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Analysis of Prey Composition in Eurasian Reed Warblers' *Acrocephalus scirpaceus* Droppings at Four Breeding Sites in Italy

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Abstract: Our aim was to investigate the among-populations diversity of prey composition in Eurasian Reed Warblers' diets via their droppings, both to assess the ecological validity of this sampling method and to test whether the prey species most abundant in fecal samples were also the most present in the Italian study site. We collected fecal samples at four sites throughout Italy, for a total of 144 samples. Within reedbeds, the breeding habitat of the Eurasian Reed Warbler, we also collected arthropods by carrying out entomological sweepings at one of the study sites. Within the fecal samples, we identified dozens of prey species, belonging mainly to Araneae, Coleoptera, Diptera, Hymenoptera and Aphidoidea taxa, whose compositions were not statistically different among sites. The commonest prey species were Coleoptera in the droppings (68.5%) and Diptera in the reedbeds (31.3%), although the latter was less numerous in the fecal samples. The diets of different Italian populations of Eurasian Reed Warbler emerging from our study underline a strong Coleopteran presence, without differences across Italy. Fecal samples appear to be an exhaustive method to study variation in Eurasian Reed Warbler diet and its ecological importance; however, although potential caveats do exist, such as the possible under-representation of Diptera.

Keywords: avian brood parasitism; bird droppings; diet composition; entomology; Eurasian Reed Warbler; insectivorous bird; ornithology

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1. Introduction

The Eurasian Reed Warbler *Acrocephalus scirpaceus* (Reed Warbler hereafter) is a transaharian migratory species breeding in the Palaearctic region and wintering mostly in tropical Africa. It is linked to reed formations, mainly dominated by *Phragmites australis*, where it is the most widespread acrocephaline species [1–3]. This bird is considered an excellent model for biological, eco-ethological and taxonomical studies [4–11].

Presently, the diet of the Reed Warbler is sufficiently known from north-central areas of Europe, while little information available from southern areas (see Table 1; [5]). This represents a serious gap in our understanding of its ecology, as a sizable part of its European population resides in the Mediterranean region [12]. Thus, our study aimed to describe the Reed Warbler diet in four different Italian localities with similar habitat. The description of a species diet is key to a proper understanding of its ecology; previous studies on the

Reed Warbler have underlined some differences between prey compositions at different sites. For example, Diptera appear to be one of the main components of the Reed Warbler diet (Table 1), but their prevalence varies from 25% to 90% across populations, without an overarching regional-level pattern (Table 1). It is also noteworthy that varying patterns in insect prevalence can, in turn, impact prey consumption in insectivore birds [13]. Sampling multiple populations in Italy is, therefore, crucial to understanding whether any eventual differences in diet between sites are due to differences in environment, local prey availability, or overall regional level climate. To this purpose, we also confronted the food items found with the insect abundance at one of the study sites. Generally speaking, a similar taxa composition in both insect abundance and prey presence in the diet signals a generalist predator, which samples prey from the environment without preferring specific taxa. On the other hand, an over-representation of a specific prey type indicates a specialist predator.

Table 1. Percentage of prey items obtained by different authors in western Palearctic countries with the method of collar-samples or fecal samples.

Reference, Country	Sample Method	N of Samples	Araneae	Coleoptera	Heteroptera	Auchenorrhyncha, Aphidoidea	Diptera	Others
[14] Chernetsov and Manukyan (1999), Russia	Collar	170	37 [1]	79 [1]	29 [1]	18.2 [1]	79 [1]	-
[15] Kazlauskas Pukas and Meldazyte (1986) in [5] Cramp (1992), Lithuania	Collar	>2000	6.9	0.7	0.4	4	83.1	4.9
[16] Dyrcz (1979), Poland	Collar [2]	2549	2.7	0	0	4.3	71.9	21.1
[4] Davies and Green (1976), UK	Collar	120	5.8	5	0	10.9	64.9	13.3
[17] Bibby and Thomas (1985), UK	Fecal	1710	18	12.5	2.1	17.6	24.2	25.6
[18] Grim and Honza (1996), Czech Republic	Collar [2]	94	6.2	1.2	2.3	13.4	67.6	9.2
[19,20] Henry (1977, 1979), France	Collar [2]	2159	5.1	0.9	0.3	44.8	35.4	8.2
[21] Bussmann (1979), France	Collar	1776	15.8	0.9	0.4	14.3	51.2	32.6
[22] Bibby and Green (1983), France	Fecal	55	7.2	13.3	3.5	19.5	23.8	10
[23] Kerbiriou et al. (2011), France	Fecal	28	6.4	4	2.3	22	53.8	12.2
[16] Dyrcz (1979), Switzerland	Collar [2]	1839	3.7	0	0	0.3	91.2	4.8
Akriotis in [5] Cramp (1992), Greece	Collar	1018	8.3	2.1	1.7	30.9	50.7	6.8
[24] Rguibi Idrissi et al. (2004) [3], Morocco	Collar	88	2.7	27.4	4.4	4.5	1.9	59.1 [4]

[1] The % refers to frequency occurrence in sample; [2] Nestling food. [3] A 1% solution of antimony potassium tartrate, according to the method of [25]. [4] In total, 28.1% of others were ants.

Various methods have been used to study the Reed Warbler diet, but undoubtedly the analysis of the droppings is the one that guarantees the least disturbance to the species, especially during the breeding phase [17]. However, studying the diet through the examination of bird droppings can present methodological limitations, as some scarcely chitinized prey are completely digested by gastric juices and leave no traces in the feces (e.g., [17,22,26,27]).

Not all insects are necessarily potential prey for an insectivore predator. Only insects that occur in micro-habitats used by the insectivorous birds can be classified as available. Various definitions of availability can be reviewed in the light of a simplified sequence of steps in catching an insect-prey: the prey is present in the area, it is met by one of its avian predators, is seen or somehow contacted by the predator, is recognized as potential prey, or can be chased, captured and eaten [28]. Each of these steps could, of course, create biases in how a specific prey taxon is represented in insectivores' diets. We hypothesize that, like their central European counterparts, the Italian populations of Reed Warblers will behave as

a generalist predator, with local differences among the sites, and that their diet pattern will be determined by prey availability. Confirming that Italian Reed Warblers are generalists, similar to their Central and Northern European counterparts, might also help indicate the degree to which they would be impacted by climate changes; one aspect of global warming that could impact all insectivorous birds is, in fact, the decline of arthropods occurring worldwide [29]. In a recent study, it was found that the diet composition of aerial insectivores in wetlands shaped how they were impacted by climate change via nutritional phenological mismatch [30]. Thus, a good grasp of the Reed Warblers' diet across its whole range could also help future studies to link warming climate conditions [31] to ecological changes [32].

Another reason that makes it interesting to monitor the provisioning diets relies on the fact that the Reed Warbler is one of the host species most frequently parasitised by the Common Cuckoo *Cuculus canorus* in its whole breeding range, including Italy [33,34]. The begging call of a cuckoo chick is able to elicit from the foster parents a provisioning rate similar to that triggered by an entire 4/5-chicks host brood [35]. Because of their larger size, cuckoo chicks have been found to be raised on larger prey types. Accordingly, monitoring their diet might provide indications of potential changes in insect availability; this is what we expect from the phenomena of climate change and the resulting habitat loss. There are, however, scant studies investigating the foster parent provision of the parasitic chick [36]. Here, we wanted to verify whether a specific investigation method (i.e., video recording at the nest) could reliably enable us to identify the prey type and to describe, although anecdotally, cuckoo chick diets.

In this study, we analyzed the prey composition of the Reed Warbler diet of four Italian populations, one in Northern Italy, the others on the island of Sicily. Our main goals were to: (i) describe the diet composition of the Reed Warbler in Italy and detect any differences among the four warbler populations, (ii) quantify the differences between the insect composition in the diet and that detected in the habitat, and (iii) describe, for the first time in Italy, the prey types with whom warblers provision avian brood parasite chicks when found in their nest.

2. Materials and Methods

2.1. Study Areas

2.1.1. Mirandola (Mortizzuolo)

Modena, Northern Italy (Figure 1), 44°51′40″ N, 11°06′25″ E. The marshland is formed by several freshwater ponds restored with EU grants. It is a protected area included in the Nature 2000 Network (SPA ITA 4040014). The vegetation along the bank and open waters is mainly constituted by thick cane beds dominated by *P. australis*, where many pairs of Reed Warblers and great Reed Warblers *A. arundinaceus* place their nests. The mist nzet transects were set in corridors created in the reed beds. Ringing activity for the study of the diet of Reed Warblers was carried out in 2019.

Figure 1. Location of study areas.

2.1.2. Pergusa Lake

Central Sicily (Figure 1), Italy, 37°30′50′′ N, 14°18′18′′ E. Pergusa is an endoreic lake in the province of Enna, 667 m a.s.l., has a perimeter of 4.5 km and is surrounded by hills between 750 and 911 m a.s.l. The vegetation along the bank is dominated by reeds *P. australis*, where many pairs of Reed Warblers and a few pairs of great Reed Warblers *Acrocephalus arundinaceus* breed. The locality is found in the center of Sicily and was established as a Special Nature Reserve in 1995 (SPA/SCI ITA 060002). Ringing activity for the study of the diet of Reed Warblers was carried out in 2012, 2018 and 2019.

2.1.3. Gela Lake

Southern Sicily (Figure 1), Italy, 37°01′17′′ N, 14°20′30′′ E. It is a retrodunal humid area, ca. 120 ha wide, in the province of Caltanissetta. Generally, it contains fresh waters, but in some summers waters may be slightly salty. The vegetation along the bank is dominated by *P. communis* and much less by *Typha* sp. In addition, *Arundo donax* and *Tamarix* sp. are present, the latter with thick formations. Since the year 2000, the area is protected and a Nature Reserve has been established; it is also a SPA/SCI PS ITA 050001. Ringing activity for the study of the diet of Reed Warblers was carried out in 2012 and 2013.

2.1.4. Vendicari

Eastern Sicily (Figure 1), Italy, 37°47′30′′ N, 15°05′29′′ E. The area is part of a complex of retrodunal marshes with salt waters, ca. 150 ha wide, in the province of Siracusa; much of the vegetation along the banks is dominated by *P. communis.* It has also been established as a Nature Reserve since 1984; it is also a SPA/SCI ITA 090029. Ringing activity for the study of the diet of Reed Warblers was carried out in 2013.

2.2. Data Collection

In this study, we analyzed adult Reed Warbler droppings collected during 2012–2019 spring–summer ringing sessions. Sicilian data were collected in July–August, during and

after the breeding season, whereas data from Mirandola was collected in June, during the breeding season. All individuals were caught during ringing sessions with 5–10 mist nets with four pockets (mesh size: 16 mm), 3 m high and 12 m long. The individual fecal samples, collected in the ringing bag and associated with the ring number of the bird, were stored in Eppendorf tubes, both dry and in alcohol. Ringing bags were changed and cleaned after each ringing session. Overall, we collected a total of 144 droppings, 66 in Gela, 21 in Vendicari, 23 in Pergusa and 34 in Mirandola. The feces were carefully dissected under the Wild 3B microscope and the individual invertebrate pieces were glued on entomological tags and then photographed. Most invertebrate pieces were identified via a comparison with entomological collections of the Department of Agriculture, Food and Forest Sciences of the Palermo University. To obtain the identification of most species, we asked for expert opinions by uploading photos of unidentified parts to the *entomologitaliani.net* forum [37].

To compare the entomological fauna detected in droppings versus warbler habitat, in Mirandola, we proceeded to capture insects with an entomological sweeping net used across the reedbed and the surrounding vegetation of tamarisks and bushes, on two days in May 2018 and another two in June 2019, from 5:30 to 18:30 (CET). A suitable net was used to mow the grass and low branches, following a 200 m transect, which was repeated several times during the day. The insects were collected and subsequently identified in the laboratory.

To test whether video recording at the parasitized nests could be a reliable method to describe and quantify provisioning to cuckoo chicks and, in the positive case, detect any potential difference between the prey items offered to their own chicks versus parasitic chicks of the Common Cuckoo, we videorecorded provisioning events at parasitized nests of Reed Warblers found at Mirandola. We analyzed a total of 3 h videos recorded at two nests during two days, showing warblers provisioning to 1-week old cuckoo chicks.

2.3. Statistical Analysis

Analyses were performed with R version 4.0.3 [38]. To investigate differences in the entomological composition of Reed Warbler fecal samples, we separated six major prey types: Aphidoidea, Coleoptera, Hemiptera, Hymenoptera Formicidae, and other Hymenoptera. Other prey types (n = 10) were discarded because of their low prevalence in the dataset. We used, as dependent variables, the presence/absence of each prey type in each dropping. We fitted as fixed factors 'site' and 'prey type' (we could not fit their interaction due to rank deficiencies); 'dropping ID' nested within 'site' was fitted as random effect. Analyses were conducted with generalized linear mixed models (GLMM, package glmmADMB; [39]) with binomial distribution (logit link) and accounting for zero-inflation. We then analyzed contrasts with post hoc Tukey tests implemented with a multcomp package [40]. Two further tests were carried out with samples from the Mirandola site only; we tested whether captured arthropod composition differed between 2018 and 2019, and if 2019 droppings' arthropod composition differed from 2019 net captures, both with Fisher's exact test.

3. Results

3.1. Diet Composition

Reed Warblers preyed on many taxa of invertebrates (Table 2). Overall, we selected 762 prey items, of which 308 were found in the feces collected at Biviere di Gela, 202 at Pergusa, 103 at Vendicari, and 149 at Mirandola. In all the feces, few vegetal remains were regularly found. Among invertebrates, the following species were identified (see also Figure 2).

Table 2. Number of prey items per study area as identified from fecal droppings of Reed Warblers, and percentage of each selected prey category out of the total prey items recorded in each area.

Prey Type	Mirandola (n = 149)		Gela (n = 308)		Vendicari (n = 103)		Pergusa (n = 202)		Total (n = 762)	
	No	%	No	%	No	%	No	%	No	%
Araneae	0	0	1	0.3	0	0	0	0	1	0.01
Coleoptera	105	70.5	220	71.4	76	73.8	121	59.9	522	68.5
Hymenoptera	0	0	24	7.8	12	11.6	5	2.5	41	5.4
Heteroptera	36	24.2	9	2.9	0	0	10	4.9	55	7.2
Aphidoidea	8	5.4	3	1.0	0	0	57	28.2	68	9
Others	0	0	51	16.5	15	14.6	9	4.5	75	9.9
Total	149	100	308	100	103	100	202	100	762	100

Figure 2. Prey found in the fecal samples of the Reed Warbler and their size: (**a**) *Coniatus* sp. (Curculionoidea, head); (**b**) *Chlaeniellus vestitus* (Carabidae, head); (**c**) *Camponotus* sp. (Formicidae, head); (**d**) *Podops curvidens* (Heteroptera, head); (**e**) Torymidae (Hymenoptera, head); (**f**) *Trichrysis cyanea* (Crysididae, head); (**g**) Bruchidae (Coleoptera, head); (**h**) *Perapion violaceum* (Apionidae, elytra and pronotum); (**i**) *Donacia* sp. (Chrysomelidae, elytra remains); (**j**) *Prasocuris junci* (Chrysomelidae, elytra); (**k**) *Drypta dentata* (Carabidae, elytra remains); (**l**) *Brachypera zoilus* (Curculionoidea, elytra remains); (**m**) *Trichrysis cyanea* (Chrysididae, abdominal segment); (**n**) *Harmonia axiridis* (Coccinellidae, elytra remains); (**o**) Alticinae (Chrysomelidae, elytra); (**p**) Talitridae? (Amphipoda, gnatopode); (**q**) Hymenoptera Ichneumonidae Cryptinae (ovipositor and one ovipositor sheath); (**r**) *Galerucella* sp. (Chrysomelidae, elytra remains); (**s**) *Coniatus* sp. (Curculionoidea, leg); (**t**) *Chlaeniellus vestitus* (Carabidae, elytra); (**u**) *Nabis* sp. (Heteroptera, head).

3.1.1. Mirandola

There were 105 Coleoptera, of which there were 22 specimens of *Drypta dentata* (Coleoptera Carabidae), 2 *Prasocuris junci* (Coleoptera Chrysomelidae), 1 *Brachypera zoilus* (Coleoptera Curculionoidea), 6 *Rhaconicha fulva* (Coleoptera Cantharidae), 1 *Donacia* sp. (Coleoptera Chrysomelidae), 8 *Galerucella* sp. (Coleoptera Chrysomelidae), 10 *Dimorphopterus blissoides* (Heteroptera Lygaeidae), 3 *Tuponia mixticolor* (Heteroptera Miridae), 2 *Megalocoroea recticornis* (Heteroptera Miridae), 8 *Hyalopterus pruni* (Aphidoidea), 3 *Pteronemobius heydeni* (Orthoptera Gryllidae). Each dropping of the Reed Warbler contained the remains of 4.5 prey items.

3.1.2. Pergusa Lake

There were 121 Coleoptera, of which there were 11 specimens of Coleoptera Carabidae Chlaeniinae, 99 Coleoptera Curculionoidea, 11 Coleoptera Chrysomelidae Alticinae, 10 Hemiptera Heteroptera, 57 Aphidoidea, 7 Hymenoptera Formicidae, 5 unidentified small Hymenoptera, 2 *Paratettix meridionalis* (Orthoptera Tetrigidae). Each dropping of the Reed Warbler contained remains of 8.8 prey items.

3.1.3. Gela Lake

There were 220 Coleoptera, of which there were 26 specimens of *Chlaeniellus vestitus* (Coleoptera Carabidae), 39 *Perapion violaceum* (Coleoptera Apionidae), 1 *Coniatus* sp., 3 *Phyllobius* sp. (Coleoptera Curculionoidea), 1 *Donacia* sp. (Coleoptera Chrysomelidae), 6 Chrysomelidae Alticinae, 3 *Harmonia axiridis* (Coleoptera Coccinellidae), 1 Coleoptera Bruchidae, 13 *Camponotus* sp. (Hymenoptera Formicidae), of which 5 winged, 1 Hymenoptera Ichneumonidae Cryptinae (ovipositor and one ovipositor sheath), 10 *Trichrysis cyanea* (Hymenoptera Chrysididae), 1 *Nabis* sp. (Heteroptera Nabidae), 7 *Podops curvidens* (Heteroptera Pentatomidae), 2 Talitridae (Crustacea Amphipoda). Each dropping of the Reed Warbler contained remains of 4.7 prey items.

3.1.4. Vendicari

There were 76 Coleoptera, of which there were 40 specimens of Coleoptera Apionidae, 11 Coleoptera Carabidae Chlaeniinae, 1 *Donacia* sp. (Coleoptera Chrysomelidae), 14 Coleoptera Chrysomelidae Alticinae, 10 Hymenoptera Formicidae, of which 2 *Craematogaster scutellaris*, 2 Hymenoptera Torymidae, 5 unidentified Coleoptera, remains of 1 Mollusca Gastropoda. Each dropping of the Reed Warbler contained remains of 4.8 prey items.

3.2. Diet Composition Analysis

Overall, the most important prey of Reed Warblers was found to be beetles of different families (70.3%), followed by hemipterans, mainly aphids (8.9%), bugs (7.0%) and small hymenopterans (3.8%); others, unidentified, represent 9.9%. Each dropping of the Reed Warbler consisted of 5.3 prey, but some of them were probably completely digested and would, thus, most likely not appear entirely in our statistics. We found a significant influence of 'prey type' on the presence of the prey in the droppings ($\chi^2 = 145.104$, $p < 0.001$), but no significant influence of site ($\chi^2 = 2.869$, $p = 0.412$). Coleoptera was significantly overrepresented in the droppings with respect to other orders (z-values ranging from $|2.884|$ to $|7.514|$, all p-values < 0.026); no other differences were significant (Figure 3).

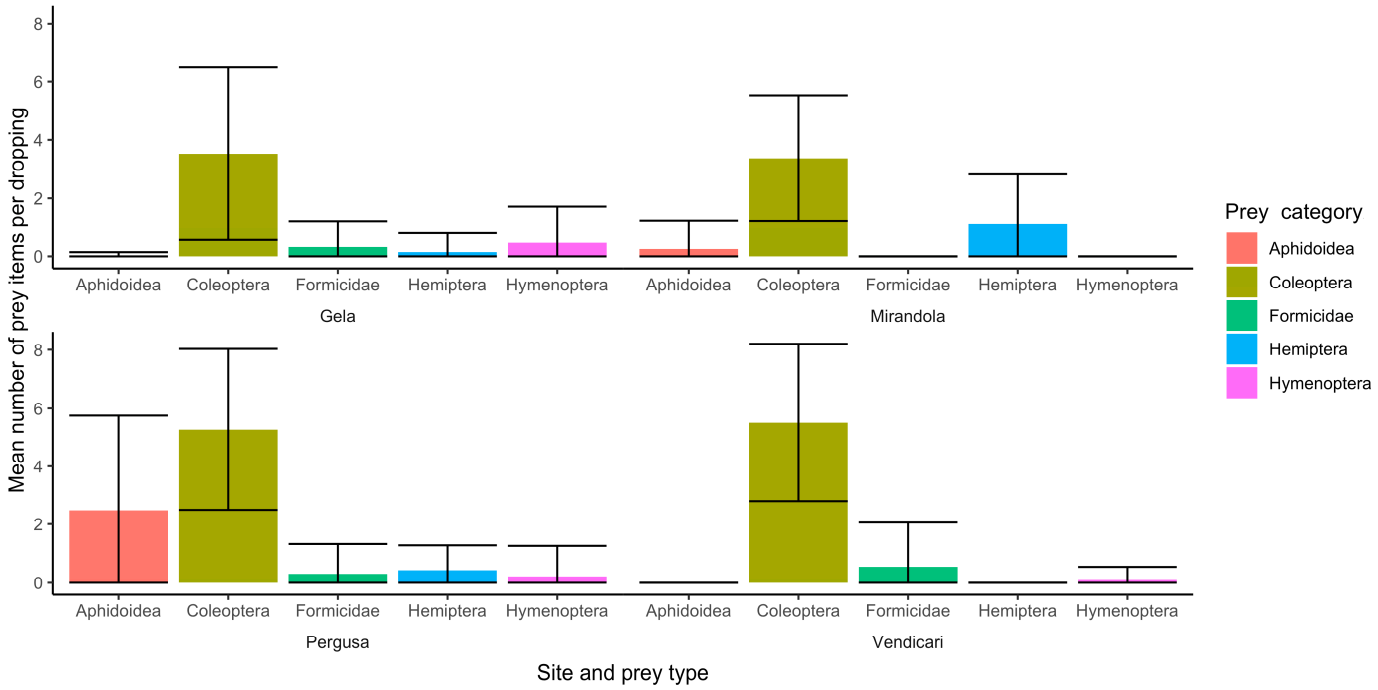

Figure 3. Mean number (±standard deviation) of prey items found in the droppings of Reed Warblers in the four Italian study areas.

3.3. Habitat Occurrences Versus Diet Composition

In Mirandola, we collected 1384 and 1366 insects and spiders in 2018 and 2019, respectively (Table 3). In 2018, 4.8% of Hemiptera were aphids *Hyalopterus pruni*, 11% of Coleoptera were Curculionoidea, 11% were Chrysomelidae Alticinae, and 29% of Hymenoptera were ants. In 2019, Coleoptera were represented by the following taxa: 91 *Anthelephila pedestris* (Anthicidae), 119 *Rhaconicha fulva* (Cantharidae), 86 Chrysomelidae Alticinae, 5 Chrysomelidae of other subfamilies (e.g., *Melasoma populi*), 29 Coccinellidae (9 adults and 20 larvae), 11 Staphylinidae (mainly *Paederus* sp.), 26 Curculionoidea, 4 Oedemeridae, 3 Cerambycidae and 9 unidentified beetles. Hemiptera were mainly aphids *Hyalopterus pruni*, and also Heteroptera, represented by *Megalocoroea recticornis*, *Lyorhyssus hyalinus*, *Megalodactylus macularubra*, *Tuponia mixticolor* linked to tamarisk, and *Dymorphopterus blissoides* to reeds. In both years, at the edge of reeds on the soil, *Pteronemobius heydeni* (Orthoptera Gryllidae) was abundantly detected by its song. The composition in the nets differed between years (Fisher's exact test, p-value = 0.0005). The difference was due to the overrepresentation of Coleoptera in 2019 and of Heteroptera in 2018 (Figure 4a). The composition of prey items inside droppings differed from the composition of captured arthropods by the entomological sweeping (Fisher's exact test, p-value = 0.0005). The greatest differences were the over representation of Coleoptera in the droppings, while Aphidoidea and Diptera were strongly underrepresented (Figure 4b).

Table 3. Number of arthropods sampled within Mirandola reedbed in 2018 and 2019.

Year	2018		2019		Total
Prey Type	**No**	**%**	**No**	**%**	**No**
Araneae	127	9.2	69	5.0	196
Coleoptera	82	5.9	383	28.0	465
Diptera	401	28.9	461	33.7	862
Hymenoptera	31	2.2	72	5.3	103
Heteroptera	675	48.7	359	26.3	1034
Aphidoidea	34	2.4	20	1.5	54
Others	34	2.4	2	0.1	36
Total	1384	99.7	1366	99.9	2750

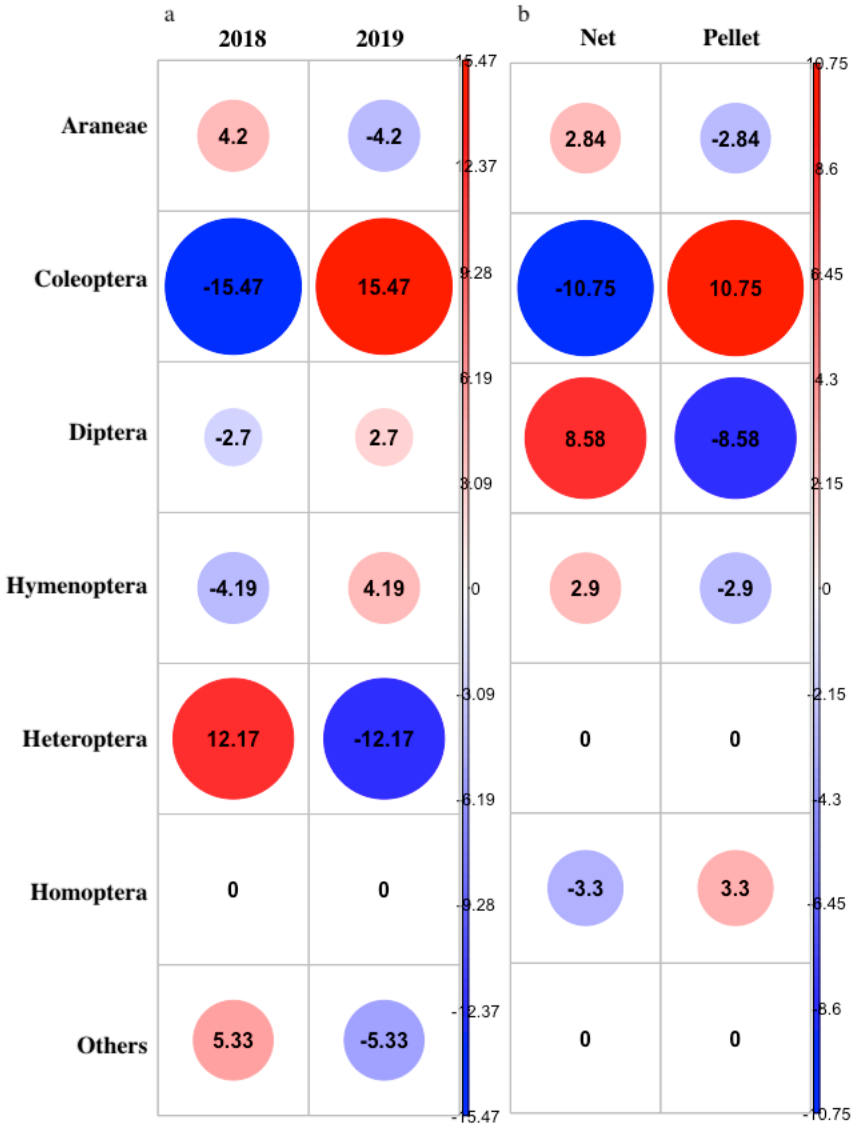

Figure 4. The charts show Pearson's Standardized Residuals (numbers within the circles), obtained through χ^2 test of independence on permuted dataset. Greater positive deviation from independence (positive association) is indicated with darker hues of RED; greater negative deviation from independence (negative association) is indicated with darker hues of BLUE. (**a**) Arthropods collected in the Mirandola breeding site of Reed Warblers in 2018 and 2019; (**b**) Comparison between the collection in 2019 and the prey items found in the bird droppings.

3.4. Prey Brought by the Reed Warbler to the Common Cuckoo

Our observation was that the diet that the Reed Warbler offered to the host cuckoo in its nest was mostly composed of larger prey (20–30 mm) than the average offered to the chicks (2–15 mm) of its own species (Table 4). Furthermore, the species brought to the cuckoo were different in percentage from those of the Reed Warbler diet at the Mirandola site (see Table 2).

Table 4. Number of prey items identified by visual analysis of three hours of video recordings of three Reed Warbler pairs provisioning cuckoo chicks at their parasitized nest in Mirandola in June 2018.

Prey Items	No.	%
Araneae	8	34.8
Heteroptera (*Dimorphopterus blissoides*)	1	4.3
Orthoptera Tettigoniidae [1]	5	21.7
Lepidoptera adults (moths)	2	8.7
Lepidoptera larvae	3	13
Others	4	17.4
Total	23	99.9

[1] Of which 3 Tettigonia viridissima nymph, 1 Tylopsis lilifolia, 1 Platycleis sp. nymph.

4. Discussion

The results of our research show that in all the Italian study sites, the main prey of Reed Warblers are Coleoptera, different from what has been detected in other parts of the breeding distribution of this bird (Table 1). In our samples, we did not find within fecal remains any evidence of flies (Diptera), although they were commonly present on the reeds and are generally reported to be preyed upon by Reed Warblers by other authors, both during the breeding season and during the migration (Table 1). This was confirmed by our netting session at Mirandola where, in fact, Diptera were much more represented than in the droppings (Figure 4b). Only a few wings of winged forms of aphids (Aphidoidea) were detected in the feces. This may depend on the high digestibility of these insects, which are very likely to be important prey for Reed Warblers (e.g., [16,19–21,41]). Indeed, aphids feeding on the plant lymph are very rich in carbohydrates, and thus they represent a highly nutritious prey. Vegetal remains, despite being regularly present in the feces of Reed Warblers, are probably not important for energy intake and, therefore, may only represent a small portion of their diet; indeed, according to [42], ca. 75% of arthropod and only 50% of vegetal energy are metabolized.

Most species in the Reed Warbler diet are typical of humid habitats; the birds capture them on plants of *P. communis*, *Tamarix* sp., *Juncus* sp. or on the ground close to the water. Examples of species captured on the ground are *Drypta dentata*, *Chlaeniellus vestitus*, *Pteronemobius heydeni*, *Paratettix meridionalis*. Torymidae found at Vendicari seem a fortuitous prey; these small hymenopterans are, indeed, parasitoids of other insects and often fly insistently over the vegetation to find their host. The ladybirds *Harmonia axiridis*, occur commonly where there are many aphids, that they prey on. The amphipods have certainly been caught on the surface of the water of the Biviere di Gela, at the edge of the reedbed. Concerning the ants, they are very abundant in all the ecosystems, included the edges of marshes; however, they appeared scarcely preyed upon, possibly because they produce unpalatable substances for the majority of vertebrates. Generally, ants are not usual prey for generalist vertebrates [43], even if [24] it has been found that 28.1% of these insects are prey to the Reed Warbler during their autumnal stopover in Morocco.

Our results show that Reed Warblers are partially generalist predators in Italy, which is consistent with their diet in north-central Europe. Their diet did not present structural differences across sites in Italy, but prey diversity was varied everywhere. Comparing our results with what is known from other countries (Table 1), it appears that the Reed Warbler greatly changes and adapts its diet across different locations. This supports the conclusion that Reed Warblers feed on the most available and palatable arthropods in the

environment in which they live. While in Italy they showed a clear preference (Coleoptera), almost a third of their diet was always composed of other miscellaneous taxa. The insects collected from the environment at Mirandola in 2018 yielded a high percentage of bugs (Heteroptera), which was lower in 2019 (Tables 2 and 3, Figures 3 and 4a). On the contrary, while beetles (Coleoptera) were found to be scarce in the warbler habitat (5.9% in 2018, 28.0% in 2019; Table 3, Figure 4), at Mirandola they were the most important prey in the warbler diet (69.1%) (Table 2, Figure 3). This may depend on the actual difference between abundance and availability from the Reed Warbler point of view, or could point to a partial specialization of the Italian population in a beetle-rich diet.

The comparison with some papers on the same subject, summarized in Table 1, shows, however, a noticeable discrepancy in our results; this is particularly evident in the high percentage of Coleoptera found across our sites, while generally they are scarcely represented in the European samples, but not in the Russian sample (79%, [14]) and the Moroccan sample (27.4%, [24]). The differences in the diet of Reed Warblers in the four Italian sites here investigated, very likely depends on the availability of certain prey species for the bird; however, it seems that these birds in our sites prefer the beetles more than other prey. One limitation of our study is that we cannot confirm that the absence of the scarcely chitinized Diptera from our samples was not due to an identification bias, given that they are otherwise well represented in European datasets. However, Diptera are almost absent from other European datasets, as well as the Moroccan study (1.9%, [24]), which unlike ours was collected via collar sample. Thus, a worthwhile venue for future studies could be to experimentally test whether fecal samples might, indeed, be biased against the detection of Diptera. On the other hand, the lack of beetles in most European samples certainly depends on a true absence, as these insects are provided with a very robust chitin and consequently they cannot disappear in the collar-samples or other sampling methods. For this reason, we argue that the prevalence of beetles in our sites is a regional-level pattern, with Italian Reed Warblers showing a slightly different diet than their central European counterparts.

Finally, the aphid *Hyalopterus pruni* is generally considered an important prey for acrocephaline birds; it lives during the winter on *Prunus* species, and moves to *Phragmites* and *Arundo* species in summer, reaching very high populations. We were able to find important populations of this species on reedbeds only at Mirandola and Pergusa. Habitat composition and use has been scarcely investigated to reveal several ecological aspects of this species' diet. This might be a promising research direction as, when it is analyzed together, the potential interference of human activity [44] upon the suitable habitat structure might indeed reveal unexpected ecological aspects of the focal species [45].

It is currently unclear how the Reed Warbler will adapt to climate change across Europe; while this species could be favored by longer breeding periods [32], it can also be expected to decline alongside its reedbed habitat [46]. Our results show that as a generalization, a shift in the arthropod composition might affect it less than other, more specialized species. However, Italian populations of Reed Warbler might still be at risk, as reedbed viability in southern Europe could be more severely impacted by severe droughts [46].

Video recording proved to be an excellent method to identify prey items delivered to cuckoo chicks in the nest. They included a high percentage of spiders and large bush crickets; further, larvae and adult moths entered the Reed Warbler diet, while they were absent in its fecal samples. This would be consistent with begging calls by cuckoo chicks being a supernormal stimulus, not only in terms of prey quantities [35], but also as prey size. Grim [36] found an unusual percentage of Diptera Syrphidae in the diet of Reed Warbler chicks and in cuckoo chicks reared by Reed Warblers (3.3 and 3.8%, respectively). If further investigations confirm the findings of our small sample size, this would represent a noticeable difference in prey provisioning. This, in turn, would suggest that Reed Warblers are shifting not only prey type choice but also foraging habitat, as bigger prey items given to cuckoo chicks, e.g., bush crickets, are commonly found in grassland, whereas the smaller prey given to Reed Warbler chicks are instead found within reedbeds [36]. In contrast to short-distance migratory species parasitized by cuckoos, Reed Warblers are among

those hosts whose parasitism rate would not change as a product of asynchrony in timing generated by climate change shifts [47]; therefore, they might be used a reliable indicator of specific insect presence.

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