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Influence of colony traits on ectoparasite infestation in birds breeding in mixed-species colonies

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Abstract

Coloniality in birds is often associated with an increase in parasite burden, but most studies have focused on mono-specific colonies. In this study, we took advantage of mixed-species colonies formed after the provision of artificial breeding structures for restoring the lesser kestrel (*Falco naumanni*) population in Portugal, to investigate the influence of colony traits on ectoparasite infestation. We sampled four groups of ectoparasites (carnid flies, haematophagous mites, louse flies, and chewing lice) in four hosts: lesser kestrels, European rollers (*Coracias garrulus*), feral pigeons (*Columba livia*), and spotless starlings (*Sturnus unicolor*). Host species was the best predictor for the composition of the ectoparasite infracommunity, with each host species having a distinct infracommunity of ectoparasites, regardless of colony traits such as size, density, or host richness. The abundance of the most common ectoparasite, *Carnus hemapterus*, was influenced by colony composition – number of nests of each host species – rather than by colony size or density, with its abundance being diluted with increasing numbers of less suitable hosts such as starlings. The increased contact between multiple species of hosts in mixed-species colonies may complexify host-parasite interactions and challenge our current knowledge on the ecological relationships between host sociality and parasitism.

Key words: host-parasite interaction; dilution effect, *Carnus hemapterus*, haematophagous mites, louse flies, chewing lice

Key Findings

- In mixed-species colonies, host identity dictated the ectoparasite infracommunity.
- Colony richness, size, and density did not shape the ectoparasite infracommunity.
- Carnid fly *Carnus hemapterus* was the most common ectoparasite in these colonies.
- The abundance of carnid flies was driven by colony host composition.
- Less suitable hosts diluted carnid fly abundance in other hosts.

Graphical abstract with the main findings on the influence of colony traits on ectoparasite infestations in mixed-species colonies breeding in artificial nest-sites in Southern Portugal.

Introduction

Avian colonial breeding is often associated with higher parasite prevalence and intensity, as the increased proximity and contact between different group members facilitates ectoparasite transmission (Brown and Brown, 1986; Côté and Poulin, 1995; Tella, 2002; Patterson and Ruckstuhl, 2013). This pattern differs according to the mode of ectoparasite transmission, with contact-transmitted parasites (e.g. mites or lice) being positively correlated with group size, whereas no correlation is expected for mobile parasites (e.g. biting flies, mosquitoes) due to encounter dilution effects (Patterson and Ruckstuhl, 2013, but see Veiga et al. 2020). Parasites can directly or indirectly affect the reproductive and survival components of individual fitness by decreasing nestling body condition and survival, or by reducing adult fecundity (Brown and Brown, 1986; Marino and Potti, 1995; Arriero and Møller, 2008; Hoi et al. 2018). This may consequently impact avian population dynamics, for example by decreasing breeding-site fidelity and foster both the formation and extinction of colonies (Brown and Brown 2004; Calabuig et al. 2010; Brown et al. 2017; Sanz-Aguilar et al. 2020). Group living may also result in increased physiological social stress due to increased competition for resources, which synergises with host susceptibility to parasite infestation (Beldomenico and Begon, 2010; Kappeler et al. 2015). However, most studies assessing the relationship between parasitism and colonial breeding targeted monospecific colonies or the interaction between a single host and a single parasite species, overlooking the likely interactions among different hosts, or different parasites within the same host – the parasite infracommunity (Bush et al. 1997; Holt et al. 2003; Poulin, 2007; but see Whiteman and Parker, 2004, Veiga and Valera, 2020). In fact, bird colonies are often composed by different species, resulting in mixed-species colonies, that reshape the structure and functioning of communities through the increased interaction between the grouping species (Gaglio et al. 2018; Catry and Catry, 2019). The formation of mixed-species colonies may allow species to acquire the benefits of group living while reducing the

costs associated with intraspecific competition (Møller et al. 1993; Campobello et al. 2012), although multi-species associations may also result in costs through factors like kleptoparasitism or interspecific competition (Gaglio et al. 2018; Catry and Catry, 2019).

In multi-species assemblages, intra and interspecific social contacts likely increase and complexify the interactions between different host and parasite species (Valera et al. 2003; Keesing et al. 2006). High host diversity could decrease infestation risk through dilution effects, as different host species may be differently susceptible and/or competent to different parasite species, but this effect may not always occur and likely depends on specific community compositions (Keesing et al. 2006; Randolph & Dobson 2012; Civitello et al. 2015; Halsey 2018; Martin et al. 2019). Mixed-species assemblages may also promote interspecific parasite exchange, especially within generalist parasite species. Whether the occurrence of several host species in bird assemblages increases or decreases the prevalence and abundance of parasites and their relationship with colony size or density remains poorly studied (Tella et al. 1998; Valera et al. 2003; Veiga et al. 2020).

Studying the ecological relationship between parasites in mixed-species colonies may be harder than in mono-specific groups due to the complexity of addressing several species and their interactions, with the solution often being to simplify the system into one-one actors. In this study, we investigate host-parasite relationships in mixed-species colonies, by considering several host and parasite species and by simultaneously looking at several colony traits that may influence the prevalence and abundance of ectoparasites. In southwestern Iberia, colonial lesser kestrels *Falco naumanni* breed in man-made structures that attract other species, including common kestrels *Falco tinnunculus*, European rollers *Coracias garrulus*, barn owls *Tyto alba*, little owls *Athene noctua*, jackdaws *Corvus monedula*, spotless starlings *Sturnus unicolor* and feral pigeons *Columba livia*; thus forming mixed-species colonies (Catry and Catry, 2019). These multi-species assemblages provide an ideal opportunity to understand the

ecological relationships between multiple host and parasite species (Valera et al. 2003). Here, we study how colony traits such as colony size, host species richness, density, and composition, influence the ectoparasite infracommunities. Specifically, we aim to: (1) describe the ectoparasite infracommunity of different avian host species breeding in mixed-species colonies; (2) determine the main colony traits potentially affecting ectoparasite infracommunity composition; and (3) assess how colony traits drive the abundance of *Carnus hemapterus*, a widespread haematophagous fly parasitising at least 64 host species from 24 avian families (Grimaldi 1997; Brake 2011) and the most common ectoparasite in our study area. For this, we study the ectoparasites in nestlings and nests of the four main common bird species in 30 mixed-species colonies.

Materials and methods

Study system: hosts and parasites

This study took place in the Castro Verde Special Protection Area, Southern Portugal (~37°43'N, 7°57'W). In the framework of European LIFE projects to recover lesser kestrel populations, more than 800 artificial nests were provided in the study area, including new cavities opened in abandoned rural buildings, artificial cavities in newly built breeding walls and towers with up to 90 cavities, wooden nest-boxes, and clay pots (Catry et al. 2009). Given the low availability of nest-sites in the area, nest-site provisioning was very effective at increasing the lesser kestrel population and attracted other bird species to nest in these structures (Catry et al. 2009; Catry and Catry, 2019; Gameiro et al. 2020).

Mixed-species colonies occur in adobe-built abandoned farmhouses (nests located in cavities that appear with building degradation – ‘natural cavities’) or in artificial nest structures. Here, ‘colony’ was defined as a site with at least two active nests - usually a single building, or a main building with few smaller, annex structures (e.g. a farmhouse with a separated shed).

Because nests occur inside cavities, the maximum size of the colony is ultimately dependent on the number of available nest-sites, which can go from just a couple to more than a hundred cavities. Larger colonies (> 40 breeding pairs) tend to have higher density of nests and a higher richness of host species and can be dominated by either lesser kestrels or spotless starlings (Table 1). Larger colonies usually also hold a few pairs of jackdaws and feral pigeons, one to three common kestrels and European rollers, and one pair of barn owl and/or little owl (Catry and Catry, 2019). Distance to nearest colony was always greater than 100 meters (min = 150 m, max = 4266 m).

This study focused on the four most common bird species in these mixed-species colonies: lesser kestrels, European rollers (hereafter rollers), spotless starlings (hereafter starlings) and feral pigeons (hereafter pigeons). Lesser kestrels and rollers are single-brooded, secondary cavity nesting birds with a trans-Saharan migration (Catry et al. 2015). Lesser kestrels are typically colonial, with colonies reaching up to 80 breeding pairs in the study area (Catry et al. 2009). They arrive at the breeding grounds in early February and typically lay 4 to 5 eggs in April-May. Nestlings are born with a down feather coat after a 28 days incubation and usually fledge at 36 days (Catry et al. 2015). Rollers (up to four breeding pairs) are usually found breeding in lesser kestrel colonies but can also nest in isolated nests in farmhouses or in nest-boxes placed on trees or telephone poles. Egg laying is asynchronous with 3 to 6 eggs laid in May-June. Nestlings are born featherless after 17-19 days of incubation and fledge at 20-25 days (Catry et al. 2015).

Starlings and pigeons are resident in the study area and lay several clutches along the breeding season. Starlings generally nest at high densities, with up to 32 breeding pairs in these mixed-species colonies/artificial nesting structures (Table 1). Starlings lay 3 to 6 eggs, incubation takes ca. 12 days and nestlings fledge at 20-22 days old (Muriel et al. 2013). Pigeons occur at lower densities, with up to eight breeding pairs in these colonies (Table 1). They lay up to two

eggs, incubation lasts around 18 days, and nestlings fledge at ca. 28 days old (Johnston and Janiga, 1995). Contrary to lesser kestrel and roller nests, which are usually just comprised of dirt and prey remains, starlings and pigeons build their nest with sticks, straws and other vegetable material, and pigeons also accumulate droppings inside the cavity.

The most common ectoparasites found in hosts' nestlings in our study area were: carnid flies (*Carnus hemapteru*, Diptera: Carnidae), haematophagous mites (Acari: Mesostigmata), louse flies (Diptera: Hippoboscidae), and feather lice (Phthiraptera); and they were considered in this study as four separate groups since: i) identification to the species level and quantification of each species in each nest and host was not feasible, and ii) working at the species level falls beyond of this study, i.e., to investigate the influence of colony traits on the general patterns of ectoparasite infestation. Yet, some information about identified species is provided at the discussion. The generalist *Carnus hemapterus* (hereafter *Carnus*) is a nidicolous, haematophagous fly infesting many bird taxa. *Carnus* loses its wings after finding a host, with peak infestations occurring during the mid-nestling stage, and with a resistance stage (pupa) that remains in the nest debris until the next breeding season when adult flies emerge (Valera et al. 2006a; Calero-Torralbo and Valera, 2008). Despite being a mobile ectoparasite, it was found to increase with colony size and host density (Hoi et al. 2010; Veiga et al. 2020; but see Liker et al. 2001). Mites are also generalist ectoparasites, feeding mostly on blood or skin tissue of nestlings and adults. Mite populations usually grow quickly in the nest during their host's breeding season and overwinter in the nest (Burt et al. 1991). Being contact transmitted parasites, they are expected to increase with colony size (Davis & Brown 1999). Louse flies are also haematophagous parasites, but imagoes do not lose their wings and can fly between nests, and they have often a more restricted host range (Veiga et al. 2019). Imagoes spend most of their time feeding on the host's body, and pupae are formed and apparently overwinter in the nest (Boyd, 1951). Louse flies are mobile parasites and should not

be affected by host coloniality (Poulin 1991). Feather chewing lice are permanent ectoparasites with a host range usually confined to species within the same family or genus (Clayton et al 2008). They live on the feathers and seldom leave their host except to transfer among individuals through direct contact (e.g., between parents and their offspring), and thus are likely influenced by host sociality. (Clayton and Tompkins 1995; Rózsa et al. 1996; Whiteman & Parker, 2004; Ortego et al. 2007).

Data collection

Ectoparasite estimation

Field work was conducted during the breeding season of 2018, from mid-April to mid-July, by sampling unclean nests occupied by the four studied host species. Colonies and nests within colonies were randomly sampled during a lesser kestrel and roller monitoring programme. A total of 30 colonies were sampled: seven with all four hosts; four with lesser kestrels, rollers and starlings; two with lesser kestrels, rollers and pigeons; six with lesser kestrels and rollers; two with lesser kestrels and pigeons; two with rollers and pigeons; five with lesser kestrels; and two with rollers (Table 1). Overall, we sampled 261 nests: 141 lesser kestrel, 33 roller, 38 pigeon and, 49 starling nests. Nests were not cleaned prior to this study for two reasons: i) removing old nest detritus was not possible for most of these nests (especially for natural cavities), ii) unclean nests provide results that better resemble the natural conditions (Møller, 1989). Soiled nests may influence the prevalence and numbers of some ectoparasites in these colonies and may cause some unaccounted variability in the data, particularly when considering ectoparasites with most of their life cycle, including long diapauses, occurring in the nests (Veiga et al. 2020). Thus, this potential bias was considered in the statistical approach (see Data analysis below) and its influence was discussed.

The presence and number of ectoparasites were assessed by examining all nestlings in

each host species nest. Each nest was sampled at two different periods: at mid-nestling stage (around 8-12 days old), and few days before fledging. During each of these two periods, each nestling was taken from its nest and placed in a transparent plastic bag to avoid losing mobile parasites (e.g. louse flies). The nestling was then taken from the bag and the number of carniid flies and louse flies on the bag and on the body surface and sheaths of the nestling were counted twice and then averaged (Roulin, 1998; Václav et al. 2008). Feather lice were sampled by carefully scanning the nestlings' sheaths and feathers (Valera et al. 2003; Ortego et al. 2007). Mites were sampled by resting the observer's hand on the bottom of the nest for one minute and then counting the number of mites on it twice (later averaged, see below). Nestlings were then carefully placed back on the nests. The number of each ectoparasite group was calculated as the sum of parasites in all nestlings from the same brood. The maximum number of parasites from both sampling periods was selected. All work was approved by the relevant Portuguese authorities (Instituto da Conservação da Natureza e das Florestas).

Mixed-species colony traits

The following variables were recorded for each sampled nest: host species identity, type of nest (natural cavities in farmhouses, artificial cavities, clay pots, wooden nest-boxes), brood size (number of nestlings), and the sampling date of the first sampling period, which was used as a proxy for ectoparasite seasonal effects (Calero-Torralbo et al. 2013). The species occupying the focal nest in the previous year was also recorded, as some ectoparasites spend most of their life cycle in the nest and their occurrence and abundance may depend on previous breeding seasons (Valera et al 2006a). Each nest was also categorized according to four main colony traits: colony host richness (number of host species in the colony); colony size (number of active nests, i.e. nests with nestlings, of all species); colony density (the inverse of the average

distance to the three active closest nests, in meters); colony composition (four variables, each with the number of active nests of each host species) (Table 1). Colony size was grouped into three categories: small (up to 20 nests), medium (21-40 nests), and large (more than 40 nests). Similarly, colony density was grouped in low (average distance to the three closest nests > 5m), medium (1-5 m), and high density (< 1m). All traits (except colony composition) acknowledged all nesting bird species, including less abundant species (common kestrels, jackdaws, little owls, and barn owls).

Data analysis

The nest was used as the sampling unit and thus the term infracommunity refers to the community of ectoparasites in a given nest (see Veiga and Valera, 2020 for a similar approach). Accordingly, the prevalence and mean intensity of each ectoparasite group for each host species was calculated, respectively, as the proportion of infested nests amongst all nests sampled and the mean number of parasites of all infested nests. Because some nests were not sampled during both periods, prevalence and mean intensity of each parasite were calculated only for nests sampled at each parasite's peak infestation stage (assessed from our own data set and the literature; see following sentences), so that comparisons could be made between the different hosts (see Results). *Carnus* infestation peaks at mid-nestling stage (prevalence of 87% and 20% for the first and second sampling period, respectively; see also Václav et al. 2008), while feather lice and louse flies are more common in fully-grown, feathered nestlings (lice prevalence of 2% and 86%, and louse fly prevalence of 26% and 47% for the first and the second sampling period, respectively; see also Muñoz et al. 1993). The prevalence of mites did not differ between the two sampling periods in our study (23% and 19% for the first and second sampling period, respectively). Accordingly, prevalence and mean intensity values of carnid flies were obtained from all nests sampled in the first period, values for lice and louse flies

from nests sampled in the second period, and values for mites were obtained from all nests (sampled both in the first and/or in the second period).

Fisher's exact tests, and Kruskal-Wallis H test followed by bootstrap 2-sample t-tests, were used for comparing parasite prevalence and mean intensity, respectively, among hosts, using 2000 replications for both estimation of confidence intervals and bootstrap t-tests (Rózsa et al. 2000; Veiga et al. 2019).

A Principal Component Analysis (PCA) was used to explore the potential correlation among the colony variables without constraints (random factors), prior to further analysis (sup. Material – Fig. S1) (Borcard et al. 2011; Václav and Valera, 2018). To examine how colony traits influence the ectoparasite infracommunity composition, a partial Canonical Correspondence Analysis (CCA) was used including all sampled nests and conditioned by brood size, sampling date and colony ID (Borcard et al. 2011). The presence-absence matrix of each parasite group in each nest was used as the response variable (nests without parasites were removed; Oksanen, 2020). Host species, nest type, the species using the nest in the previous year, and colony host richness, size, density, and composition, were used as the predictive variables. The variance inflation factor (VIF) was used to assess collinearity between predictive variables (all VIFs <10, min = 1.01, max = 6.61) and forward and backward selection were performed to choose the best CCA model (Borcard et al. 2011; Václav and Valera, 2018).

To assess how colony traits (richness, size, density, and composition) influence the abundance (i.e. including both infested and non-infested nests) of *Carnus*, the most common ectoparasite in our mixed-species colonies, a generalized linear mixed models (GLMMs) was used. The model followed a negative binomial distribution of errors to account for the aggregated distribution of *Carnus* among hosts (Václav and Valera, 2018). *Carnus* abundance in each nest was used as the response variable and colony ID as a random variable. The sample-to-variable ratio and the high correlation between some colony traits did not allow the

inclusion of all variables in a single model (Fig. S1). Because colony size, density, composition, and host richness were correlated, the number of variables were reduced by choosing the most meaningful traits. Colony size was removed in favour of colony density because the latter reports about the nest density around each focal nest, while colony size attributes the same value to all nests within the same colony. Colony composition was selected instead of colony host richness because it contains information about the identity of the host and its abundance, rather than just the number of species found in a given colony. Nonetheless, different combinations of variables were tested, but did not provide additional or different outputs. The results shown here refer to the most biologically meaningful model, that includes the following, non-correlated, explanatory variables: host species, brood size, sampling date, type of nest, colony density (categorical), number of lesser kestrel nests, and number of starling nests (highest VIF = 5.07, for type of nest). The number of roller and pigeon nests were also discarded as their abundance was considerably lower than the one of lesser kestrels and starlings and both were correlated with the number of starling nests (Fig. S1). A model-averaging approach based on the Akaike Information Criterion (AIC) was used to obtain weighted average estimates for fixed parameters (Grueber et al. 2011). Model averaging was performed on models with the cumulative sum of corrected AIC (AICc) weights > 0.95.

All continuous explanatory variables were scaled and centred prior to analysis. All analyses were conducted with R software 3.6.1 (R Core Team, 2016), using the packages Vegan 2.5-6 (Oksanen, 2020), nlme 3.1-148 (Pinheiro et al. 2020), lme4 1.1-23 (Bates et al. 2020) and MuMIn 1.43.17 (Bartón, 2020).

Results

Prevalence and intensity of ectoparasites in mixed-species colonies

The composition of the infracommunity of ectoparasites differed among the various host

species breeding in mixed-species colonies: lesser kestrels and pigeons had all four types of ectoparasites whereas starlings had no louse flies and rollers had no lice and almost no louse flies (Table 2).

Carnus hemapterus was the most common ectoparasite in mixed-species colonies but its prevalence differed among the four host species (Fisher test $p < 0.001$), being highest in lesser kestrels and rollers and lowest in pigeons. *Carnus* mean intensity was more than double in rollers than in lesser kestrels (bootstrap 2-sample t-test: $t = 4.219$, d.f. = 29.65, $p < 0.001$) and lowest in pigeons (Table 2). Mites were the second most common ectoparasite in mixed-species colonies, reaching the lowest prevalence and intensity in lesser kestrels (Table 2). Rollers, pigeons, and starlings had similar mite prevalence and intensity (prevalence; Fisher test: $p = 0.969$; intensity: Kruskal-Wallis H: 1.11, d.f. = 2, $p = 0.57$). Lice were almost exclusively found in lesser kestrels and pigeons, with similar prevalence and intensity (prevalence: Fisher test: $p = 1.00$; intensity: bootstrap 2-sample t-test: $t = -1.45$, d.f. = 22.65, $p = 0.160$) and louse flies were mostly found in pigeon nests (Table 2).

Influence of colony traits on ectoparasite infracommunity in mixed-species colonies

The partial Canonical Correspondence Analysis (CCA), conditioned by sampling date, brood size, and colony ID, revealed that host species was the only predictor of parasite infracommunity (in terms of presence/absence), being the only variable selected following both forward and backward selection processes (final CCA adjusted R-squared = 0.18; $X^2 = 0.2$, $F = 18.2$, p -value = 0.001). Conditioned and constrained partitioning explained 27.0% and 16.7% of the total inertia (“variance”, total inertial = 1.11, respectively). The ectoparasite infracommunity of each host species was best described by the presence of louse flies, followed by lice and mites (Fig. 1). Louse flies were clearly associated with pigeons and lice commonly infested pigeons and lesser kestrels. Mites showed a positive association with starlings, rollers,

and pigeons, whereas *Carnus* was negatively associated with pigeons (see also Table 2).

Influence of colony traits on Carnus hemapterus abundance in mixed-species colonies

Host species had a significant influence on *Carnus* abundance, being highest in rollers and followed by lesser kestrels, starlings, and pigeons (the reference level) (Table 3). Model averaging revealed that *Carnus* abundance increased significantly with brood size and marginally significantly with increasing numbers of lesser kestrel nests in the colony. In contrast, it decreased significantly with increasing numbers of starlings' nests. The abundance of carnid flies also decreased, although marginally significantly, in clay pots when compared to other types of nests. Colony density had no influence on *Carnus* abundance. The models with $\Delta AICc < 2$ after model averaging and the best two models can be found in tables S1 and S2, respectively, in the Supplementary Material.

Discussion

This study provides information on the ecology of avian parasitism in mixed-species colonies. To our knowledge, there is little information on the factors influencing host infestation by ectoparasites in colonies formed by multiple species of hosts and even less at the parasite infracommunity level. Four main ectoparasite groups – carnid flies, louse flies, lice, and mites – in four different host species were analysed, including the conservation reliant lesser kestrel and European roller (Gameiro et al. 2020). It was found that the main driver for the differences in composition of the ectoparasite infracommunity was the identity of the host species, regardless of colony traits such as size, density, or richness. Accordingly, variation in the abundance of the most common ectoparasite (*Carnus hemapterus*) in these mixed-species assemblages was explained by colony composition (number of nests of each host species), rather than by overall colony size or density.

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351 The importance of host species identity for ectoparasite ecology has already been described in
352 small mammals (Krasnov et al. 2008; Lareschi and Krasnov, 2010). These results suggest that
353 the four host species vary in their susceptibility (sensu Martin et al. 2019) to different
354 ectoparasite groups (Keesing et al. 2006). Even the prevalence and intensity of the most
355 common and broadly considered generalist species, *Carnus hemapterus* (Veiga et al. 2019)
356 varied considerably between hosts. Nest- and host-related characteristics may account for some
357 of the differences found, as suggested by our results on *Carnus*. Pigeons and starlings were the
358 least attractive hosts. These bird species use vegetable material when building their nests,
359 which was found to be avoided by *Carnus* (Valera et al. 2006b; Valera et al., 2018). Pigeons
360 also accumulate droppings inside the cavity that may limit the suitability of the detritus for the
361 larval and pupal stages of this ectoparasite (Veiga et al. 2019). Carnid flies seemingly also
362 prefer rollers to lesser kestrels since the former bird species holds more than twice as many
363 *Carnus* as lesser kestrels. Nestling kestrels are born with a down feather coat that may hinder
364 the access to blood by ectoparasites, when compared to the naked body of roller nestlings.

365 Mites also infested all host species but were less prevalent and abundant on lesser kestrels.
366 In a dry climate like the one of the study area, the humidity required by mites (Nordenfors et
367 al. 1999) may be secured by the vegetable material used in starling or pigeon nests, when
368 compared to a mostly sandy substratum of lesser kestrel or roller nests. However, as rollers
369 arrive later to the breeding grounds from their spring migration, they are likely to re-use a nest
370 previously occupied by starlings or pigeons (hence more humid), potentially explaining why
371 rollers had higher prevalence of mites than lesser kestrels (see below and Veiga et al. 2020).

372 The factors accounting for differences in prevalence of lice and louse flies among host
373 species are not so clear and their identification goes beyond the aims of our study. Nonetheless,

374 it is worth reporting such differences in mixed colonies where the contact among species and
375 individuals can facilitate transmission. Lice were mostly present on lesser kestrels and pigeons,
376 with just a single louse found on one starling nestling and none on rollers. Although lice were
377 not identified to the species level, these ectoparasites are considered species-specific and no
378 studies were found reporting the same lice species infecting both pigeons and lesser kestrels
379 (Rózsa, 1990; Ortego et al. 2007; Calabuig et al. 2010; Galloway and Lamb, 2015). Factors
380 such as gregariousness can explain why species like pigeons or lesser kestrels have many lice.
381 In contrast, species usually breeding in low densities (e.g. rollers) could be less appropriate for
382 lice. Because chewing lice are mostly found on feathers, they may be more common and
383 abundant on post-fledging juveniles or adults, when compared to young nestlings (Liker et al.
384 2001; Ortego et al. 2007). Louse flies were found almost exclusively on pigeons, with only
385 three lesser kestrel and one roller nest infested and no starling nest infested. Examination of six
386 louse flies demonstrates that at least three different species occur in our study area:
387 *Pseudolynchia canariensis* in pigeons and lesser kestrels, *Ornithophila gestroi* in lesser
388 kestrels, and *Hippobosca longipennis* in rollers. *P. canariensis* has a wide host range and is
389 common in pigeons and in the *Falco* genus (Maa, 1966; Veiga et al. 2019), although to the
390 authors' knowledge this the first time *P. canariensis* is reported in lesser kestrels. *Ornithophila*
391 *gestroi* has already been reported in lesser kestrels, with similar abundances but at higher
392 prevalence than the one found in this study (Tella et al. 1997). *Hippobosca longipennis* is an
393 ectoparasite typically found in carnivores (Maa, 1969; Mihalca et al. 2019). Besides anecdotal
394 references, there are no clear reports of *H. longipennis* on birds, so that this finding can be also
395 considered circumstantial. Yet, the clear pattern found (preference of louse flies for pigeons)
396 could be explained either by a higher abundance of the louse fly species typical of this particular
397 host and/or interspecific differences in the suitability of each host species and their nests
398 characteristics for the co-existing louse fly species (e.g. Veiga et al. 2019). Understanding the

pattern of occurrence of these parasites and what factors contribute to the interspecific differences found here requires detailed knowledge of the natural history and host choice criteria of these species and goes beyond the aims of this study. Nonetheless, it is important to remark that even in situations favouring intra and interspecific parasite transmission, clear preference patterns are evident.

Ectoparasite abundance in mixed-species colonies is mostly driven by colony composition rather than colony size or density

It has been widely acknowledged that social breeding incurs in higher risk of parasite and pathogen transmission, with larger, denser colonies, having higher prevalence and intensity of ectoparasites, even in mobile, non-contact-transmitted species (Brown and Brown, 1986; Côté and Poulin, 1995; Hoi et al. 1998; Kleindorfer and Dudaniec, 2009; Veiga et al. 2020). Most of these studies have focused on mono-specific colonies or on single parasites species, overlooking the complex multi-species associations that often occur in nature (Valera et al. 2003). This study's results on mixed-species colonies suggest that colony size, density or richness, common characteristics used to measure host sociality, do not affect the infracommunity of ectoparasites in each host, nor the abundance of the generalist *Carnus hemapterus*. Although the correlation among these variables prevented the examination of the isolated effect of each trait, none were selected using a model-averaging approach, suggesting a lack or weak effect of these variables in our study system. Instead, host species was the main predictor for *Carnus* abundance. Besides colony composition, which will be discussed below, *Carnus* abundance was found to increase with brood size. More nestlings in a nest means more food resources for ectoparasites and higher heat and CO₂ emissions that may facilitate nest detection. Thus, an increase in *Carnus* abundance with larger broods was expected (see also Veiga et al. 2020). In contrast to previous results (Calero-Torralbo et al. 2013; Veiga et al.

2020), the abundance of *Carnus* was not influenced by sampling date - a proxy of *Carnus* emergence patterns. But, contrary to these studies, the results reported here consider simultaneously different host species, so the lack of a global effect of date may be related with merging host species with different breeding phenologies, number of clutches and nestling development rates. There was also no influence of type of nest except for a marginally significant decrease in clay plots. Clay pots are shallower than cavities or nest-boxes, and some of them have a low (sometimes almost inexistent) amount of detritus, which may inhibit egg deposition or larvae development by *Carnus*.

The main finding of this study is that in mixed-species assemblages, rather than colony size or density, it is the composition of the colony – the number of nests of the various host species – that influences the abundance of the most common ectoparasite. *Carnus* abundance decreased with increasing number of starling nests and increased with increasing numbers of lesser kestrels. Because *Carnus* mean intensity varied considerably between hosts, with lesser kestrels hosting more than three times more *Carnus* than starlings, starling-dominated colonies will have lower *Carnus* loads than lesser kestrel dominated ones. If the proportion of less competent hosts like starlings increases in a mixed colony, then the total amount of ectoparasites will decrease. A dilution effect is occurring by “adding” less competent host species, starlings (or pigeons), to the colony (Johnson and Thieltges, 2010; Civitello et al. 2015). Because most *Carnus*’ life stages occur in the nest (Valera et al. 2006a), *Carnus* will produce less offspring in a colony dominated by less suitable hosts than in a colony with more suitable hosts. In parallel, the number of *Carnus* will increase in colonies with increasing number of preferred hosts such as lesser kestrels and rollers. This suggests that increasing colony diversity would only result in a dilution effect – decreasing parasitism – if the added host species are less suitable for a given parasite. This dilution effect may also depend on the parasite species. For instance, pigeons are avoided by *Carnus* but are preferred by *P.*

canariensis (Veiga et al. 2019). Thus, having pigeons in colonies may decrease *Carnus* abundance but increase *P. canariensis* in lesser kestrel nests.

The findings of this study must be considered in the light of some limitations. Colony size, density, and host richness were correlated so that larger colonies also tended to have higher nest density and higher richness of hosts. This prevented the investigation of the effect of each colony trait, only allowing the comparison between smaller colonies (less dense and less rich) from larger ones (denser and richer). Increasing the sample size (which was not possible for this study due to logistics constraints) could potentially allow for the investigation of the correlation/interaction between colony traits and reveal other effects we have been unable to detect. However, because large colonies had different host species composition – some dominated by lesser kestrels and others by starlings – this study was able to untwine the effects of colony composition on ectoparasite infestation patterns. Also, as opposed to similar works (Veiga et al. 2019), nests in this study were not sanitized before sampling. As such, ectoparasites that spend most of their life cycle in the nest, such as carnid flies, were not removed, and so the prevalence and abundance (and their variability) found in this study may have been at least partially influenced by the outcome of previous breeding seasons (Valera et al 2006a). Nonetheless, results from this study suggest that the host species occupying the focal nest in the previous year had no effect (variable not selected) on the ectoparasite infracommunity nor on *Carnus* abundance. The influence of the abundance of ectoparasites during the previous season may be diluted for several reasons. For instance, *C. hemapterus* – arguably the most nest-based ectoparasite from the ones addressed – was found to rapidly colonize new (and clean) nests and to discriminate between potential hosts, favouring some of them (Veiga et al. 2019, 2020). Moreover, insect predation after the breeding season may decrease the abundance of carnid pupae (Salido et al. in press). These facts suggest that our approach may have had little impact on the results found for mobile ectoparasites (carnid flies

and louse flies) and for parasites spending all their life cycle on the host's body (e.g., lice). On the other side, by examining uncleaned nests, this study reflects more closely the natural conditions than studies using sanitized nests, and thus our results are likely to apply to birds occurring in natural assemblages (nest-sites; Møller, 1989). Lastly, this study examines ectoparasite groups instead of individual species. This limits our capacity to establish species-specific parasite-host relationships, but it does not preclude disentangling the effects of colony traits on the general patterns of ectoparasite infestations.

Final considerations

Previous studies on monospecific colonies have found a positive correlation between colony size and density and *C. hemapterus* infestations (Hoi et al. 1998, 2010; Veiga et al. 2020, but see Liker et al. 2001). The present study in mixed-species colonies revealed that colony traits such as size, density, richness, or composition, had no effect on ectoparasite presence, with each avian host maintaining a distinct ectoparasite infracommunity. It also revealed that colony composition, rather than colony size or density, influenced the infestation of the most common ectoparasite in these assemblages. *Carnus hemapterus* can be found from North America to Europe and Asia infesting a wide range of bird species, so the implications of this study go beyond our study area (Grimaldi, 1997; Brake, 2011; Ganbold et al. 2020). The association between farmland buildings or artificial nests and secondary cavity nesters such as lesser kestrels and rollers is common in other European countries, such as Spain and Italy (Campobello et al. 2012; Negro et al. 2020). Similar natural species assemblages occur in sandstone cliffs and bridges (e.g., in south Spain), where many species, including the ones studied here (and others such as common kestrels, little owls, jackdaws, bee-eaters *Merops apiaster*, and rock sparrows *Petronia petronia*) coexist and re-use the same natural and man-made cavities (Valera et al. 2003; Veiga et al. 2020). Lesser kestrel colonies throughout Europe that rely on man-

made buildings are also likely comprised by other species (Blanco & Tella 1997; Campobello et al. 2012). Densities in these “natural scenarios” are likely lower than in artificial breeding towers, but it may depend on ecological circumstances like availability of nest-sites). Our results that larger colonies do not necessarily translate into higher risk of ectoparasite transmission, and that this effect is influenced by the species composition of the colonies, are likely applicable to other mixed-species colonies of birds or even other non-bird mixed-species groups (Goodale et al., 2020), but the exact outcome may vary depending on the specific parasites and hosts forming the assemblages.

The interpretation of the ecological relationships between parasites and hosts should consider the interactions occurring among different hosts and their parasites and should be studied as they occur in nature, i.e. considering mixed-species assemblages, which are often overlooked despite being widespread. More studies on the natural history of ectoparasites and on host-parasite interactions in multi-species group living are clearly needed to fully understand the complex relationships between parasites and sociality.

Supplementary material

Submitted online as a separate file.

Data

none

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Author Contribution

All authors were responsible for conceiving and designing the study. JG and IC conducted data collection. JG and JV performed statistical analyses. JG wrote the manuscript. All authors reviewed and contributed to the final article.

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539 **Conflicts of Interest**

540 The authors declare there are no conflicts of interest.

541

542 **Ethical Standards**

543 Trapping and handling of birds during this study was approved by Instituto da Conservação da
544 Natureza e Florestas (License 547/2018/CAPT).

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784 **Appendix**

785 None

786

Tables & Figures

Table 1: Colony traits of small (<20 pairs), medium (21-40 pairs) and large (> 40 pairs) mixed-species colonies sampled for ectoparasites. Median (min-max) values are shown for host richness, colony size, density (average distance to the 3 active closest nests and colony composition (number of active nests).

	Small	Medium	Large
Colony host richness	3 (1 – 5)	4 (4 – 5)	5 (3 – 6)
Colony size*	6 (2 – 11)	29 (27 – 30)	49 (46 – 62)
Colony density*	6.2 (2.0 – 22.6)	2.6 (0.8 – 5.6)	0.9 (0.8 – 4.4)
Colony composition*			
Lesser kestrel	4 (0 - 10)	20 (17 – 24)	22.5 (8 – 47)
Roller	1 (0 – 2)	2 (1 – 3)	1 (1 – 3)
Pigeon	0 (0 – 5)	5 (0 – 5)	4 (0 – 8)
Starling	0 (0 – 1)	3 (0 – 3)	21.5 (1 – 32)
Colonies sampled	19	5	6
Nests sampled	56	61	144

*Also includes other, less abundant host species (common kestrels, jackdaws, little owls, and barn owls).

793 Table 2: Prevalence (percentage of infested nests) and mean intensity (mean number of parasites found in infested
794 nests) (with 95% confidence intervals in round brackets and number of infested nests/ number of sampled nests
795 in square brackets) of ectoparasites found on nests of mixed-species colonies in southern Portugal.

Parasite	Roller	Lesser Kestrel	Pigeon	Starling
Carnid fly	100%	97.7%	41.9%	76.9%
	Prevalence (87.7 – 100)	(93.5 – 99.5)	(24.5 – 60.9)	(60.7 – 88.9)
	[28/28]	[129/132]	[13/31]	[30/39]
	58.3	21.4	2.9	5.6
	Mean intensity (40.1 – 75.8)	(17.7 – 25.2)	(1.3 – 4.6)	(3.9 – 7.3)
Louse fly	3.0%	2.6%	47.4%	0.0%
	Prevalence (0 – 15.8)	(0.5 – 7.4)	(24.4 – 71.1)	(0.0 – 21.8)
	[1/33]	[3/116]	[9/19]	[0 /15]
	1*	1	1.8	0
		(1 – 1)	(1.1 – 2.4)	
Lice	0.0%	84.5%	94.7%	6.7%
	Prevalence (0 – 10.6)	(76.6 – 90.5)	(74.0 – 99.9)	(0.0 – 31.9)
	[0/33]	[98/116]	[18/19]	[1/15]
	0	24.3	37.9	1*
		(17.4 – 31.1)	(19.5 – 56.3)	
Mite	33.3%	15.6%	31.6%	30.6%
	Prevalence (18.0 – 51.8)	(10.0 – 22.7)	(17.5 – 48.7)	(18.3 – 45.4)
	[11/33]	[22/141]	[12/38]	[15/49]
	18	5.0	18.3	19.1
	Mean intensity (0 – 39.9)	(1.4 – 8.6)	(4.2 – 32.3)	(9.4 – 28.7)

797 Table 3: Results of model averaging on the effect of colony traits on *Carnus hemapterus* abundance in mixed-
798 species colonies using nests from all four host species (lesser kestrels, rollers, pigeons, and starlings).

Parameter	Estimate	Adjusted SE	Z value	P-value	Relative Importance
Intercept	0.725	0.310	2.342	0.019	
N lesser kestrel nests	0.137	0.077	1.777	0.076	0.63
N starling nests	-0.200	0.089	2.237	0.025	0.74
Brood size	0.274	0.091	3.023	0.003	0.99
Host: roller	3.117	0.380	8.204	<0.001	1.00
Host: lesser kestrel	2.185	0.316	6.921	<0.001	
Host: starling	0.917	0.313	2.930	0.003	
Sampling date	-0.006	0.092	0.066	0.947	0.24
Artificial cavities	-0.128	0.268	0.477	0.633	0.19
Clay pots	-0.612	0.332	1.843	0.065	
Wooden nest-boxes	-0.177	0.265	0.667	0.505	
Medium density	-0.114	0.213	0.534	0.593	0.11
High density	-0.141	0.276	0.512	0.609	

- Entry model: *Carnus* abundance ~ host species + brood size + sampling date + nest type + colony density (Cat) + number of lesser kestrel nests + number of starling nests + (1|colony ID); Sample size = 230 nests (132 lesser kestrels, 28 rollers, 31 pigeons, 39 starlings).

- Values reported are conditional averages and adjusted SE. *Carnus* abundance was studied using GLMMs following a negative binomial distribution of errors to account for its aggregated distribution among hosts. Colony ID was used as random factors. N, number of; Cat, categorical; ID, identity.

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800

801 **Figures**

802 (Figure supplied in a separate file. No colour required in print)

803 *Figure 1: Biplot (scaling 3) of the partial Canonical Correspondence Analysis (CCA), showing the relationship*
804 *between the occurrence of ectoparasites and host species, the only explanatory variable selected after forward*
805 *and backward selection. Statistics: CCA1: $X^2 = 0.10$, $F = 29.0$, $p\text{-value} = 0.001$; CCA2: $X^2 = 0.08$, $F = 24.9$, $p\text{-}$*
806 *value = 0.001. Sample size = 240 nests (139 lesser kestrels, 29 rollers, 33 pigeons, 39 starlings).*

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