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Influence of colonial nesting on host-parasite compatibility of the hematophagous ectoparasite *Carnus hemapterus* on two bird species in Southeast Portugal

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Acknowledgments

Abstract

Ectoparasites are widespread in wildlife populations and are known for their diverse impacts on avian hosts. They can impair their hosts by depleting resources that could otherwise be used for growth, immune system and several behaviours.

In southeast Portugal, the generalist hematophagous ectoparasite *Carnus hemapterus* feeds on two endangered bird species: the colonial lesser kestrel (*Falco naumanni*) and the European roller (*Coracias garrulus*; hereafter roller). Rollers are typically solitary breeders, but here the two species can be often found breeding together, forming mixed-species colonies. Most studies of host-carnid fly interactions have focused on the parasite infective phase and solitary or monospecific colony breeding host species. However, a better comprehension of non-infective phases is needed for a more comprehensive knowledge of *C. hemapterus* epidemiological dynamics. Also, the complex host-parasite interactions in mixed-species colonies require more attention, with parasite infestation patterns and its impacts likely to differ between host species, influencing the epidemiological and evolutionary aspects of these associations.

The main goals of this study were to: 1) assess whether carnid flies (infective phase) have some preference for lesser kestrels or rollers in mixed-species colonies; 2) investigate if the social breeding context of rollers (colonial versus solitary) influences the infestation pattern (i.e. the prevalence, abundance and intensity) of carnid flies; 3) describe the infestation pattern of the pupal phase (non-infective phase) of *C. hemapterus* on roller and lesser kestrel nests and compare it with the infective phase; and 4) determine the relationship between carnid fly abundance and lesser kestrel and roller nestlings' body condition, in different nest types.

Lesser kestrel and roller nestlings were sampled during two consecutive breeding seasons (2018 and 2019). Carnid fly abundance on nestlings was estimated and nestlings body measurements taken to calculate a body condition index (the scaled mass index). In 2019, nest detritus were collected for the quantification of carnid pupae.

Our results suggest that carnid flies prefer rollers over lesser kestrels, with rollers (in mixed-species colonies) having three times more flies. Fly abundance was also positively related to brood size and decreased in nests previously occupied by spotless starlings (*Sturnus unicolor*). Rollers breeding in mixed-species colonies had a similar prevalence of flies but were twice as much parasitized as solitary rollers. The host species identity seems to have a more profound effect on parasite infestation patterns than their social context, as even solitary rollers had higher intensities than lesser kestrels. The host social context (colonial versus solitary) appears to have a stronger influence in intraspecific comparisons. Moreover, the abundance and intensity of the pupal phase did not differ between host species or correlated with the infective phase. An incompatibility between host preferences and their suitability to the parasite may be leading to lower parasite fitness, thus influencing epidemiological dynamics. The results also suggest a negative association between nestling body condition and carnid fly abundance, but only on lesser kestrels growing in nest-boxes and rollers in artificial cavities. Therefore, allowing for a better understanding of the relationship between *C. hemapterus* with these host species and how synergetic factors may be aggravating parasite impacts.

In general, this study contributes to the better understanding of the determinants regulating *C. hemapterus* infestation patterns and help disentangle the effects of host suitability and host density, which is important for evaluating the effectiveness of pre-existent conservation measures and design future management plans.

Keywords: *Carnus hemapterus*; lesser kestrel; European roller; host–parasite interactions; host preference

Resumo

Os ectoparasitas são capazes de exercer uma grande diversidade de impactos sobre os seus hospedeiros. Influenciam aspetos imunológicos, fisiológicos e morfológicos destes, com impactos na sua distribuição, dinâmica populacional, comportamento social e evolução. Em sistemas com múltiplas espécies de hospedeiro, como colónias multiespecíficas, as dinâmicas hospedeiro-parasita são extremamente complexas, uma vez que os padrões de infestação e os efeitos dos parasitas variam em magnitude entre espécies de hospedeiro. O aumento de frequência e duração de contactos sociais entre potenciais hospedeiros e o reuso de ninhos em anos alternados conduzem a um aumento do risco e níveis de parasitismo e à transmissão de doenças, sendo o parasitismo reconhecido como um dos principais custos da nidificação colonial em aves.

Carnus hemapterus é um ectoparasita hematófago generalista que se alimenta predominantemente de crias de aves que utilizam cavidades para nidificar. Este parasita detém a capacidade de localizar e colonizar os seus hospedeiros de forma ativa não necessitando, por isso, de contacto entre indivíduos para a sua transmissão. A sua abundância aparenta ser influenciada por características relacionadas com o hospedeiro (por exemplo, substrato de nidificação, imunocompetência e fenologia de nidificação) e pelo seu contexto socioambiental. Não existe uma concordância generalizada quanto aos seus impactos, mas entre os reportados encontram-se a redução da condição corporal e taxa de crescimento das crias e, em casos extremos, o aumento da taxa de mortalidade.

No Sudeste de Portugal, nomeadamente na Zona de Proteção Especial (ZPE) de Castro Verde, *C. hemapterus* é um dos ectoparasitas mais abundantes em espécies de aves, incluindo o francelho (*Falco naumanni*) e o roleiro (*Coracias garrulus*). Ambas as espécies são migradoras estivais de longa distância que invernam em África e nidificam na região Paleártica, apresentando também requisitos de alimentação e nidificação semelhantes. Em Portugal, são consideradas de conservação prioritária, estando o francelho atualmente classificado como "Vulnerável" e o roleiro como "Criticamente em Perigo". Na ZPE de Castro Verde, a implementação de medidas de conservação direcionadas para o francelho, como a disponibilização de locais de nidificação, levaram ao aumento da população nacional de francelho e de roleiro. Atualmente, a região alberga mais de 80% das populações nidificantes portuguesas destas espécies. Os roleiros nidificam geralmente em solitário, no entanto a escassez de locais de nidificação e consequente recuperação de cavidades em edifícios abandonados e disponibilização de ninhos artificiais (por exemplo, paredes e torres de nidificação) conduziram à formação de colónias multiespecíficas compostas por francelhos e roleiros, entre outras espécies. Assim, na região de Castro Verde, ambas as espécies podem ser encontradas a nidificar nas mesmas estruturas e a utilizar os mesmos ninhos em anos alternados. As dimensões das colónias podem variar de 1 a 80 casais de francelhos e normalmente 1 a 3 casais de roleiro. Os roleiros podem, também, ser encontrados a criar em ninhos solitários, como caixas-ninho em árvores, postes telefónicos ou de eletricidade. Nestas colónias multiespecíficas, o aumento de densidade de ninhos e proximidade entre diferentes espécies de hospedeiro pode aumentar as oportunidades de transmissão para *C. hemapterus* e conduzir a interações agonísticas que, por sua vez, intensificam o stress social dos indivíduos, aumentando a sua suscetibilidade a doenças e infeções. Estas complexas interações hospedeiro-parasita, promovidas em parte pela disponibilização de ninhos artificiais, têm consequências ainda desconhecidas nas populações de francelho e roleiro. Além disso, a maioria dos estudos sobre *C. hemapterus* e os seus hospedeiros tem-se concentrado em espécies solitárias ou formadoras de colónias monoespecíficas.

O nosso principal objetivo foi fornecer um estudo abrangente das relações hospedeiro-parasita num contexto particular de nidificação social, considerando diferentes espécies de hospedeiro, diferentes fases do ciclo de vida de *C. hemapterus* e as consequências para as espécies hospedeiras. Deste modo, esta dissertação visou: 1) avaliar se a fase infeciosa de *C. hemapterus* apresenta preferências entre francelhos e rolieiros em colónias multiespecíficas; 2) investigar se o contexto social de nidificação (solitário ou colonial) em rolieiros influencia o padrão de infestação deste ectoparasita; 3) descrever o padrão de infestação da fase de pupa (fase não-infeciosa) nos ninhos de francelho e rolieiro e compará-lo com o padrão de infestação da fase infeciosa; 4) determinar a relação entre a abundância de *C. hemapterus* e a condição corporal de crias de francelho e rolieiro, em diferentes tipologias de ninhos.

Neste estudo, foram amostradas crias de francelho (em colónias multiespecíficas) e rolieiro (tanto em ninhos solitários como em colónias multiespecíficas) em duas épocas de nidificação consecutivas, em 2018 e 2019. Nas crias amostradas, foi quantificado o número de *C. hemapterus* e realizadas medições corporais de forma a calcular o índice de condição corporal ("scaled mass index"). Em 2019, foram recolhidos detritos de ninhos de ambas as espécies e quantificado o número de pupas existentes.

Os resultados sugerem que *C. hemapterus* prefere crias de rolieiro a francelho, tendo os rolieiros (em colónias multiespecíficas) uma abundância três vezes maior. A sua abundância foi também positivamente influenciada pelo tamanho da ninhada e diminuiu em ninhos previamente ocupados por estorninho-preto (*Sturnus unicolor*). Rolieiros em colónias multiespecíficas obtiveram prevalências semelhantes a rolieiros solitários, mas uma maior intensidade. A identidade da espécie hospedeira aparenta ter uma maior influência nos padrões de infestação deste parasita, com o contexto social a adquirir uma maior relevância em comparações intraespecíficas. Além disso, o padrão de infestação da fase de pupa não diferiu entre rolieiros e francelhos ou esteve correlacionado com o padrão de infestação da fase infeciosa, revelando uma possível discordância entre fases do desenvolvimento do parasita. Esta aparente incompatibilidade entre preferências por espécies de hospedeiro e a sua adequação enquanto hospedeiro pode diminuir o fitness do parasita, influenciando as suas dinâmicas epidemiológicas. Os resultados sugerem também uma associação negativa entre a condição corporal das crias e a abundância de *C. hemapterus*, no entanto esta relação apenas se verificou em crias de francelho em caixas-ninho e crias de rolieiro em cavidades artificiais (em paredes de nidificação). Estes resultados providenciaram, assim, uma melhor compreensão da relação entre *C. hemapterus* e estas espécies de hospedeiro e como fatores sinergéticos podem estar a agravar os seus efeitos.

Em geral, este estudo permitiu aprofundar o conhecimento sobre os determinantes que regulam os padrões de infestação de *C. hemapterus* e deslindar os efeitos da adequação e densidade dos seus hospedeiros. Contribuindo, deste modo, com informação relevante para a avaliação da eficácia de medidas de conservação já implementadas e para o delineamento de futuros planos de gestão.

Palavras-chave: *Carnus hemapterus*; Francelho; Rolieiro; interações hospedeiro-parasita; preferência de hospedeiro

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C:N – The interaction between carnid fly abundance and nest type

cE3c – Centre for Ecology, Evolution and Environmental Changes

GLMM – General Linear Mixed Model

ICNF – Institute for Nature Conservation and Forests

LMM – Linear Mixed Model

LPN – League for the Protection of Nature

QGIS – Quantum Geographic Information System

SMI – Scaled mass index

SPA – Castro Verde Special Protection Area

Species t – The species occupying the nest in the year t

Species t-1 – The species occupying the nest in the previous breeding season

Introduction

<u>Avian coloniality and parasitism</u>

Avian coloniality, defined as the gregarious breeding of several individuals with more or less spatial clustering of nests, is a complex and common type of avian social organization (Brown and Brown 2001).

Colonial breeding in birds results in multiple fitness costs and benefits, the magnitude and balance of which may vary according to the species, populations and individuals (Danchin and Wagner 1997). Identified benefits are variable and may include enhanced foraging efficiency (exchange of information), reduced predation risk (e.g. dilution effect, cooperative vigilance) and increased thermoregulation and mate choice (Danchin and Wagner 1997; Brown and Brown 2001). On the other hand, several possible costs can also be listed such as increased competition for limited resources (e.g. nest sites, food and mates; Burger 1981), increased probability of misdirected parental care (Brown and Brown 2001), enhanced conspicuousness and consequent attraction of predators (Burger 1981; Brown and Brown 2001), intensified social stress (Minias et al. 2015) and exacerbation of parasite risk and disease transmission (Brown and Brown 2001).

The relationship between coloniality and parasitism depends on the mode of transmission of the parasite. Contact-transmitted ectoparasites, which need host-to-host contact for transmission, are predicted to have higher opportunities for infestations in colonies, due to the increased number of hosts, higher nest density and frequency of hosts interactions, as well as under higher nesting synchronization (Poulin 1991). On the other hand, mobile parasites are expected not to differ between solitary and colonial breeding since they can actively locate and colonize host nests (Poulin 1991). However, mobile parasites can still increase in colonies if the probability of host detection increases with host density (colonies are potentially more conspicuous), consequently increasing the probability of finding a host, or when an individual parasite can take advantage of multiple hosts (Mooring and Hart 1992; Kleindorfer and Dudaniec 2009; Veiga et al. 2020).

Avian mixed-species colonies

Mixed-species colonies offer the same advantages as conspecific aggregations while possibly decreasing intraspecific competition for partners and essential resources (Burger and Gochfeld 1995). By lowering the species' ecological niche overlap, the benefits associated with larger colony sizes (e.g. enhance predator vigilance, foraging opportunities) increase at a faster rate than the costs (Burger 1981). Moreover, different species may provide complementary contributions (e.g. species-specific traits regarding information detection or transmission), which would not be available in a monospecific colony (Burger 1981). For instance, a more submissive species may benefit from enhanced predator deterrence by nesting closer to a more aggressive one.

In mixed-species aggregations, the increase of frequency and duration of intra- and interspecific social contacts increases and nest re-use among coexisting potential host species might enhance parasite transmission, increasing the probability of parasite exchange and possibly exposing successive generations of a parasite to different species that vary in their suitability as hosts (Valera et al. 2003).

Host-ectoparasite interactions and their impacts

Ectoparasites are extremely diverse and widespread in wildlife populations (Price 1980). They live on the surface of their hosts and possess the capacity to separate themselves from them for short periods of time (e.g. moving between hosts). Nevertheless, they are entirely dependent on their hosts for nutritional resources (Nelson et al. 1975), at least for some phases (e.g. infective phase). Based on their host range (number of host species a parasite is capable to explore), parasites can be classified as specialists or generalists (Barrett and Heil 2012).

It has become increasingly acknowledged that ectoparasites can impact their hosts in many ways. In avian hosts, ectoparasites can decrease nestlings' growth rate, reduce their body mass and size by depleting their energy reserves, leading to an inferior body condition and mobility and increasing their mortality rate (Cannings 1986; Avilés et al. 2009; Wiebe 2009). Parasites can also impair their hosts by stimulating nutritionally demanding and potentially costly immune responses (Graham et al. 2011). In addition, parasites can influence the attractiveness of breeding sites for hosts (Brown and Brown 1996; Loye and Carroll 1998), increase natal dispersal (Brown and Brown 1992), play a key role in determining the outcome of biological invasions (Hatcher et al. 2006) and influence species competitive and predatory interactions (Price 1986; Hatcher et al. 2006). For instance, a generalist parasite shared by two competitor species may reduce the competitive strength of the most susceptible or less tolerant species, possibly modifying the outcome of the interaction (Price 1986; Hatcher et al. 2006). Some ectoparasites have also been shown to cause tissue damage (directly or indirectly, e.g. hosts injuring themselves by scratching) and be vectors of disease agents (Lesko and Smallwood 2012).

Birds respond to parasitic exposure with a variety of behavioural (e.g. grooming, nest sanitation) and physiological adaptations which may be time and resource-consuming (Clayton et al. 2010). Some species incorporate fresh plants with insecticidal and antipathogenic properties in their nests (Clark and Mason 1985). Birds may also respond by decreasing clutch size, delay clutch initiation, laying fewer second clutches, and increasing nest desertion rates, resulting in reduced reproductive success (Oppliger et al. 1994). Parents, may alter their investment towards parasitized offspring, either increasing their provisioning rates to compensate for the depletion of resources caused by parasites or reducing their parental effort (Christe et al. 1996; Richner and Tripet 1999).

Overall, parasites may play a significant role in shaping or modifying various aspects of the host population and evolutionary dynamics, distribution, social behaviour and ecology (Price 1980; Gómez and Nichols 2013). Parasite-host interactions may function as a generator of multiple adaptations, by the host as well as the parasite, engaging in a coevolutionary arms race (Decaestecker et al. 2007; Clayton et al. 2010). A more comprehensive understanding of these systems is needed since significant knowledge gaps about the great variety of responses and consequences resulting from host-parasite relationships remain (Poulin 2007).

Introduction to *Carnus hemapterus*

  The carnid fly *Carnus hemapterus* Nitzch, 1818 (Diptera: Carnidae) is an easy to detect and count ectoparasite that has been documented parasitizing 64 avian host species (Grimaldi 1997; Roulin 1998; Brake 2011). It is a 2 mm long, mobile hematophagous fly with a geographic range that includes the Holarctic region and India (Capelle and Whitworth 1973; Brake 2011). This nest-dwelling ectoparasite feeds predominately on unfeathered nestlings of cavity-nesting species (Capelle and Whitworth 1973; Marshall 1981; Grimaldi 1997).

  The life cycle comprises an adult infective phase, three larval phases and a pupal phase (Figure 1.1.; Guiguen et al. 1983). During winter, pupae diapauses in the hosts' nests and flies usually emerge the following spring with the hatching of hosts nestlings (Guiguen et al. 1983; Valera et al. 2003), though prolonged diapause up to 3 years has been reported (Valera et al. 2006a). Evidence of bivoltinism has also been found, with shorter diapauses giving rise to multiple generations during a breeding season (Amat-Valero et al. 2012). Diapause allows for synchronization of emergence with resource availability (hosts), pupae accumulation and perpetuation in the nest, unless it is not occupied or it has been used by an unsuitable host (Calero-Torralbo et al. 2013; Veiga et al. 2019a). If so, winged adults, capable of flying, are forced to disperse (Liker et al. 2001; Veiga et al. 2019a). The winged morph has less than four days to actively encounter and colonize a suitable host (Veiga et al. 2019b). Afterwards, most flies lose their wings and spend the rest of the cycle in their hosts' nests (Grimaldi 1997; Roulin 1998). Flies mainly parasitize nestlings but have been seen attacking incubating birds (López-Rull et al. 2007; Hoi et al. 2010). The diet of adult flies consists of hosts' blood, cutaneous secretions and epidermal cells, usually concentrating on axillary and inguinal areas (Marshall 1981; Kirkpatrick and Colvin 1989). Copulation takes place on the host. Then, gravid females lay their eggs in the nest substrate. Larvae are saprophagous – feed upon decaying organic matter – and thrive in the nest debris for approximately 21 days before pupating (Guiguen et al. 1983).
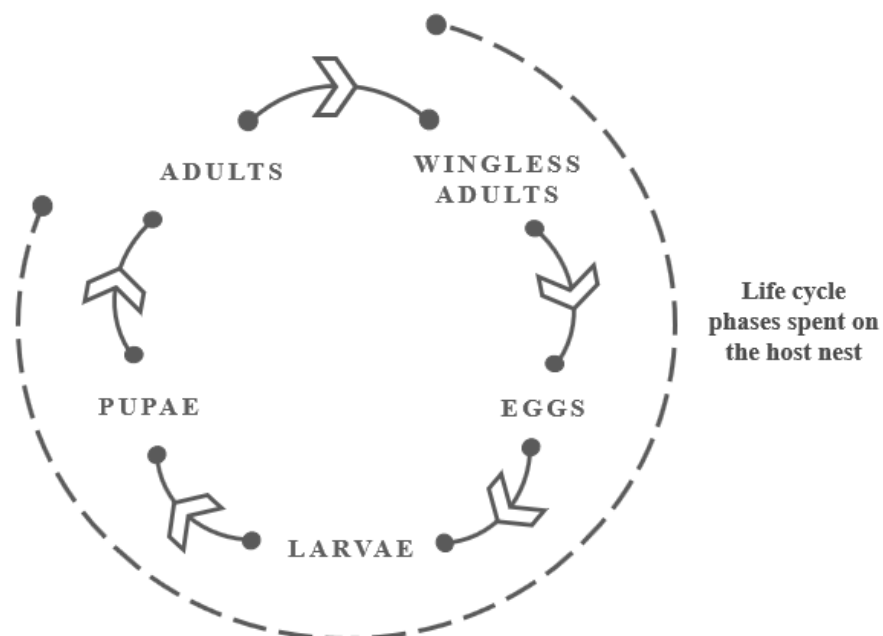
Figure 1.1. *C. hemapterus* life cycle.

  Possible factors influencing *C. hemapterus* occurrence, infection levels and reproductive success include hosts' social breeding context (solitary, inter- or intraspecific aggregations), brood size, host density (Veiga et al. 2020), host-parasite life-cycle synchronization (Calero-Torralbo and Valera 2008), nest site characteristics – e.g. age (Brown and Brown 1996), type (Kal'avský and Pospíšilová

2010) and microclimatic properties (Martínez-de la Puente et al. 2010) – and environmental conditions (Amat-Valero et al. 2013). Carnid fly abundance seems to follow an increase after nestlings hatched peaking with nestlings at an intermediate development stage and following a seasonal declining trend thereafter (Liker et al. 2001; Calero-Torralbo et al. 2013). The lower abundance in late-hatched nests probably reflects the lack of suitable hosts at the end of the breeding season (Calero-Torralbo et al. 2013). In addition, parasite abundance appears to be related with the type of nest substratum – carnid flies seem to avoid nest with abundant vegetable matter (Valera et al. 2006b) – and higher levels are found in re-used nest sites (Roulin 1998).

No general agreement exists regarding the impact of this generalist ectoparasite on their hosts, yet an increased in nestling mortality has been reported (Cannings 1986).

In southeast Portugal, two known hosts of this carnid fly – the European roller *Coracias garrulus* Linnaeus, 1758 (Václav et al. 2008; hereafter roller) and the colonial lesser kestrel *Falco naumanni* Fleischer, 1818 (Ganbold et al. 2020) – often breed in the same nesting structures, forming mixed-species colonies along with other cavity-nesting species (such as the spotless starling *Sturnus unicolor*, jackdaw *Corvus monedula*, feral pigeon *Columba livia* or common kestrel *Falco tinnunculus*; Catry et al. 2015, 2016). Most studies of host-carnid fly interactions have focused on solitary or monospecific colony breeding species (but see Valera et al. 2003; Veiga et al. 2019a). In these mixed-species colonies, the number and density of potential host species is high and the re-use of nests by different host species facilitates the exposure of successive generations of a parasite to different host species. The complex multispecies interactions may create the potential for higher transmission opportunities, possibly leading to higher abundances than previously reported, with unanticipated consequences (Valera et al. 2003).

Past conservation efforts have provided lesser kestrels and rollers with numerous artificial nests (e.g. wooden nest-boxes, clay-pots, cavities in walls of existing buildings, new cavities in breeding walls and towers) but did not acknowledge the consequences of host-parasite interactions. Nest-provisioning of breeding walls and towers has promoted the attraction and subsequent aggregation of these cavity-nesting species. Thus, it is important to understand whether these artificial nesting structures lead to a higher prevalence and abundance of *C. hemapterus*. Rollers are typically solitary breeders, but in our study area they also breed in mixed-species colonies. Therefore, it is a suitable study species to comprehend how the host social breeding context influences parasite infestation.

In complex communities involving multiple host species, knowledge of host preferences and their suitability through all parasite life phases are key to better understand the coevolution of host–parasite interactions and the epidemiological consequences of such associations (Veiga et al. 2019a). Differences in lesser kestrel and roller host-related factors may result in differential host suitability and, consequently, differences in the prevalence and abundance of *C. hemapterus*. This ectoparasite may impose distinct selective pressures on these species, thus influencing the community structure and dynamics.

Objectives

Our main goal was to provide a comprehensive study of host-parasite relationships in a particular social breeding context, considering different host species, different phases of the parasite cycle and the consequences for the host species. Therefore, we aim at: 1) assessing whether carnid flies (infective phase) have some preference for lesser kestrels or rollers in mixed-species colonies; 2) investigating if the social breeding context of rollers (solitary versus colonial) influence the infestation pattern (i.e. the prevalence, abundance and intensity) of carnid flies; 3) describing the infestation pattern of the pupal phase (non-infective phase) on roller and lesser kestrel nests and compare it with the infective phase on both species; and 4) determine the relationship between carnid fly abundance and lesser kestrel and roller nestlings' body condition, in different nest types.

We hypothesized that differences in host-related factors (e.g. nesting substrate, immunocompetence and breeding phenology) between lesser kestrels and rollers can lead to differences in prevalence and abundance. Also, we predicted solitary roller nests to be less parasitized when compared to roller nests in mixed-species colonies. Furthermore, we expected the infestation patterns observed for the flies (infective phase) to be consistent with the ones obtained for the pupal phase. In addition, we expected a negative relationship between carnid flies and lesser kestrel and roller nestlings'

body condition since these are endangered species under a combination of stressors that may exacerbate the effects of carnid flies.

Material and methods

<u>Study species</u>

Lesser kestrel and roller are single-brooded, long-distance Afro-Palearctic migratory species that forage in open landscapes with short vegetation (Rodríguez et al. 2010; Kiss et al. 2014). Both species prey predominantly on invertebrates, mainly Coleoptera and Orthoptera, and occasionally on small mammals and reptiles (Cramp 1980, 1985; Cramp and Simmons 1988; Del Hoyo et al. 2001a, b). They are obligate secondary cavity-nesting species, meaning that they do not build their own cavities. Lesser kestrels breed in natural holes cliffs, cavities in old human constructions and artificial nests (Catry et al. 2009). Rollers use abandoned woodpecker holes or natural cavities in rocks and sandstones, as well as artificial nests and cavities in human constructions in southern latitudes, where natural cavities are scarce (Cramp and Simmons 1988).

The lesser kestrel is a small-sized colonial raptor (Bobek et al. 2018; Podofillini et al. 2018). Typically it lays 4-5 eggs in april-may, hatching occurs 28 days later, and nestlings are able to fledge when 36 days old (Del Hoyo et al. 2001a). The western European population underwent a dramatic decline and range contraction since 1950 (Bobek et al. 2018). These declines led to the implementation of several conservation initiatives, which often included the provision of artificial nesting structures as compensation for the loss of suitable breeding sites. Such initiatives resulted in an improvement of the lesser kestrel global conservation status to Least Concern (BirdLife International 2020a). In Portugal, a European LIFE-Nature Project (LIFE2002/NAT/P8481) led to an increase in the national population. However, the species was classified as Vulnerable in the last Portuguese Red Data List (Cabral et al. 2005).

The roller is a medium-sized Coraciiform that breeds in open habitats from Iberia and northwest Africa to Central Asia (Cramp and Simmons 1988; BirdLife International 2020b). Rollers usually lay 3-6 eggs in may, which are incubated for approximately 17 to 19 days, and nestlings fledge when 25 to 30 days old (Del Hoyo et al. 2001b). The European population has undergone drastic declines in size and range in the past. Currently, it is still declining, although at a less severe rate, leading to its global classification as Least Concern (BirdLife International 2020b). In Portugal, the number of breeding pairs increased recently (Catry et al. 2011; Gameiro et al. 2020), but the national population distribution is still limited and considerably fragmented (Equipa Atlas 2008) being listed as Critically Endangered (Cabral et al. 2005).

<u>Study area</u>

This study was carried out in the Castro Verde Special Protection Area (SPA), 37° 41'N, 8°05'W (Figure 1.2.), created under the European Union Birds Directive (2009/147/EC) to protect wild populations of threatened steppe bird species, including lesser kestrel and roller, one of the most endangered bird groups through Europe (Catry et al. 2015; Ribeiro et al. 2018).

Castro Verde is a High Nature Value farming landscape in southeast Portugal (Ribeiro et al. 2018) that includes the most representative cereal-steppe area in the country (Catry et al. 2015). The climate is typical Mediterranean, with hot dry summers and moderately rainy cold winters (Ribeiro et al. 2018). Characterized by extensive plains and a undulating topography, the Castro Verde landscape is dominated by an agricultural mosaic of cereal fields and fallow lands that serve as grazing for

livestock (low-intensity rotating system), sporadically interrupted by trees (oaks, eucalyptus and olive plantations) and small natural patches of herbaceous-shrub vegetation (Catry et al. 2016).

Conservation efforts in the Castro Verde SPA increased the number of breeding pairs for lesser kestrels and rollers from 155–158 in 1996 to 577–625 in 2017 and from 35–40 in 2004 to 58–60 in 2017, respectively (Catry et al. 2009, 2011; Gameiro et al. 2020). Presently, the area harbours more than 80% of the Portuguese breeding populations of both species. Here, lesser kestrels breed colonially in wall cavities or under roof tiles of abandoned rural buildings and in artificial nest structures (Catry et al. 2009). Rollers, which are generally solitary breeders, are often found in mixed-species colonies, so the two species may use the same cavities in consecutive years (Catry et al. 2015, 2016). These colonies usually range from 1 to 80 lesser kestrel and 1 to 3 roller breeding-pairs (Gameiro et al. 2020). Both species can be parasitized by lice, mites and louse flies, but *C. hemapterus* is the most prevalent ectoparasite (Gameiro et al., in prep.).
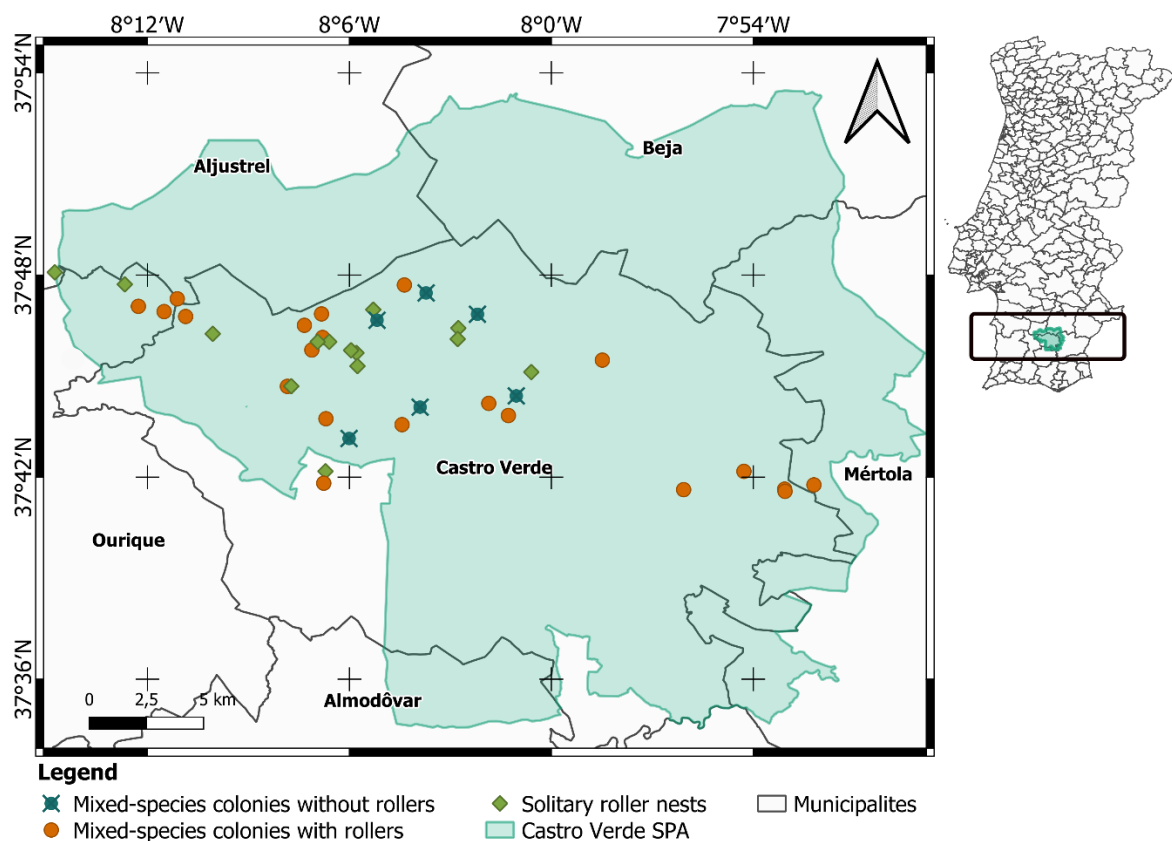
Figure 1.2. Map of Portugal with the location of the study area (Castro Verde SPA), displaying the sampled mixed-species colonies (with and without roller nests) and solitary roller nests (data from 2018 and 2019).

Data collection

In this study we sampled lesser kestrel and roller nestlings in their nests during two consecutive breeding seasons (2018: april–july; 2019: june–july). Colonies and nests (Figure 1.2.) were sampled during an ongoing monitoring programme of lesser kestrel and roller nests. We attempted to sample every roller nest (solitary and in mixed-species colonies) and a few lesser kestrel nests from each colony (mean: 6.12, range: 1-13). A total of 153 lesser kestrel nests from 27 mixed-species colonies (121 from 2018 and 32 from 2019), 50 roller nests from 22 mixed-species colonies (24 form 2018 and 26 from 2019) and 18 solitary nests (13 from 2018 and 5 from 2019). Carnid fly abundance on lesser kestrel and roller nestlings was estimated and nestlings body measurements taken to calculate a body condition index (the scaled mass index). In 2019, nest detritus was collected for the quantification of carnid pupae.

Carnid fly estimation on nestlings

Carnid fly peak abundance happens when host offspring are 8 to 12 days old (Dawson and Bortolotti 1997; Václav et al. 2008), so sampling occurred within this age range. Each sampled nestling was carefully removed from its nest and searched for carnid flies on the body surface and sheaths, which would be counted twice and, subsequently, averaged. This method has been found reliable by previous studies for this ectoparasite species (Roulin 1998; Václav et al. 2008). Animal handling was authorized by the Institute for Nature Conservation and Forests (ICNF).

For every nest, we also recorded the number of nestlings (brood size), nest type and the species occupying the nest in the previous breeding season (species t-1). Nest type comprises wooden nest-boxes (n=34), adobe cavities (on abandoned buildings; n=72), clay-pots (n=14), stone cavities (n=4), artificial cavities (breeding walls; n=92), and tiles (roofs; n=5). Previous species occupying the nests include rollers, lesser kestrels, spotless starlings, jackdaws, feral pigeons, common kestrels, little owls (*Athene noctua*) or unoccupied nests. Flies that emerge in unoccupied nests are forced to disperse in search of an adequate host, which probably lowers the abundance of flies in the nest during the following breeding season. As such, previously unoccupied nests were also considered in the analyses.

Carnid pupae in hosts' nest

In July 2019, we sampled occupied nests by our study species after nestlings had fledged. In each nest, we collected the detritus by hand. The amount of extracted material varied with its availability and accessibility. We stored the samples in properly identified plastic bags, which were initially left open to expedite the drying process. Nest detritus consisted essentially of soil and organic substrate, such as excrements, invertebrates remains and vegetable matter. We did not used insecticide, nor did we remove the nesting substrate from the cavities before the breeding season. As such, detritus can contain diapausing pupae from previous breeding seasons (Valera et al. 2006a).

We sieved each sample through a column composed of 8 mm, 4 mm, 2 mm, 1 mm and 0.5 mm sieves. Next, we mixed the material on 1 mm and 0.5 mm (where carnid pupae is collected) and selected two subsamples of 5 g (Valera et al. 2018). Then, we examined the subsamples with a Leica S6E Microscope to search and quantify the carnid pupae. *C. hemapterus* has a barrel-shaped reddish-brown puparium (i.e. protective outer covering enclosing the pupae) with two divergent spiracles, each with three curved projections (see Valera et al 2018 for a more detailed description and photographs). We classified carnid puparia in closed (apparently viable pupae), open, or broken. For statistical analyses, we only considered the average of viable pupae between the two subsamples for each nest (Veiga et al. 2019a).

Nestling body condition index

To study the relationship between this nestling-based ectoparasite with lesser kestrels and rollers body condition, we weighted each nestling using a Pesola dynamometer (maximum capacity=300 g, division=2 g) and measured its wing length with a 0.1 mm precision ruler. Measurements were taken once in each breeding season when nestlings were 8 to 12 days old.

For each species separately, we used the scaled mass index (SMI) as a body condition index, which accounts for the scaling relationship between body mass and a body length measurement (wing length in our case; Peig and Green 2009). The SMI was calculated as:

Equation 1.1.:

$$\text{Scaled mass index of body condition: } \hat{M}_i = M_i \left[\frac{L_0}{L_i}\right]^{b_{SMA}};$$

where M_i represents the body mass of nestling i. L_0 is the mean wing length of all sampled nestlings and L_i is the wing length of nestling i. Finally, b_{SMA} represents the scaling exponent estimated by the standardized major axis regression of body mass on wing length. Then, we calculated the mean SMI per nest (mean SMI of all nestlings in the nest for each sampled nest) since flies can move between sibling nestlings, which, therefore, are not independent of each other.

Rollers are non-dimorphic birds (Demongin 2016) and lesser kestrels are known for being sexually size dimorphic, however, this is not yet apparent during the early nestling stage (Podofillini et al. 2018), so we did not considered sex in our analyses.

Statistical analyses

Prevalence of *C. hemapterus* (proportion of infested nests among all examined) with Clopper-Pearson 95% confidence intervals (using the BinomCI function in the DescTools package; Signorell et al. 2020) were estimated for each host species (both for the infective and pupal phases) and in relation to rollers' social context (solitary versus colonial). Mean intensity (the mean number of parasites found across infected nests) and mean abundance (the mean number of parasites across all nests examined, including non-infected ones) with respective standard deviations (± SD) were also calculated. The nest was considered our sample unit for all analyses.

Host preferences by the carnid fly

A Fisher's exact test ('fisher.test' function) was used to compare the prevalence of carnid flies between lesser kestrels and rollers in mixed-species colonies (Rozsa et al. 2000)

A General Linear Mixed Model (GLMM) with a negative binomial error distribution ('glmer.nb' function from the 'lme4' R package; Bates et al. 2015), to account for the aggregated distribution of parasites among hosts (Rozsa et al. 2000; Liker et al. 2001), was used to analyse host preferences by carnid flies between lesser kestrels and rollers (species t, i.e. species occupying the nest in the year t). The response variable was the canid fly abundance per nest (total number of parasites per nest, including infected and non-infected ones). Also, other predictor variables that could potentially influence carnid fly abundance were included: nest type, brood size, year (2018, 2019) and species t-1 (species occupying the nest in the previous year, t-1). For the analysis, we excluded 18 nests (lesser kestrel: 14; roller: 4), comprising tiles and stone cavities as nest types and nests occupied in the previous breeding season (t-1) by jackdaw, feral pigeon, common kestrel or little owl, owing to their low representation (n≤5).

Three random factors were considered – nest identity, colony identity and Julian date (sampling date in Julian format) – to avoid pseudo-replication of sampling the same nests in both years, sampling nests from the same colonies and sampling nests along the breeding season. To obtain the combination of random effects that better explains the variation in our data, GLMMs including all predictor variables and different combinations of random factors were ranked according to AICc (Akaike Information Criterion corrected for small sample sizes), using the 'model.sel' function from the 'MuMIn' R package (Barton 2019). The full-fixed effects model with the highest support (lowest AICc and the highest Akaike weight, ωi; Burnham and Anderson 2002) was selected and included Julian date as a random factor. Then, using the 'dredge' function ('MuMIn' R package) all possible subsets of the global model

(different combinations of predictor variables) were created and ranked based on their AICc. Goodness-of-fit of the best-fitted model was determined by calculating marginal (variance explained by the fixed factors) and conditional (variance explained by fixed and random factors) R^2 values using the 'r2_nakagawa' function ('performance' R package; Lüdecke et al. 2020). Subsequently, a model-averaging approach with a subset of ΔAICc<7 (AICc difference between a particular model and the best-fitting model) was used to obtain the most parsimonious models (Burnham and Anderson 2002), by applying the 'model.avg' function ('MuMIn' R package). Conditional model-averaged estimates are presented, predictor variables are ranked according to their relative importance ($\sum \omega i$, sum of ωi for all top-models in which the predictor variable was included) and confidence intervals were used to assess the magnitude of each predictor estimate.

Comparison of carnid fly infestation pattern on rollers in different social contexts

We did not compare the prevalence or abundance between social contexts since all roller nests were parasitized (100% prevalence). We performed bootstrap 2-sample *t*-test analyses (Rozsa et al. 2000), using the 'boot.t.test' function with the default 9,999 iterations ('MKinfer' R package; Kohl 2020), to investigate differences in carnid fly intensity between social contexts (solitary versus colonial). Bootstrap 2-sample *t*-test analyses (9,999 iterations) were performed to examine possible inter-annual differences in carnid fly intensity for each social context. To check for possible confounding effects due to differences in Julian date and brood size between social contexts, we carried out two-sample Wilcoxon tests with the 'wilcox.test' function.

Infestation patterns of the pupal phase and concordance between the pupal and infective phase

A Fisher's exact test was used ('fisher.test' function) to compare the prevalence of pupae per 5 g of detritus between lesser kestrels and rollers in colonies. Abundances and intensities were compared by applying bootstrap 2-sample *t*-tests (9,999 iterations). Spearman's rank correlation tests ('cor.test' function) were used to examine the relationship between carnid fly abundance per nest (infective phase) and pupae abundance per 5 g of detritus (pupal phase). This analysis was performed for lesser kestrels and rollers in colonies, but not for solitary roller nests since the sample size was too small (n=4).

Relationship between carnid fly abundance and nestling body condition index

A body length variable that is strongly correlated with body mass is likely to be a good predictor of the fraction of mass associated with structural size (Peig and Green 2009). To assess this, Spearman's rank correlations were performed between body mass and wing length (log-transformed). Regarding roller, no significant differences between weight (Wilcoxon rank-sum test: $W=7405.5$, *p*-value=0.842, $n_c=230$, $n_s=67$) and wing length (Wilcoxon rank-sum test: $W=7650.5$, *p*-value=0.544, $n_c=230$, $n_s=67$) were found between social contexts (colonial versus solitary), so all roller nestlings were grouped for the SMI estimation. The b_{SMA} was calculated by applying a standardized major axis regression using the 'sma' function ('smatr' R package; Warton et al. 2012) to log-transformed body mass and wing length values.

Linear Mixed Models (LMMs) were used to assess the relationship between ectoparasite abundance and nestling body condition index. The response variable used was the mean SMI per nest. The predictor variables were: carnid fly abundance per nest, nest type, year (2018, 2019) and brood size. Plus, the interaction between carnid fly abundance per nest and nest type, to assess how the increase of parasite abundance relates to nestling body condition within different nest types. For roller, we excluded

clay-pot from the analysis, owing to its low representation (n=3) and added an additional predictor, the social context (solitary versus colonial). Before the analysis, brood size and fly abundance were scaled and centred with the 'scale' function. The previously described model selection procedure was performed for both species. For lesser kestrels, colony identity and Julian date were included as random factors. For rollers, the selected random factor was Julian date. Then, model-averaging was performed on the subset models with $\Delta AICc<7$, as previously described.

The 'ggplot' (ggplot2 R package; Wickham 2016), 'interact_plot' (jtools R package; Long 2020) and 'ggscatter' (ggpubr R package; Kassambara 2020) functions were used for graphic visualizations. All statistical analyses were conducted in R Version 3.6.2 (R Core Team 2019; RStudio version 1.2.5033) with statistical significance established at p-value<0.05. The study area map (Figure 1.1.) was developed using QGIS version 3.12.2 software (QGIS Development Team 2020).

Results

We found 2784 carnid flies on 542 lesser kestrel nestlings (mean fly abundance per nestling $\pm$ SD$=5.70 \pm 6.50$ and 3.28 ± 4.16 for 2018 and 2019, respectively), 2934 on 230 roller nestlings in mixed-species colonies (mean carnid fly abundance per nestling $\pm$ SD$=13.83 \pm 14.73$ and 11.80 ± 17.44 for 2018 and 2019, respectively) and 586 on 67 roller nestlings in solitary nests (mean fly abundance per nestling $\pm$ SD$=8.84 \pm 12.46$ and 8.44 ± 8.53 for 2018 and 2019, respectively). Fly abundance among nestlings varied greatly in both species (lesser kestrel: range=0-46; rollers in colonies: range=0-108; solitary rollers: range=0-55).

Table 1.1. Sample size (n), prevalence and Clopper-Pearson 95% confidence intervals (CI) of carnid flies and pupae per 5 g of detritus. Mean abundance ($\pm$ SD), mean intensity ($\pm$ SD) and range of carnid flies per nest and pupae per 5 g of detritus for roller (solitary and in colonies) and lesser kestrel nests. Carnid pupae' values are reported for 2019 and flies for 2018 and 2019.

	Flies		Pupae/5 g
	2018	**2019**	**2019**
Solitary rollers			
n	13	5	4
Prevalence	100 (13/13)	100 (5/5)	100 (4/4)
95% CI	75.29-100	47.82-100	39.76-100
Mean abundance $\pm$ SD	—[a]	—[a]	—[a]
Mean intensity $\pm$ SD	34.69 ± 35.60	27.00 ± 10.84	8.88 ± 9.59
Range	8-128	18-45	—
Rollers in colonies			
n	24	26	30
Prevalence	100 (24/24)	100 (26/26)	83.33 (25/30)
95% CI	85.75-100	86.77-100	65.27-94.36
Mean abundance $\pm$ SD	—[a]	—[a]	6.3 ± 12.16
Mean intensity $\pm$ SD	62.25 ± 45.54	55.38 ± 66.38	7.56 ± 12.99
Range	5-198	1-255	—
Lesser kestrels			
n	121	32	79
Prevalence	97.52 (118/121)	90.63 (29/32)	60.76 (48/79)
95% CI	92.93-99.49	74.98-98.02	49.13-71.56
Mean abundance $\pm$ SD	19.6 ± 17.76	12.91 ± 12.06	8.80 ± 20.54
Mean intensity $\pm$ SD	20.09 ± 17.71	14.24 ± 11.89	14.48 ± 24.82
Range	0-82	0-58	—

[a] When prevalence is 100% mean abundance equals mean intensity

Host preferences by the carnid fly

No significant differences in prevalence were found between species within each sampled year (Fisher's exact test: p-value=1, p-value=0.245, respectively).

The most parsimonious models (ΔAICc<7) on the variation of carnid fly abundance incorporated species t, brood size, nest type (Figure A.1.), year and species t-1 (Table 1.2.), with Julian date as a random factor. The marginal and conditional R^2 for the best-fitted model reached 0.37 and 0.47, respectively.

Table 1.2. Top-ranking (ΔAICc<7) GLMM with a negative binomial distribution of predictors influencing the carnid fly abundance in lesser kestrel and roller (in mixed-species colonies) nests (data from 2018 and 2019).

	Brood size	Species t	Year	Species t-1	Nest type	AICc	ΔAICc	ωi
1	✓	✓	✓	✓		1552.12	0.00	0.54
2	✓	✓	✓			1553.71	1.59	0.25
3	✓	✓	✓	✓	✓	1555.89	3.77	0.08
4	✓	✓		✓		1556.22	4.10	0.07
5	✓	✓	✓		✓	1557.47	5.35	0.04
6	✓	✓				1559.74	6.62	0.02

Akaike information criterion corrected for small sample sizes (AICc). AICc difference between a particular model and the best-fitting model (ΔAICc). Akaike weight (ωi), the contribution of each model to the average of all candidate models. "✓" means that the predictor was included in the model.
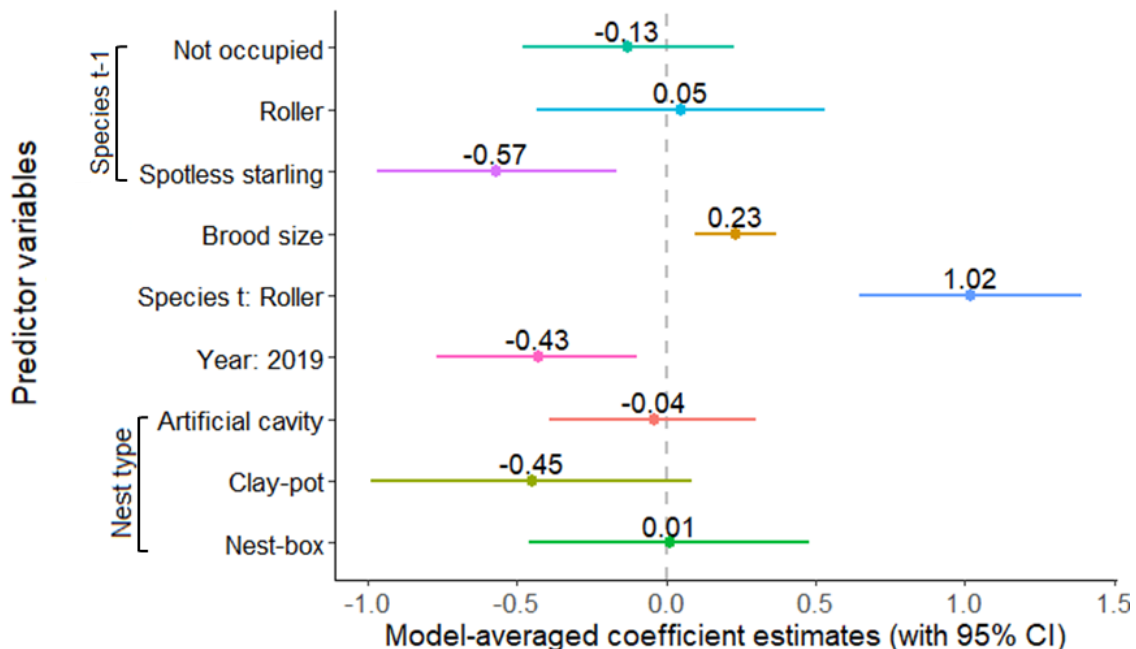
Figure 1.3. Model-averaged conditional coefficient estimates and 95% confidence intervals (CI) for the predictor variables on carnid fly abundance per nest. The intercept (species t: lesser kestrel; species t-1: lesser kestrel; year: 2018; nest type: adobe cavity) is not shown but was estimated as 2.15 (1.63-2.67).

Host species (species t), brood size ($\sum\omega i$=1.00), and year ($\sum\omega i$=0.91) were the most important predictors, followed by previous species occupying the nest (species t-1; $\sum\omega i$=0.70) and nest type ($\sum\omega i$=0.12). Carnid fly abundance was higher in rollers (estimate $\pm$ SE= 1.02 $\pm$ 0.19, CI: 0.65-1.39) than in lesser kestrels, decreasing from 2018 to 2019 (estimate $\pm$ SE= -0.43 $\pm$ 0.17, CI: -0.77-(-)0.10). Brood size positively influenced fly abundance (estimate $\pm$ SE= 0.23 $\pm$ 0.07, CI: 0.10-0.37). When spotless starling was the previous occupant of the nest, the parasite abundance in the next year was lower (estimate $\pm$ SE= -0.57$\pm$ 0.20, CI: -0.97-(-)0.16; Figure 1.3.; Table A.1.).

Comparison of carnid fly infestation pattern on rollers in different social contexts

No inter-annual differences in carnid fly intensity were found for nests in mixed-species colonies or solitary nests (bootstrap 2-sample *t*-test: *p*-value=0.710, *p*-value=0.399, respectively). Julian date did not significantly vary between social contexts (Wilcoxon rank-sum test: *W*=384.5, *p*-value=0.365, n_c=50, n_s=18). Also, brood size differed between social contexts (Wilcoxon rank-sum test: *W*=589, *p*-value=0.046, n_c=50, n_s=18), with solitary nests having lower brood sizes than nests in colonies (mean $\pm$ SD=3.70 $\pm$ 1.60 and 4.60 $\pm$ 1.30, respectively). Roller nests in mixed-species colonies were significantly more parasitize than solitary nests (bootstrap 2-sample *t*-test: *p*-value=0.013; Figure 1.4.).

Figure 1.4. Boxplot of the carnid fly intensity per roller nests in mixed-species colonies and solitary nests including both sampled years (data from 2018 and 2019). Respective means (black dots) and sample sizes (n) are shown. Boxplots illustrate the median (horizontal bold bar), 25th and 75th percentile (box), the extension of 1.5 times the interquartile range from the 25th and 75th percentiles (whiskers) and the outlying points (coloured dots).

<u>Infestation patterns of the pupal phase and concordance between the pupal and infective phase</u>

Carnid pupae prevalence was significantly higher in roller than in lesser kestrel nests (Fisher's exact test: p-value=0.039). No significant differences were found in pupae abundance and intensity between hosts (bootstrap 2-sample t-test: p-value=0.471, p-value=0.101, respectively). In both species, the pupae abundance per 5 g of detritus grew slightly with carnid fly abundance per nest, but the trend was not statistically significant (lesser kestrel: r_s=0.16, p-value=0.446; adobe cavity: n=8, artificial cavity: n=19, clay-pot: n=3, nest-box: n=1; roller: r_s=0.20, p-value=0.288; adobe cavity: n=9, artificial cavity: n=9, clay-pot: n=1, nest-box: n=5; Figure A.2.)

<u>Relationship between carnid fly abundance and nestling body condition index</u>

In both species, wing length was strongly correlated with body mass after log-transformation (lesser kestrel: r_s=0.93, p-value<0.001; roller: r_s=0.96, p-value<0.001; Table A.2.). For lesser kestrels, mean wing length (L_0) was 45.35 mm (Table A.2.) and the regression slope was 0.89 (confidence intervals: 0.86-0.92; Table A.3.). Regarding rollers, L_0 was 55.23 mm (Table A.2.) and the regression slope was 0.94 (confidence intervals: 0.91-0.98; Table A.3.). The SMI successfully controlled for body size differences among individuals since it was not correlated with wing length (lesser kestrel: r_s= -0.24, p-value<0.001; roller: r_s= -0.46, p-value<0.001).

Lesser kestrel

The full model on lesser kestrel nestlings' body condition included colony identity and Julian date as random factors. The marginal and conditional R^2 for the best-fitted model reached 0.05 and 0.68, respectively. Model averaging was performed on the top-models, which included nest type, carnid fly abundance, year, brood size and the interaction between nest type and fly abundance (Table 1.3.).

Table 1.3. Top-ranking (ΔAICc<7) LMMs on mean body condition index (SMI) per lesser kestrel nest (data from 2018 and 2019).

	Nest type	Carnid fly abundance	Year	Brood size	C:N	AICc	ΔAICc	ωi
1	✓	✓	✓		✓	1105.24	0.00	0.38
2	✓	✓	✓	✓	✓	1105.72	0.48	0.30
3	✓	✓			✓	1106.82	1.57	0.18
4	✓	✓		✓	✓	1107.32	2.07	0.14

Akaike information criterion corrected for small sample sizes (AICc). AICc difference between a particular model and the best-fitting model (ΔAICc). Akaike weight (ωi), the contribution of each model to the average of all candidate models. Interaction between carnid fly abundance and nest type is represented by "C:N". "✓" means that the predictor was included in the model.

Nest type, carnid fly abundance (Figure A.3.) and the interaction between these variables (Figure A.4.) were the best predictors (∑ωi=1.00), followed by year (∑ωi=0.69) and brood size (∑ωi=0.44). The interaction between carnid fly abundance and nest-box had a negative influence on nestlings' body condition index (estimate ± SE= -9.01 ± 3.32; CI: -15.58-(-)2.45; Figure 1.5.; Table A.4.).
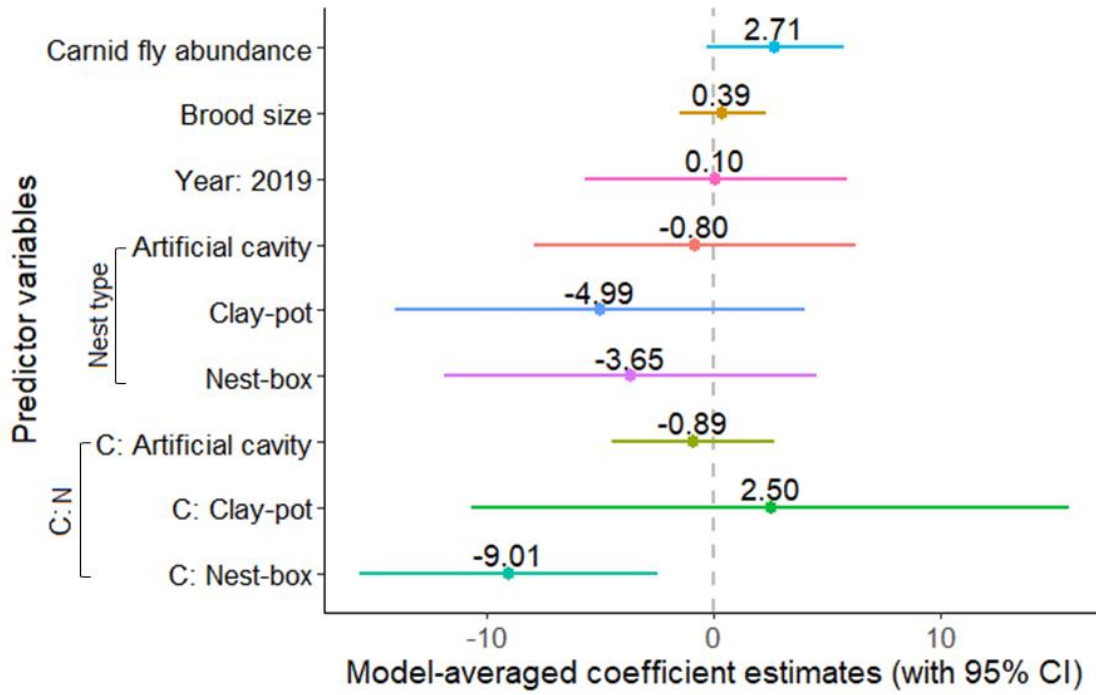
Figure 1.5. Model-averaged conditional coefficient estimates and 95% confidence intervals (CI) for the predictor variables on mean body condition index (SMI) per lesser kestrel nest. The intercept (year: 2018, nest type: adobe cavity, C:N: C:adobe cavity) is not shown but was estimated as 80.30 (73.76-86.84). The interaction between fly abundance and nest type is represented by "C:N".

Roller

The full model included Julian date as random factor. Model averaging was performed on the top-models, which included the predictors: nest type, carnid fly abundance, year, brood size, social context and the interaction between nest type and fly abundance (ΔAICc<7; Table 1.4.). The marginal and conditional R^2 for the best-fitted model reached 0.27 and 0.45, respectively.

Table 1.4. Top-ranking (ΔAICc<7) LMMs on mean body condition index (SMI) per roller nest (data from 2018 and 2019).

	Nest type	Carnid fly abundance	Brood size	Year	Social context	C:N	AICc	ΔAICc	ωi
1	✓	✓		✓	✓	✓	432.04	0.00	0.38
2	✓	✓	✓	✓	✓	✓	432.70	0.66	0.28
3	✓	✓		✓		✓	433.39	1.36	0.19
4	✓	✓	✓	✓		✓	433.97	1.93	0.15

Akaike information criterion corrected for small sample sizes (AICc). AICc difference between a particular model and the best-fitting model (ΔAICc). Akaike weight (ωi), the contribution of each model to the average of all candidate models. Interaction between carnid fly abundance and nest type is represented by "C:N". "✓" means that the predictor was included in the model.

Year, carnid fly abundance (Figure A.5.), nest type and the interaction between fly abundance and nest type (Figure A.6.) were the best predictors ($\sum\omega i$=1.00), followed by rollers social context ($\sum\omega i$=0.66) and brood size ($\sum\omega i$=0.42). In 2019, nestlings had lower body conditions (estimate $\pm$ SE= -10.03 $\pm$ 2.50, CI: -15.06-(-)5.01) than in 2018. Also, the interaction between carnid fly abundance and artificial cavity had a negative influence on nestlings' body condition index (estimate $\pm$ SE= -7.24 $\pm$ 3.43; CI: -14.12-(-)0.36; Figure 1.6.; Table A.5.).

Figure 1.6. Model-averaged conditional coefficient estimates and 95% confidence intervals (CI) for the predictor variables on mean body condition index (SMI) per roller nest. The intercept (social context: colonial, year: 2018, nest type: adobe cavity, C:N: C:adobe cavity) is not shown but was estimated as 104.26 (98.85- 109.66). The interaction between fly abundance and nest type is represented by "C:N".
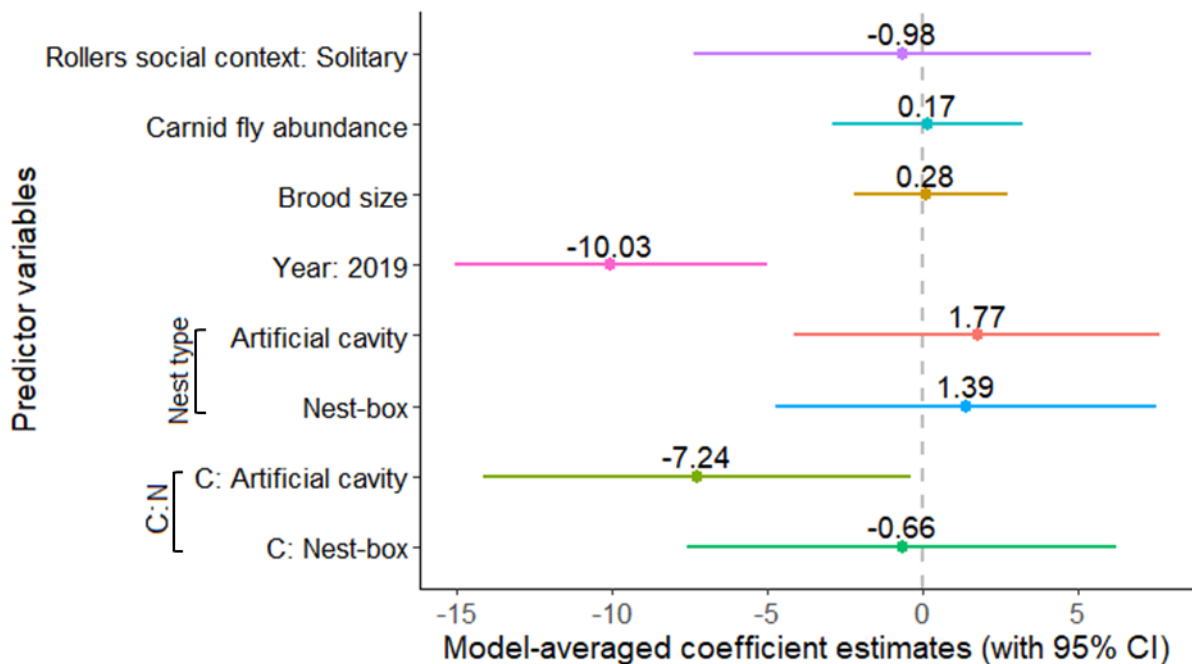
Discussion

This study allowed us to assess host preference of the carnid fly *C. hemapterus* between rollers and lesser kestrels, two sympatric species with similar ecological niches. The results suggest that carnid flies preferred rollers over lesser kestrels, even when the latter breed in higher densities. But host sociality seemed to influence the abundance of carnid flies in roller nests, with rollers breeding in mixed-species colonies, having approximately twice as many parasites as solitary rollers. Moreover, the infestation pattern of the pupal phase (non-infective phase) revealed an imperfect concordance with the infective phase as the host preference for rollers inferred by carnid flies did not relate with the occurrence of pupae in hosts' nests. Our results also suggest a negative association between carnid flies and nestling body condition, however, solely within specific nest types (with lesser kestrels in nest-boxes and rollers in artificial cavities).

Host preferences by the carnid fly

All roller nests were infected by carnid flies in both years. High prevalences in roller nests have already been reported in several studies (Václav et al. 2008; Veiga et al. 2019a, 2020). However, our intensity levels (Table 1.1.) are one of the highest reported so far for this species (Václav et al. 2008: mean intensity per nest ± SE=29.62 ± 4.11; Veiga et al. 2019a: mean intensity per nest=20.87; 95% CI=16.45–27.19, see Veiga et al. 2020 for similar values). For lesser kestrel, Ganbold et al. (2020) observed a mean of 4.6 carnid flies per nestling with 9-12 days of age, a value comparable to ours (mean abundance per nestling ± SD=5.70 ± 6.50 and 3.28 ± 4.16 for 2018 and 2019, respectively). Similarly, in common kestrel, Roulin et al. (2003) recorded a moderate parasite intensity ± SE of 15 ± 3 per nest. Yet, other studies on closely-related species showed greater carnid fly abundances (Kal'avský and Pospíšilová 2010: common kestrel, mean abundance per nestling=9.56; Lesko and Smallwood 2012: American kestrel *Falco sparverius*, in previously cleaned nests, mean abundance per nest ± SD=54.7 ± 67.3).

Carnid fly abundance in roller nests was approximately three times higher than in lesser kestrel nests when both species breed in mixed-species colonies, suggesting a preference for rollers over lesser kestrels. In fact, even solitary rollers were more parasitized than lesser kestrels, possibly indicating that host species identity has a more profound effect on parasite infestation patterns than their social breeding context. A generalist parasite may differ in its' fitness (survival and reproductive success) between host species, with some hosts being more suitable (optimal hosts) than others (suboptimal hosts). Lesser kestrel nestlings are born covered by white down feathers, whereas newly hatched nestling rollers are entirely naked, having their body nearly all covered with closed feather sheaths at 13 days of age and with fledging taking place around 25-30 days post-hatching (Del Hoyo et al. 2001a). It is known that the increase of feather density and layering hampers carnid flies access to the host skin (Kirkpatrick and Colvin 1989; Dawson and Bortolotti 1997). In addition, Marshall (1981) found that carnid flies may feed on the base of growing feathers, so the keratinization and retraction of blood vessels nourishing the feathers may lead to the avoidance of older nestling (Calero Torralbo 2011). The plumage of nestling kestrels probably decreases foraging efficiency leading to a selection for naked nestling rollers by immigrant flies. Although several studies have shown nestling kestrels infected until 25 days post-hatching, fly numbers are particularly low (lesser kestrel: Ganbold et al. 2020; common kestrel: Kal'avský and Pospíšilová 2010; American kestrel: Dawson and Bortolotti 1997). Host-related factors, such as immunocompetence or nest substrate, may also contribute to the disparities found in carnid fly abundance. Rollers and lesser kestrels appeared to breed in similar nest substrates, mainly composed of earthy, rotting material with prey remains, faeces and feathers debris. However, we did not perform a thorough assessment of their nest substrates, so these disparities may in part result from differences in species-specific accumulated detritus amongst these species, with roller nests being more beneficial for

carnid flies. Moreover, the probability of a ectoparasite finding a particular host species is influenced by that species local availability (Veiga et al. 2019a). Lesser kestrel was the better represented species in our sampled colonies, so the higher parasite abundance in roller nests does not reflect a higher probability of finding a roller nest, indicating that a preference is occurring.

Our results showed that carnid fly abundance was lower when spotless starlings were the previous occupant of the nest. Spotless starlings are common breeders in the studied mixed-species colonies and are known to add green plants as a nest lining material, which can have antiparasitic effects on blood-sucking arthropods (Clark and Mason 1985). Valera et al. 2006b proposed that carnid flies avoid nests with abundant vegetable matter (e.g. straw, sticks, leaves). Thus, the added material by starlings in the previous breeding season may influence parasites' nest choice in the present season. Liker et al. 2001, however, recorded high abundances (mean abundance of 54 carnid flies per nest) for a closely related species, the common starling *Sturnus vulgaris*.

We found a positive association between carnid fly abundance and brood size, which was expected as more nestlings in the nest translates to higher food resources and cues (see Veiga et al. 2020 for a similar effect of rollers brood mass). Variations in nest thermal environment and gases' concentration are well-known attractants for many blood-feeding insects and likely to occur in relation to brood size. For instance, nest temperature and CO_2 concentrations have been shown to positively relate to brood size (Andreasson et al. 2016; Castaño-Vázquez et al. 2019). Consequently, a nest with more nestlings may be easier to detect, thus showing greater abundances of ectoparasites (Castaño-Vázquez et al. 2019). However, other studies found carnid fly abundance unrelated to brood size (Dawson and Bortolotti 1997; Liker et al. 2001; Wiebe 2009).

Carnid fly abundance in hosts' nests varied between study years with a significant decrease in 2019. The inter-annual disparity might be related to differences in the sampling effort of lesser kestrel nests (n=121 and 32 for 2018 and 2019, respectively) or environmental factors, such as temperature and humidity (Dudaniec et al. 2007; Veiga et al. 2019b). The summer (june, july, august) of 2019 was drier and colder than normal and was preceded by a heatwave at the end of may (IPMA 2019). Temperature has long been recognized as an abiotic factor influencing flying insects emergence patterns and diapause duration (Calero-Torralbo and Valera 2008; Amat-Valero et al. 2013). However, little is known about the effects of environment-related factors in carnid fly abundance.

Comparison of carnid fly infestation pattern on rollers in different social contexts

Our results revealed that rollers in mixed-species colonies were twice as much parasitized by carnid flies as solitary nests.

According to Poulin (1991), the abundance of mobile ectoparasites is not expected to differ between group-living and solitary individuals since flies can actively search and colonize hosts' nests without needing physical host-to-host contact for transmission. In agreement, Liker et al. (2001) found no significant evidence of a relationship between residuals of mean carnid fly abundance per nest and spatial position of nests within a common starling colony. However, the opposite pattern may occur if the effects of higher local density lead to enhanced sensory cues increasing the probability of hosts' detection and parasites can take advantage of multiple hosts (Mooring and Hart 1992). For example, smaller inter-nest distances were associated with a higher carnid fly intensity in the European bee-eater *Merops apiaster* nestlings (Hoi et al. 2010). Also, *Philornis downsi* – a mobile, non-contact-transmitted blood-sucking fly – total intensity increased with hosts' nest density (Kleindorfer and Dudaniec 2009).

Our findings support the hypothesis that mobile parasite numbers increase with host density, with parasite intensity higher on roller nests in mixed-species colonies than on solitary nests. Veiga et al. (2020) also showed that the abundance of colonizing carnid flies in roller nests was positively related to hosts' density. This suggests that high breeding density may facilitate the colonization of nests by dispersing carnid flies. Flies host-seeking behaviour is likely to rely on a combination of sensory cues, such as host body heat, carbon dioxide concentration, host specific odours, visual and auditory cues (Kleindorfer and Dudaniec 2009; Tomás and Soler 2016; Veiga et al. 2020). Host aggregations may convey enhanced stimulus, leading to higher conspicuousness and consequent attraction of parasites (Brown and Brown 2001), possibly explaining the increase intensity of carnid flies in roller nests in mixed-species colonies. Mixed-species colonies could also allow *C. hemapterus* to raise multiple generations, through a multivoltine cycle, within a breeding season. If so, flies could exploit early breeders (e.g. spotless starling), followed by resident species with later phenology (e.g. common kestrels, little owl) and, finally, late breeding migratory species (e.g. roller, lesser kestrel; Amat-Valero et al. 2012). Moreover, solitary rollers had smaller brood sizes than rollers in colonies (possibly due to differences in the sampling effort, n=18 and 50, respectively) and, as previously reported, the brood size appears to positively influence carnid fly abundance. Therefore, the lower intensity in solitary nests could be partially explained by the smaller brood sizes.

The similar high prevalence between roller nests in solitary and colonies, may be due to diapausing pupae in the nest and not from immigrant flies since we did not clean the nests before the breeding season. Nonetheless, carnid fly prevalence reached almost 100% in cleaned – new or fumigated –, isolated nest-boxes of rollers, in four separate years (Veiga et al. 2020). Podofillini et al. (2018) found higher carnid fly abundance on lesser kestrel nestlings in dirty nest-boxes than those in clean nest-boxes soon after hatching (0-3 days), but this effect became non-significant during the rest of the season. Also, no differences were found in carnid fly intensity on one-week-old nestlings of northern flicker (*Colaptes auratus*) between freshly excavated and re-used cavities (Wiebe 2009). Several studies have suggested a high colonization capacity of the winged morph (Liker et al. 2001; Veiga et al. 2020), perhaps due to highly efficient host detection mechanisms, potentially explaining why we did not observe differences in prevalence between roller nests in different social contexts, despite solitary roller nests being at least 100 meters apart from the nearest colony. Though, it is still unclear which sensory cues are used by carnid flies when searching for potential hosts (Liker et al. 2001; Veiga et al. 2020).

Infestation patterns of the pupal phase and concordance between the pupal and infective phase

Our results showed that inferences based on the pupal phase were not consistent with the ones on the infective phase. Roller nests in mixed-species colonies had a significantly higher prevalence of carnid pupae compared to lesser kestrels. However, both abundance and intensity did not significantly differ from lesser kestrels' nests. Additionally, carnid fly abundance per nest correlated weakly with the pupae abundance per 5 g of detritus.

In a previous study, Veiga et al. (2019a) reported a mean carnid fly intensity per nest of 20.87 and a mean intensity of carnid pupae per 5 g of detritus of 10.51. Although our results on pupae intensity for roller (mean intensity of pupae per 5 g of detritus $\pm$ SD=7.56 $\pm$ 12.99) are comparable to those found by Veiga et al. (2019a), our fly intensity was much higher (mean intensity $\pm$ SD=55.38 $\pm$ 66.38), so we expected a higher abundance and intensity of pupae. The results found for the pupal phase were not consistent with the ones observed for the infective phase. It is possible that different phases of *C. hemapterus* differ in their requirements and levels of specialization, which may lead to an imperfect concordance between host preference and host suitability (Veiga et al. 2019a). By lowering parasite fitness, this incompatibility may influence *C. hemapterus* epidemiological dynamics.

Other hypotheses can be proposed to explain the mismatch between these development phases of *C. hemapterus*. According to Veiga et al. (2019a), nest sanitation behaviour by rollers is common. Adults and/or juveniles may have played a role in keeping pupae levels low by removing the nesting material along with eggs, larvae and/or pupae. In blue tits (*Parus caeruleus*), parasite density significantly increased the time devoted to nest sanitation (Tripet et al. 2002). Moreover, host species-related differences in nest predation pressure by arthropods could contribute to the mismatch found between infective and pupal phases (Veiga et al. 2019a). Eskafi and Kolbe (1990) found evidence of mediterranean fruit fly *Ceratitis capitata* larvae and pupae predation by the fire ant *Solenopsis geminata* and coleopterous predators. Also, egg, larval and pupal predation could happen more intensely in some types of nests in comparison to others, possibly due to microclimatic differences. On the other hand, it is known that parasite populations tend to exhibit aggregated distributions (Rozsa et al. 2000), so intraspecific competition amongst parasites could limit parasite fitness (Veiga et al. 2020). The fitness of blood-sucking flies depends on their feeding success, so a higher parasite density on a host will increase intraspecific competition, thus decreasing the feeding success and, consequently, parasite fitness (Krasnov et al. 2007). The quantity of blood consumed by *Xenopsylla conformis* was found to differed between rodent host species and the number of conspecifics fleas on a particular host, plus egg production and survival varied amongst host species (Krasnov et al. 2004). The host body size may act as a limiting factor when parasitic flies feed meanly on particular areas of the host, as it is the case of carnid flies (Marshall 1981). *C. hemapterus'* fitness may also be influenced by the microclimate inside different types of nests, leading some to be unsuitable for egg, larval and/or pupal development (Krasnov et al. 2001). However, this seems unlikely to explain the lack of differences between rollers and lesser kestrels, since both species tend to use the same type of nests with similar frequency. The amount and composition of the nest substrate may also influence parasite fitness since some nest materials, such as vegetable matter, may jeopardize egg, larvae and/or pupae survival (Veiga et al. 2019a). It could also be that, in some nests, carnid larvae did not have enough time to pupate before the sampling of the detritus.

Regarding the weak correlation between fly abundance per nest and pupae abundance per 5 g of detritus, the correlation coefficient may differ for each host species in each nest type, and so the type of nest could be hiding the real relation. Yet, no conclusion can be drawn due to the extensively small and unequal representation of each nest type (roller: adobe cavity: n=9, artificial cavity: n=9, clay-pot: n=1, nest-box: n=5; lesser kestrel: adobe cavity: n=8, artificial cavity: n=19, clay-pot: n=3, nest-box:

n=1). Preferably we would have extrapolated the number of pupae per 5 g for the totality of the nest through the nest weight. However, in most of the cases, especially on natural cavities, it was impracticable to extract the nest from the nest cavity and weight it.

Relationship between carnid fly abundance and nestling body condition index

Our results revealed a negative association between carnid fly abundance and mean body condition index (SMI) per nest, but only on lesser kestrels growing in nest-boxes and rollers in artificial cavities.

No general agreement exists relating carnid flies impacts on its hosts. Several studies revealed no evidence of detrimental effects by this ectoparasite on breeding success, nestlings mass, growth and survival (Dawson and Bortolotti 1997; Lesko and Smallwood 2012; Sumasgutner et al. 2014). In common starlings, carnid fly abundance was not associated with nestling condition, growth and mortality rates (Liker et al. 2001; Hornsby et al. 2013). In fact, some researchers have even found a preference by carnid flies for nestlings in better condition (roller: Václav et al. 2008; Václav and Valera 2018; European bee-eater: Valera et al. 2004; Hoi et al. 2010; American kestrel: Dawson and Bortolotti 1997). On the other hand, various studies have shown adverse effects by this ectoparasite on the infected nestlings: lower growth rate in the European bee-eater (Hoi et al. 2010) and the northern flicker (Wiebe 2009); inferior body mass in spotless starlings (Avilés et al. 2009) and the northern flicker (Wiebe 2009); decrease of nestling body condition, immune response, haematocrit levels in the European bee-eater (Hoi et al. 2018); and nestling mortality in northern saw-whet owl *Aegolius acadicus* (Cannings 1986). Hematophagous parasites may maximize their fitness by balancing the quality and quantity of nutritive resources (blood meals) extracted against hosts' immune defences (Bize et al. 2008; Václav and Valera 2018). Poor condition, immunodeficient hosts may provide smaller blood meals of lower nutritional quality to parasites and, as such, be avoided. Likewise, high-quality hosts, which may offer larger amounts and richest resources, are expected to resist parasite exploitation through an efficient immune system. Therefore, it is plausible that the choice for intermediate condition individuals is favoured (Bize et al. 2008; Václav and Valera 2018). On the other hand, it is possible that parents increase their food provision rate in order to compensate for the depletion of resources by parasites. Christe et al. (1996) found a 50% increase in food provisioning rate by great tit *Parus major* males of infected nests.

Our mean abundances and intensities (Table 1.1.) on lesser kestrel and roller seem to be only moderate levels when compared to the nest mean abundance of 98 ± 0.6 for bee-eaters (Hoi et al. 2018) and the mean intensity of 40 flies per nestling for barn owl *Tyto alba* (Roulin 1998). The considerable variations amongst previous studies may be due to differences in the host species investigated and their densities, numbers and suitability of host species present or environmental and ecological factors. Moreover, nestling age when sampled, nest substrate, competitive interactions among flies and carnid fly abundance in the previous year can also influence ectoparasite abundance patterns. Dealated adults (without wings), even with limited dispersal ability, may actively walk between nestlings in the same brood and neighbouring nests if not too far away (Hoi et al. 2010). Considering the ectoparasite dispersal capacity, perhaps flies move to another host when its condition starts to deteriorate, preventing strong detrimental effects (Hoi et al. 2018). Conceivably, a negative association may only occur by high parasite abundances combined with particular circumstances, such as an already weakened host. Nestling susceptibility can vary between species and be affected by an adverse year (e.g. limited resources), environmental and social stressors and poor parental care, especially in large asynchronized clutches with competition (Hoi et al. 2010; Kappeler et al. 2015; Catry and Catry 2019). Potential synergetic effects, for instance, the interaction between parasite abundance and other stress factors (such as nest microclimatic conditions) may differ in its magnitude between species. Our results support a

negative association between carnid flies and rollers' body condition when growing in artificial cavities. On the other hand, lesser kestrels showed significantly lower body conditions when parasitized in nest-boxes. Microclimatic conditions inside the nest (e.g. temperature, humidity) can influence the development and survival of altricial nestlings and affect their interaction with parasites (Martínez-de la Puente et al. 2010). Nest microclimate may vary considerably, yet our knowledge is scarce and biased towards some nest types, as nest-boxes (Amat-Valero et al. 2014). In our study area, in extremely warm years, nest-boxes reach high temperatures and deficient ventilation levels, which may lead to reduced nestling growth rates and increased mortality (Catry et al. 2011). Catry et al. (2015), found that nest-boxes warmed at a faster rate than adobe cavities, creating unfavourable nest microclimatic conditions. Also, lesser kestrels revealed lower physiological and immunological conditions due to heat stress, in contrast with rollers in nest-boxes that exhibited higher resilience to heat (Catry et al. 2015). By extracting resources and possibly inducing nutritionally demanding immune responses, parasites may exacerbate the consequences for already debilitated hosts.

Final considerations

Previous conservation efforts for lesser kestrel and roller have mainly focused on nest-site provisioning. In our study area, this has fostered a remarkable recovery of their Portuguese breeding populations (Catry et al. 2009). However, conservation efforts have seldom considered host-parasite interactions and their consequences. The higher nesting density and proximity of different species promoted by breeding walls and towers may enhance intra- and interspecific agonistic interactions (e.g. predation, competition) leading to social stress, which is known to increase individual susceptibility to disease and infection (Kappeler et al. 2015; Catry and Catry 2019). Also, the re-use of nests by different host species is facilitated, increasing the opportunities for intra- and interspecific parasite transmission, which may lead fly numbers to be higher than expected. Parasites lead to the depletion of resources that could otherwise be used for nestling growth, immune system development and behaviour. As such, parasites may reduce nest quality, attractiveness, cause birds to reject or abandon nests (Loye and Carroll 1998). However, we were not able to determine if these factors affect in any way the reproductive success of the studied species.

We found higher ectoparasite abundances on roller nests in comparison to lesser kestrel nests when both species breed in mixed-species colonies. The species social context also affected carnid fly abundance, with rollers breeding in mixed-species colonies being significantly more parasitized than solitary rollers. Thus, it is important to assess how colony traits (e.g. colony size, inter-nest distance, host density and colony richness) influence flies numbers. The previous occupation of the nest by spotless starlings (species t-1) had a negative influence on the parasite abundance of the following year (t). Moreover, brood size contributed positively to explain fly abundance. Future studies should investigate which cues are used by immigrant flies (e.g. heat, humidity and CO_2 emissions) to locate their hosts. Additionally, the infestation patterns observed for the carnid fly (infective phase) were not consistent with the ones obtained for the pupal phase. Plus, carnid fly abundance per nest correlated weakly with the pupae abundance per 5 g of detritus possibly revealing an imperfect concordance between host preference by the infective phase and its suitability through the parasite life cycle. Thus, this study contributed to the better understanding of the determinants regulating *C. hemapterus* infestation patterns and heterogeneity in host suitability through the parasite life cycle. Also, a negative association was found between carnid flies and nestlings' body condition in particular nest types (lesser kestrels in nest-boxes and rollers in artificial cavities). These results allowed us to better understand how these two endangered species are responding to fly abundance and how synergetic effects may be aggravating the parasite effects. However, future studies should assess parents' provisioning rate since parents with more parasitize nests may increase their provisioning rates concealing carnid flies' effects

on nestlings. Also, determine if differences in artificial nest constructions (e.g. nest internal size, shape, placement and microclimatic properties) are influencing the outcome of host-parasite interactions, which is important to assess the effectiveness of pre-existent conservation measures.

In the light of climate change, the Mediterranean region mean and extreme temperatures are predicted to change, plus heat waves and drought events are expected to increase in its frequency, intensity and duration (Meehl and Tebaldi 2004; Zittis et al. 2019). Climate change is predicted to disrupt established patterns, differentially alter species phenology, and shift the geographic range of both hosts and parasites, potentially leading to novel interactions. A more thorough assessment of the role of environmental variation on parasite epidemiology and host-parasite interactions is required to improve predictions of infection risk in response to global climate change.

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Appendix
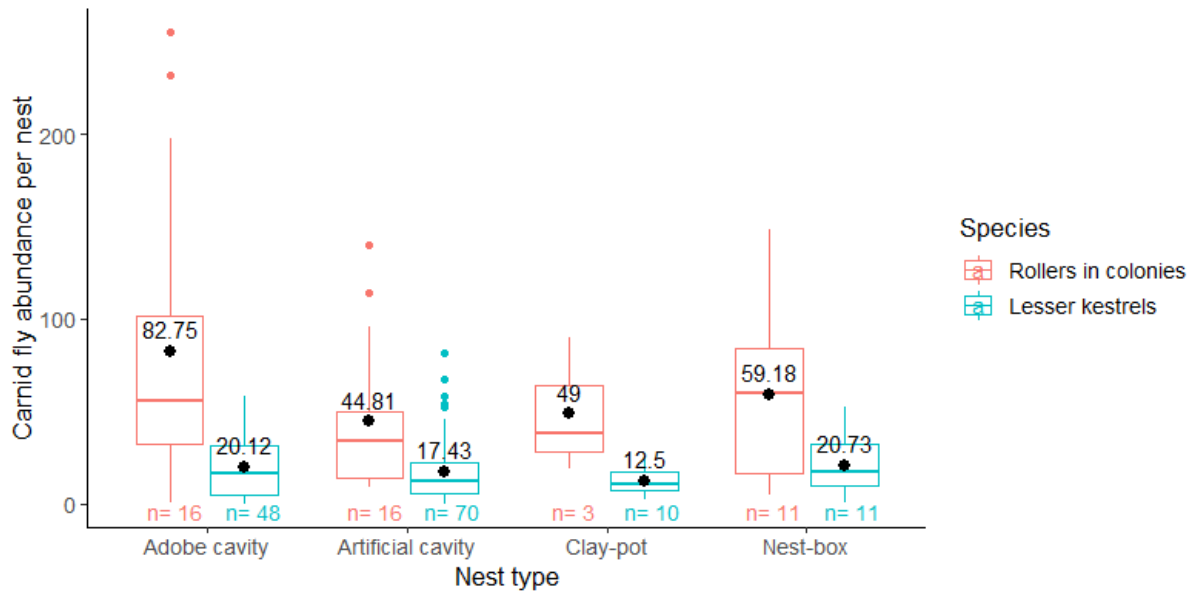
Figure A.1. Boxplot of carnid fly abundance per nest within different nest types on roller and lesser kestrel nests in mixed-species colonies (data from 2018 and 2019). Respective means (black dots) and sample sizes (n) are shown. Boxplots illustrate the median (horizontal bold bar), 25th and 75th percentile (box), the extension of 1,5 times the interquartile range from the 25th and 75th percentiles (whiskers) and the outlying points (coloured dots).

Table A.1. Model averaging results (ΔAICc<7) on the carnid fly abundance in lesser kestrel and roller (in mixed-species colonies) nests (data from 2018 and 2019). Predictors are ranked according to their relative importance ($\sum \omega i$, sum of ωi for all possible models in which the predictor variable was included). Estimate, standard errors (SE) and 95% confidence intervals (CI) of each predictor variable are reported.

GLMM - Negative Binomial	**Estimate**	**SE**	**Z**	**95% CI**	$\sum \omega i$
Random effect:					
Julian date					
Intercept	2.15	0.26	8.12	1.63-2.67	
Brood size	0.23	0.07	3.34	**0.10-0.37**	1.00
Species t					1.00
Roller	1.02	0.19	5.35	**0.65-1.39**	
Year					0.91
2019	-0.43	0.17	2.54	**-0.77-(-)0.10**	
Species t-1					0.70
- Not occupied	-0.13	0.18	0.71	-0.48-0.23	
- Roller	0.05	0.24	0.20	-0.43-0.53	
- Spotless starling	-0.57	0.20	2.76	**-0.97-(-)0.16**	
Nest type					0.12
- Artificial cavity	-0.04	0.18	0.25	-0.39-0.30	
- Clay-pot	-0.45	0.27	1.66	-0.99-0.08	
- Nest-box	0.01	0.24	0.05	-0.46-0.48	

The results reported are conditional averages after model averaging
Variables with confidence intervals that exclude zero have well-supported effects and are shown in boldface
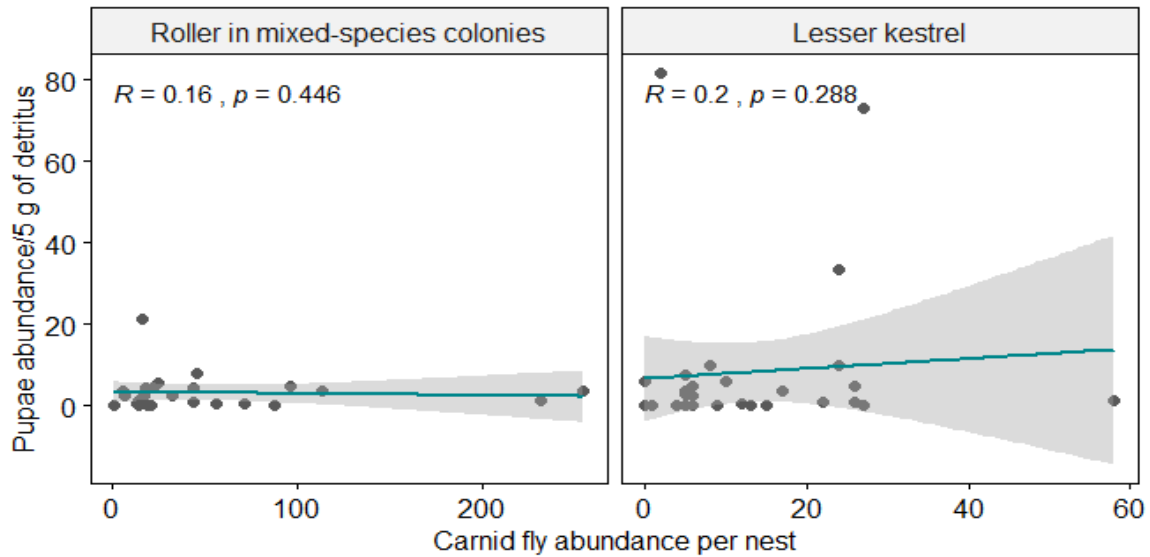
Figure A.2. Variation of carnid pupae abundance per 5 g of detritus in relation to carnid fly abundance in lesser kestrel and roller nests, separately. The grey shaded area corresponds to the 95% confidence interval. Spearman's rank correlation coefficient (R) and *p*-value (p) are displayed on the top left (lesser kestrel: n=31 nests, roller: n=24 nests; data from 2019).

Table A.2. Mean weight (g), mean wing length (mm), Spearman's rank correlation (r_s) and respective *p*-value for lesser kestrel and roller, separately (data from 2018 and 2019).

|	Mean weight	Mean wing length	r_s	*p-value*
Lesser kestrel	78.78	45.35	0.93	**<0.001**
Roller	98.04	55.23	0.96	**<0.001**

p-values<0.05 are shown in boldface

Table A.3. Slope b_{SMA} (with 95% confidence intervals, CI) and test statistics of standardized major axis regression between body mass and wing length (log-transformed) for lesser kestrel and roller, separately (data from 2018 and 2019).

SMA regression	b_{SMA} **(95% CI)**	R^2	*p-value*
Lesser kestrel	0.89 (0.86-0.92)	0.86	**<0.001**
Roller	0.94 (0.91-0.98)	0.89	**<0.001**

p-values<0.05 are shown in boldface
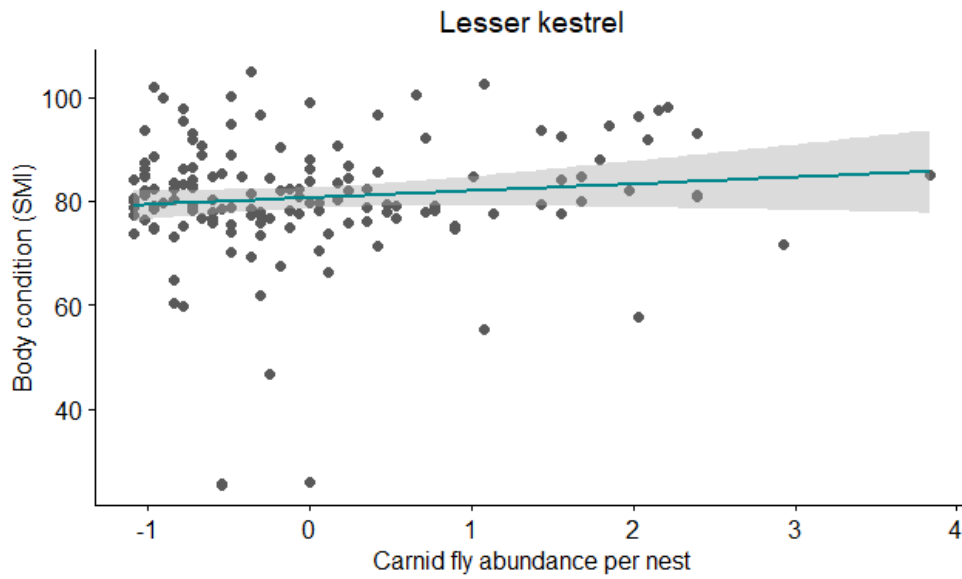
Figure A.3. Variation of mean body condition index (SMI) per lesser kestrel nest according to carnid fly abundance per nest in different nest types (n=147 nests, data from 2018 and 2019). The grey shaded area corresponds to the 95% confidence interval.
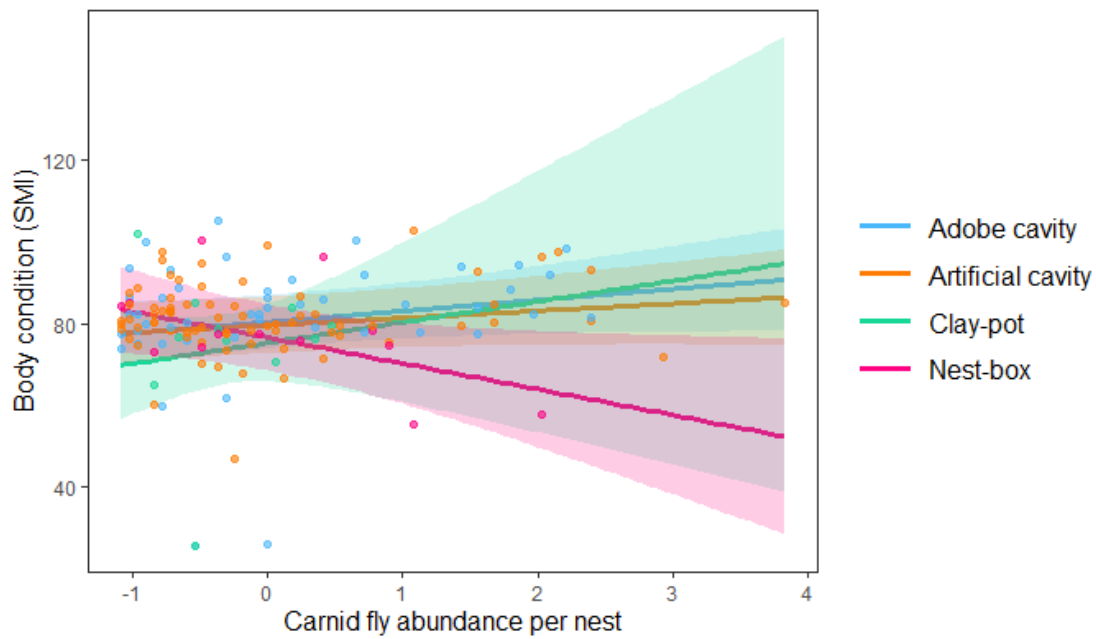
Figure A.4. Variation of mean body condition index (SMI) per lesser kestrel nest according to carnid fly abundance per nest in different nest types (adobe cavity, artificial cavity, clay-pot and nest-box). The shaded areas correspond to the 95% confidence intervals.

Table A.4. Model averaging results (ΔAICc<7) on mean SMI per lesser kestrel nest (data from 2018 and 2019). Predictors are ranked according to their relative importance ($\sum \omega i$, sum of ωi for all possible models in which the predictor variable was included). Estimates, standard errors (SE) and 95% confidence intervals (CI) of each predictor variable are reported.

Lesser kestrel	Estimate	SE	z	95% CI	$\sum \omega i$
Random effect:					
Julian date; Colony ID					
Intercept	80.30	3.31	24.06	73.76-86.84	
Nest Type					1
- Artificial cavity	-0.80	3.58	0.22	-7.89-6.28	
- Clay-pot	-4.99	4.56	1.09	-14.01-4.02	
- Nest-box	-3.65	4.13	0.88	-11.82-4.52	
Carnid fly abundance	2.71	1.52	1.76	-0.30-5.73	1
C:N					1
- C: Artificial cavity	-0.89	1.82	0.48	-4.48-2.71	
- C: Clay-pot	2.50	6.65	0.37	-10.66-15.65	
- C: Nest-box	-9.01	3.32	2.69	**-15.58-(-)2.45**	
Year: 2019	0.10	2.91	0.03	-5.65-5.85	0.69
Brood size	0.39	0.96	0.40	-1.52-2.30	0.44

The results reported are conditional averages after model averaging
Variables with confidence intervals that exclude zero have well-supported effects and are shown in boldface
Interaction between carnid fly abundance (C) and nest type (N) is represented by "C:N"
ID, identity
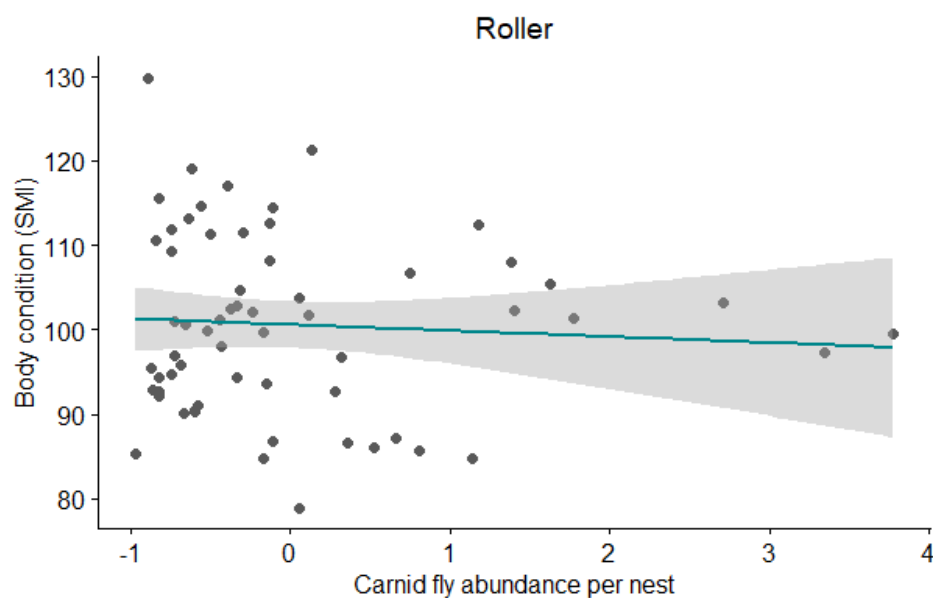
Figure A.5. Variation of mean body condition index (SMI) per roller nest according to carnid fly abundance per nest (n=61 nests, data from 2018 and 2019). The grey shaded area corresponds to the 95% confidence interval.
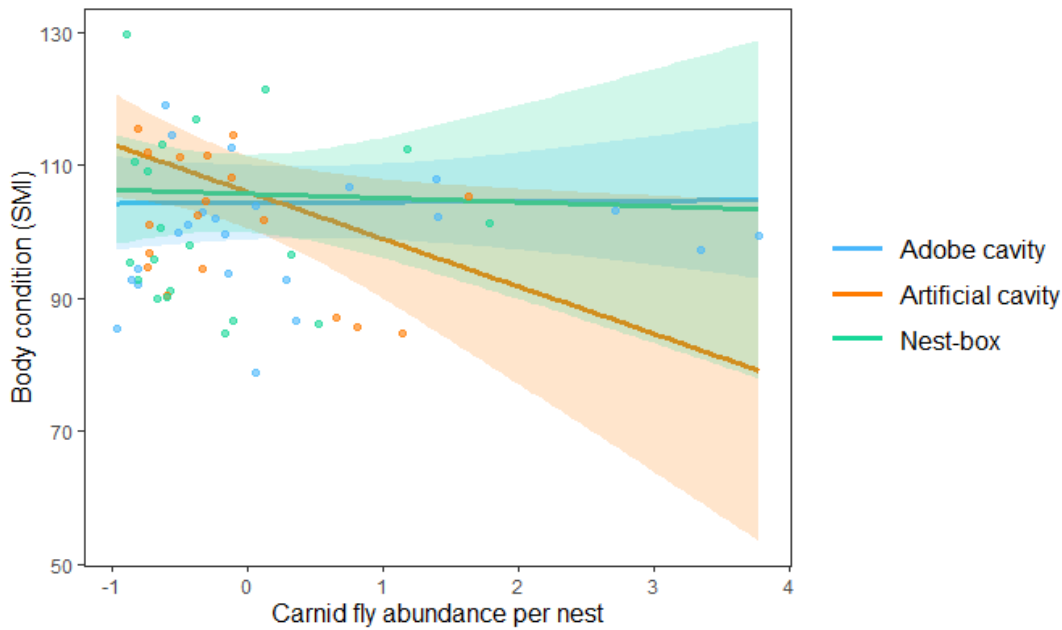
Figure A.6. Variation of mean body condition index (SMI) per roller nest according to carnid fly abundance per nest in different nest types (adobe cavity, artificial cavity and nest-box). The shaded areas correspond to the 95% confidence intervals.

Table A.5. Model averaging results (ΔAICc<7) on mean SMI per roller nest (data from 2018 and 2019). Predictors are ranked according to their relative importance (∑ ωi, sum of ωi for all possible models in which the predictor variable was included). Estimate, standard errors (SE) and 95% confidence intervals (CI) of each predictor variable are reported.

Roller	Estimate	SE	z	95% CI	∑ωi
Random effect:					
Julian date					
Intercept	104.26	2.69	37.81	98.85-109.66	
Nest Type					1
- Artificial cavity	1.77	2.93	0.59	-4.12-7.65	
- Nest-box	1.39	3.06	0.44	-4.76-7.54	
C:N					1
- C: Artificial cavity	-7.24	3.43	2.06	**-14.12-(-)0.36**	
- C: Nest-box	-0.66	3.44	0.19	-7.56-6.25	
Year: 2019	-10.03	2.50	3.91	**-15.06-(-)5.01**	1
Carnid fly abundance	0.17	1.52	0.11	-2.88-3.22	1
Solitary rollers	-0.98	3.19	0.30	-7.39-5.43	0.66
Brood size	0.28	1.24	0.22	-2.22-2.78	0.42

The results reported are conditional averages after model averaging
Variables with confidence intervals that exclude zero have well-supported effects and are shown in boldface
Interaction between carnid fly abundance (C) and nest type (N) is represented by "C:N"