes that were considered lab contamination based on published reference lists of species occurring in Azores. When the same haplotype matched more than one species or genera with similar probabilities, we only considered species or genus known to occur at Graciosa, or in other islands of the Azores.

Results

We collected and analysed for SIs 57 Madeiran wall lizard blood samples; mean values and standard deviation for carbon and nitrogen isotopes for the different sampling locations and times are given in Table 1. The food reference material collected on the islet showed clear differences in carbon isotope ratios between terrestrial and marine items with lower $\delta^{13}\text{C}$ values for terrestrial items (Table 2), but an overlap in nitrogen

isotope ratios. During the incubation period, values of $\delta^{13}\text{C}$ isotopes were slightly higher in the location “Nests” than in the location “House” but the difference was not significant (Wilcoxon rank sum test with continuity correction, $W = 120.5$, $P = 0.265$; $n_{\text{house}} = 16$, $n_{\text{nests}} = 12$). During the chick-rearing period, values of $\delta^{13}\text{C}$ isotopes were again higher in the location “Nests” and there was no significant difference between locations ($W = 135$, $P = 0.181$; $n_{\text{house}} = 13$, $n_{\text{nests}} = 16$). $\delta^{13}\text{C}$ isotope values were higher during chick-rearing for the sampling location ‘Nests’, but also not significant ($W = 61$, $P = 0.109$; $n_{\text{house}} = 12$, $n_{\text{nests}} = 16$). Around the ‘Nests’ site, there was also a much greater variance in the stable carbon isotope values (Table 1, Fig. 2). Some individuals ($n = 5$) from this study area reached as high a carbon value as the Monteiro’s storm petrel chicks — stable carbon isotope values between -21.49 and -20.10 ‰. The arthropod samples collected at the islet show an enrichment in $\delta^{13}\text{C}$ values compared to plants (Table 2), but the values were still lower than the ones found for Monteiro’s storm-petrel chicks, giving support to the hypothesis that some lizards may be feeding on seabirds.

Out of the nine faecal samples analysed, only seven worked for most markers used and two samples failed to be sequenced. In total, 43 molecular operational taxonomic units (MOTU) were identified: 17 plants, 23 invertebrates and 3 vertebrates (supplementary Table S2 and dataset 1). Most faecal samples included invertebrates and/ or plants (seven and six out of seven, respectively), and some of them were composed of only one of these groups. Three out of the seven samples contained vertebrate remnants. The vertebrates included two birds that breed at Praia Islet, the rock pigeon *Columba livia* and *Hydrobates castro*, which most likely corresponds to *H. monteiroi* considering the small fragment size, the recent taxonomic split and phylogenetic proximity, and the absence of 12S sequences of the latter in public databases. It also included the Madeiran wall lizard itself, indicating a potential failure of the blocking primer, cannibalism or scavenging.

Discussion

Our study indicates that the values for stable carbon isotopes are higher in lizards caught around nest sites than in those caught around the support house, suggesting a possible higher reliance on marine-derived items in the diet of lizards caught in proximity to breeding seabirds. The marine-derived items on the lizards’ diet can include remains of

hatched eggs (eggshell or membrane), seabird prey dropped in the colony and/ or seabirds (resulting from either scavenging or predation). There was also a higher marker for marine items after the hatching of the chicks than during incubation around the nests, as indicated by the higher carbon isotope levels. Although not significant, this difference is higher than the difference between the two study areas. However, this analysis does not allow distinguishing between marine items consumed and that could have been seabirds in the form of eggs (either whole or remains of eggshell and membrane after hatching), chicks, dead birds, faeces or dropped fish and regurgitate.

The SI analysis also revealed one individual with a stable carbon isotope ratio almost as high as was found in the chicks a month before the hatching started, an indication that marine food sources, other than Monteiro's storm-petrel chicks, are available. These can probably be attributed to Macaronesian shearwater and band-rumped storm-petrel, species that breed during the winter period. In general, there was a wider variance in the stable carbon ratios for the lizards at the nests which indicates that some individuals specialize more in terrestrial food and some more in marine food. Previous studies on lizards and SIs have shown that soil lithology may affect the values of stable carbon isotopes (Martín et al. 2017), but in our case the study islet is small and there is no variation in soil type.

The faecal sample analysis using metabarcoding indicated that these lizards are highly reliant on invertebrates and plants as was found for this species at Selvagens Islands (Sadek 1981; Gil et al. 2020). In addition, that analysis provided a good complement to the SI results and confirmed the presence of seabirds in the diet of Madeiran wall lizards. During fieldwork, we could confirm the deaths of three Monteiro's storm-petrel chicks in the nesting area. Two chicks showed no injuries and the third one was dragged to the nest's entrance and lizards were eating from it, but it is unclear if the bite marks were the cause of death or if the lizards were only taking advantage of the carcass. The next-generation sequencing also showed the presence of seabirds, but we could not confirm if that resulted from scavenging or predation.

It is important to consider that Praia Islet only holds an estimated 150–183 pairs of

Monteiro's storm-petrel (Monteiro et al. 1999; Oliveira et al. 2016). The restricted distribution and small breeding numbers of this endemic species make it much less resilient to any impact factors, such as putative predation by lizards. Recently Lopes et al. (2019) found evidence that the main prey of an endemic and "Endangered" lizard, the Cabo Verde giant wall gecko *Tarentola gigas*, is the endemic and "Critically Endangered" bird Raso lark *Alauda razae*, confirming the importance of monitoring the impact of these trophic interactions, even when considering endemic species, especially during drought years when the population of Raso lark can be severely reduced (Lopes et al. 2019).

This study provides a first step towards understanding the trophic links at one of the main seabird sanctuaries in the Azores. Our results also confirm the potential of next-generation sequencing to further our understanding of the lizard/ storm-petrel trophic interactions. Marine-derived food items are not ubiquitous in lizard's diet and to assess lizards' impact on the breeding success of Monteiro's storm-petrel it would be necessary to increase the sample size and to sample in different seasons. Further studies should also attempt to use wildlife cameras on the nests to study the interactions between lizards and storm-petrels and clarify if the presence of marine items in the diet of the Madeiran wall lizard result from direct predation of viable eggs and nestlings or from scavenging of chicks that died for other reasons or remains of the membrane from hatched eggs.

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Table 1. Results of the isotopic analyses from lizard blood samples. Sample size (n), stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios (mean \pm standard error in permillage, ‰) for the different sampling locations and periods. Check Fig. 1 for sample locations.

Location_period	n	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
House_incubation	16	-24.53 ± 0.12	12.63 ± 0.12
House_chick-rearing	13	-24.27 ± 0.07	12.90 ± 0.23
Nests_incubation	12	-24.14 ± 0.27	11.46 ± 0.25
Nests_chick-rearing	16	-23.11 ± 0.41	12.03 ± 0.17

Table 2. Results of the isotopic analyses from reference food items samples. Stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope ratios (mean \pm standard error in permillage, ‰) for reference food resources. Terrestrial items include two plant and six arthropod species, and the marine items consist of blood samples from Monteiro’s storm-petrel chicks.

Food items	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)
Terrestrial	-24.71 ± 0.63	9.62 ± 1.19
Plants	-26.94 ± 0.42	12.74 ± 2.92
Arthropods	-23.97 ± 0.53	8.58 ± 1.09
Marine	-20.23 ± 0.06	11.08 ± 0.07

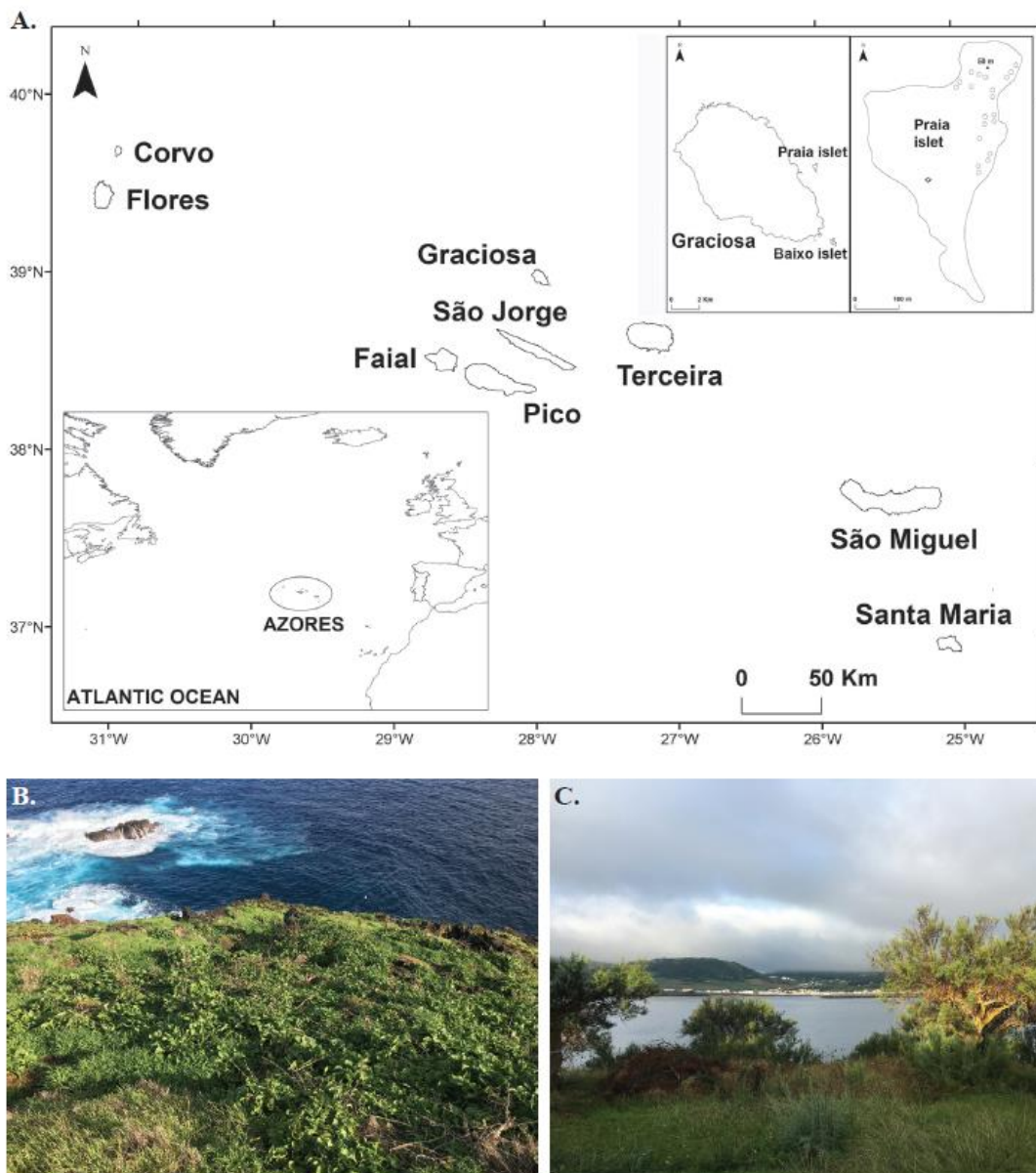


Figure 1. A) Location of Praia Islet in the Azores Archipelago. Squares marked in the east and north-east of the islet indicate the location of artificial nests of Monteiro’s storm-petrel, *Hydrobates monteiroi*; the diamond symbol in the west of the islet indicates the location of the house. Adapted from Bried and Neves (2014). B) Study area - location “Nests”. C) Study area - location “House”.



Figure 2. A) Monteiro's storm-petrel (*Hydrobates montei*) in an artificial nest at Praia Islet with a recently hatched chick and remnants of eggshell. B) Madeiran wall lizard (*Teira dugesii*) eating a chick of Monteiro's storm-petrel in an artificial nest at Praia Islet.

Supplementary material

Table S1. Forward and reverse primers used in this study. The amplified region, the fragment length (in base pairs, bp), the sequences, and the reference for each primer set is given.

Amplified region	Fragment length (bp)	Primer sets	Primer sequence	Reference
V5-loop (12s)	73–110	12sv5F 12sv5R	TAGAACAGGCTCCTCTAG TTAGATACCCCACTATGC	Riaz et al. 2011
16S rRNA	~ 110	IN16STK–1F–mod IN16STK–1R–mod	TRAACTCAGATCATGTAA TTAGGGATAACAGCGTWA	Pinho et al. 2018 (based on Kartzinel et al. 2015)
P6-loop (chloroplast trnL)	10–143	g_F h_R	GGGCAATCCTGAGCCAA CCATTGAGTCTCTGCACCTATC	Taberlet et al. 2007

References

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- Taberlet P, Coissac E, Pompanon F, Gielly L, Miquel C, Valentini A, Vermat T, Corthier G, Brochmann C, Willerslev E (2007) Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. *Nucleic Acids Resources*. 35: 14. <https://doi.org/10.1093/nar/gkl938>

Table S2. Results of the metabarcoding analyses. List of Operational Taxonomic Units (OTUs) sequenced from the faecal pellets of Madeiran wall lizard for the different taxonomic groups and the incidence (n) of the identified taxa.

Taxonomic group (# faecal samples)	OTUs	n
Plants (n = 6)	<i>Tamarix africana</i>	4
	Asteraceae	3
	Caryophyllales	2
	Apiaceae	1
	<i>Atriplex</i> sp.	1
	Brassicaceae	1
	<i>Frankenia</i> sp.	1
	Malvaceae	1
	<i>Oxalis</i> sp.	1
	<i>Pinus pinaster</i>	1
	Poaceae	1
	<i>Populus</i> sp.	1
	<i>Quercus</i> sp.	1
	<i>Ruta chalepensis</i>	1
	Solanaceae	1
	<i>Solanum</i> sp.	1
<i>Urtica membranacea</i>	1	
Invertebrates (n = 7)	<i>Armadillidium vulgare</i>	6
	<i>Opsius stactogalus</i>	6
	Tachinidae	3
	Diplopoda	2
	<i>Lampides boeticus</i>	2
	<i>Pollenia rudis</i>	2
	<i>Tenuiphantes tenuis</i>	2
	Acrididae	1
	Mesostigmata	1

	Braconidae	1
	Carabidae	1
	Curculionidae	1
	Diptera	1
	Insecta	1
	<i>Lasius grandis</i>	1
	Lepidoptera	1
	<i>Piezodorus lituratus</i>	1
	Salticidae	1
	<i>Spodoptera</i> sp.	1
	Staphylinidae	1
	Tetragnathidae	1
	<i>Trechus</i> sp.	1
	<i>Trox scaber</i>	1
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Vertebrates (n = 3)	<i>Teira dugesii</i>	3
	<i>Columba livia</i>	2
	<i>Hydrobates</i> sp.	1
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