UNIVERSITY OF
GLOUCESTERSHIRE

Blunsden, Thomas P and Goodenough, Anne E ORCID logoORCID: https://orcid.org/0000-0002-7662-6670 (2023) Influence of nest box design and nesting material on ectoparasite load for four woodland passerines. Bird Study, 70 (1-2). pp. 25-36. doi:10.1080/00063657.2023.2190081

Influence of nest box design and nesting material on ectoparasite load for four woodland passerines

Thomas Blunsden and Anne E. Goodenough*

School of Natural and Social Sciences, Francis Close Hall, University of Gloucestershire, Cheltenham, UK

* corresponding author aegoodenough@glos.ac.uk

Running title: Factors affecting nest ectoparasite loads

Keywords: Cavity-nesting, Hen Flea, Blowfly, Tit, Flycatcher, Nuthatch

Summary

Capsule: Abundance of haematophagous ectoparasites in woodland passerine nests is influenced by complex interactions between nest box design, bird species, amount of nesting material, and nest composition.

Aims: To analyse ectoparasite abundance relative to nest box design (old wooden nest boxes present for ≥ 3 years vs new wooden nest boxes of the same dimensions vs deep wooden nest boxes designed to reduce predation risk) and bird species (Blue Tit *Cyanistes caeruleus*, Great Tit *Parus major*, Pied Flycatcher *Ficedula hypoleuca*, and European Nuthatch *Sitta europaea*). The potential influence of amount of nesting material and nest composition was also studied.

Methods: After fledging, nests were collected from nest boxes. Ectoparasites and nest materials were identified and quantified. Generalised Linear Modeling was used to examine the influence of nest box design, bird species, amount of nest material, and nest composition on ectoparasite loads. Akaike's Information Criterion was used to select optimal models.

Results: Abundance of Hen Fleas *Ceratophyllus gallinae* and parasitic Blowfly *Protocalliphora* was significantly higher for deep nest boxes than nest boxes of standard dimensions. Old nest boxes had significantly higher loads than new nest boxes, despite thorough cleaning between breeding seasons. Hen Flea abundance was highest in Nuthatch nests. Blowfly abundance was highest in Pied Flycatcher nests. Abundance of both fleas and blowfly was positively related to nest mass and amount of animal hair in the nest, and, for parasitic Blowfly, was negatively related to amount of tree bark.

Conclusion: Ectoparasite load depends not only on bird species but also nest box design and nesting material. We recommend: (1) nest boxes are regularly replaced to reduce parasite load; (2) deep nest boxes are not used as the large nests constructed not only removes anti-predator benefits of eggs/chicks being harder to reach but are also associated with high haematophagous ectoparasite loads.

Introduction

Nest ectoparasites constitute a threat to the fitness and survival of chicks and parents. Haematophagous parasites can cause anaemia, lack of weight gain for chicks, and weight loss for adults (Richner *et al*. 1993, Merino & Potti 1995, Dudek *et al*. 2021). Some ectoparasites can also cause disease, either because they act as vectors or because puncture wounds or scratches become infected (Warren 1994, Tomás *et al*. 2007, Atkinson *et al*. 2009). The effects of nest-based ectoparasitism can manifest either during the nesting period itself (Moore 2002, Bush & Clayton 2018) or immediately afterwards via carry-over effects that reduce post-fledging survival of young or post-breeding survival of parents (Wesołowski 2001, Dudek *et al*. 2021). Impacts of parasitism can be situation- or condition-dependent, for example, becoming more evident during times of severe weather (e.g. rainfall for Peregrine Falcons *Falco peregrinus* (Lamarre *et al*. 2018); high temperatures for Blue Tits *Cyanistes caeruleus* (Castaño-Vázquez & Merino *et al.* 2021)) or in highly human-modified landscapes (e.g. Cliff Swallows *Petrochelidon pyrrhonota* (Benedict *et al*., 2021)). Recent work on Cliff Swallows has also shown that high parasitic loads in nests can affect carotenoid-derived mouth colouration of chicks, thereby potentially signalling reduced offspring quality to parents and creating a positive feedback loop (Dugas & Border 2022).

For passerines nesting in temperate environments, probably the two most important nest ectoparasites are Hen Fleas *Ceratophyllus gallinae* and parasitic Blowfly *Protocalliphora* spp (López-Rull & Macías Garcia, 2015). Both these ectoparasites undertake key stages of their life cycle within nest material alongside their avian host. Hen Fleas synchronise their reproduction with that of birds to complete two generations during the avian nesting period so that larvae can feed on blood-rich faeces of adult Hen Fleas (Tripet & Richner 1999). Parasitic Blowfly lay their eggs when chicks are ~30% grown so that larvae can take direct blood meals from growing chicks and then pupate within the nest structure (Bennett & Whitworth 1991). Because of the potential cost of these parasites on breeding success, Great Tits *Parus major* in Switzerland actively chose nest sites without Hen Fleas when they were available in an experimental setup; when only infested nest sites were available clutch initiation was delayed, desertion was higher, and hatching success was lower (Oppliger *et al.* 1994). A similar study on Blue Tits in Spain showed that when Hen Fleas and parasitic Blowfly were abundant, breeding success and parental condition was lower compared to when parasites had been artificially removed (Tomás *et al.,* 2007).

A number of different parameters can influence the presence and abundance of nest ectoparasites. Bird species is important as some ectoparasites are host specialists, for example, Hen Fleas are particularly abundant in nests of tits (Tripet & Richner 1997), while other species such as the avian vampire fly *Philornis downsi* are generalists (Common *et al.* 2021). The type of nest plays a key role too: open nests (i.e. those

constructed on the ground, or elevated structures on platforms or in vegetation) generally have a low ectoparasite load but a high predation risk, whereas birds using natural cavities and nest boxes tend to have a high parasitic load but low predation risk (Tripet & Richner 1999). Within natural cavities and nest boxes, the tendency for birds to use the same nest site over successive years can be a major determinant of ectoparasite load, especially for ectoparasites that pupate within the nest or use nest sites as a location in which to overwinter (Oppliger *et al*. 1994, Rendell & Verbeek 1996, Mazgajski 2007, Tomás *et al.* 2007). Nest composition and amount of nest material can also influence ectoparasites (López-Rull & Macías Garcia, 2015). The materials used to construct a nest varies between bird species (Britt & Deeming 2011, Dickinson *et al.* 2022), with climate conditions, geographic location, and local availability of materials also having an impact (Hilton *et al.* 2004, Deeming *et al*. 2012, Biddle *et al.* 2018; Briggs & Deeming 2021, 2022). Vegetative material, especially twigs, bark and dead leaves, is often used to form a structural layer to provide the shape of the nest, while dry grass, feathers, wool and animal hair can provide an insulative lining (Collias & Collias 2014, Briggs & Deeming 2016). Fresh plant material, such as green leaves, can also be used by species, possibly because they contain chemical aromatic compounds that fill the headspace air of the nest box (Gwinner & Berger 2005) and act as repellents or natural fumigants (Mennerat *et al.* 2009, Banbura *et al*. 1995, Dubiec *et al.* 2013, Scott-Baumann & Morgan 2015). The effectiveness of this is demonstrated by a study on European Turtle Doves *Streptopelia turtur* in Morocco, where the inhibition capacity of aromatic plants was associated with very low levels of infestation by *Columbicola columbae* and *Dermanyssus gallinae* (Mansouri *et al*. 2021). However, it should not be assumed that nest materials always affect ectoparasite load; Cantarero et al. (2014) experimentally altered materials within European Nuthatch *Sitta europaea* nests and this had no effect on ectoparasite abundance. Even in these cases, though, the amount of nesting material can be important. Rendell & Verbeek (1996) found positive correlations between the volume of nest material used by Great Tits and the number of Hen Fleas and Fowl Mite *Ornithonyssus sylviarum*; a similar pattern was found for artificially enlarged nests of Common Starling *Sturnus vulgaris* in relation to the abundance of *Carnus hemapterus* flies (Tomás *et al*. 2020).

Nest boxes are often used as a way to conserve and monitor avian populations but there are many ways that nest box construction, maintenance or management could affect ectoparasite loads – and thus the effectiveness of nest box schemes. For instance, older nest boxes could have higher ectoparasite loads if parasites overwinter in the nest box (Oppliger *et al*. 1994, Tomás *et al.* 2007). Moreover, nest box dimensions can affect nest volume as larger nests are typically built in larger nest boxes (positive relationship between

nest box basal area and nest size shown by Møller *et al*. (2014); positive relationship between nest box depth and nest size shown by Tomás *et al*. (2020)). Such relationships have concomitant effects on parasite load, which tends to increase with nest volume (Rendell & Verbeek 1996). Use of predator-proof nest boxes appears, anecdotally, to be increasing. Predator-proof nest boxes tend to be made of stronger materials such as woodcrete (a mixture of woodchip and cement) or to be extra deep to increase the distance between eggs/chicks and the entrance hole. They are used in areas where predator pressure from non-native mammals such as Grey Squirrel *Sciurus carolinensis* is high or where native predators such as Pine Marten *Martes martes* are being reintroduced (Kaliński *et al*. 2014, Griebel *et al*. 2020). Nest box design could co-occur with, or interact with, underlying relationships between bird species, nesting materials, and ectoparasite abundance within nests (López-Rull & Macías Garcia, 2015).

Here, we consider the complex interrelationships between ectoparasite abundance, bird species, nest box design, amount of nesting material, and nest composition. We are analysing multiple factors in a single study to replicate the complexity of reality whereby several co-occurring factors might influence nest ectoparasite community simultaneously and/or interact with one another. Our aim is to advance understanding of avian ectoparasite relationships and inform optimisation of nest box design for conservation and management, especially for declining woodland specialists such as the Pied Flycatcher. The nest boxes being studied are "old" wooden nest boxes (present for ≥ 3 years before the study), "new" wooden nest boxes of the same dimensions (erected shortly before the start of the study), and "deep" wooden nest boxes (also erected shortly before the start of the study). The nest boxes were used by Blue Tit *Cyanistes caeruleus*, Great Tit *Parus major*, Pied Flycatcher *Ficedula hypoleuca*, and European Nuthatch *Sitta europaea.* The specific hypotheses being tested are: (1) abundance of Hen Fleas and parasitic Blowfly will differ between avian species, with the former likely being higher in tit nests than non-tit nests; (2) for wooden nest boxes of standard dimensions, ectoparasite abundance will be higher in old nest boxes than it is in new nest boxes; (3) nest mass will be greater, and ectoparasite abundance will be higher, in deep wooden nest boxes than wooden nest boxes of standard dimensions; and (4) there will be relationships between abundance of specific nesting materials and ectoparasite abundance (direction and magnitude to be elucidated).

Study site and field methods

Nagshead Nature Reserve (Gloucestershire, UK), covers 308 hectares centred on 2°34'0"W, 51°47'0"N and has been used as a study site for ornithological research since the 1940s (Campbell 1968). Just under half of the reserve (120 hectares) was designated as a Site of Special Scientific Interest in 1972. Since 1974, the site has been managed as a reserve by the Royal Society of Protection of Birds (RSPB), with specific focus on the regionally-important breeding population of Pied Flycatchers.

Before the start of the 2019 breeding season, 100 new deep nest boxes and 100 new wooden nest boxes were placed next to pre-existing old wooden nest boxes, giving 200 trees with a nest box dyad (i.e. two nest boxes to allow birds a direct choice, territoriality preventing both nest boxes on a single tree being used). This followed the experimental design of Oppliger *et al.* (1994) and Pidifillini *et al.* (2018). This was done systematically in an alternating pattern to ensure good distribution of all nest box designs across the reserve. There were also 200 old wooden nest boxes affixed to trees individually (i.e. where an additional new nest box was not added to create a dyad). The internal dimensions of the old and new wooden nest boxes were 110 mm width by 170 mm depth with a roof that sloped from the back (higher) to the front (lower) of the nest box with an internal height at the midpoint of 210 mm. The deep nest boxes had the same width and depth measurements as the standard dimension boxes but had an internal height measurement of 265 mm at the midpoint of the roof. All nest boxes had a 32 mm hole in the front panel and were affixed to mature English oak *Quercus robur* approximately 3m above ground level (mean = 3.1 m above the ground ± 0.20 m S.E.M.). The rationale for using the deep nest boxes was to mitigate potential increases in predator pressure due to the imminent reintroduction of Pine Marten to the area (following release, and as expected, this site now falls within the territory of at least one Pine Marten (Cat McNicol, Gloucestershire Wildlife Trust Reintroduction Officer, unpublished radiotracking data)), as well as existing predation pressure by Grey Squirrel.

In total, 78 nests were collected post-fledging while complying with the relevant legislation (Wildlife and Countryside Act 1981). All nests were checked for addled eggs and dead chicks before being placed in a ziplock bag with excess air removed before sealing. They were then weighed, with the pre-determined weight of each bag being deducted (López-Arrabé *et al.* 2014). Nests were frozen at -18°C as soon as possible to kill ectoparasites and preserve them until nests could be processed (Rogers et al., 1991; Goodenough et al., 2011). Collected nests were subdivided between species and nest box designs thus: 24 Blue Tit (Old = 19, New = 5, Deep = 0), 26 Great Tit (Old = 18, New = 4, Deep = 4), 12 Nuthatch (Old = 8, New = 2, Deep = 2) and 16 Pied

Flycatcher (Old = 16, New = 0, Deep = 0). It is recognised that some of these sample sizes are lower than would be considered ideal, especially when they are subdivided, but this reflects 100% of nests constructed that were ultimately successful (to ensure that all nest structures had been used for a full breeding cycle such that parasite loads could be meaningfully compared, nests were not removed where breeding had failed). This meant that the sample size was fundamentally limited by avian breeding population and UK Covid-related lockdowns prevented extension of the project into the 2020 breeding season as had initially been planned.

Nest processing and ectoparasite identification

To remove feather dust and separate most arthropods from the main nest material, a 2mm sieve was used. Sieved material was searched using a soft-tipped paint brush and angled seekers as per Goodenough et al. (2011). To locate any remaining arthropods, including Blowfly larvae/pupae, the remaining nest material was carefully dissected using tweezers (Reynolds *et al*. 2016). All arthropods were extracted using entomological forceps and examined using a Nikon SMZ800 dissection microscope at 10x magnification. Ectoparasites were identified using Whitaker (2007) for fleas and Falk (2016) for blowflies, counted, placed in clear tubes to be preserved in 95% ethanol.

Nest composition

To allow for the analysis of interrelationships between nest material and ectoparasites, all nest material was catalogued. Moss identification was conducted using the keys in Atherton *et al*. (2010), and a reference collection was established to ensure consistency throughout nest processing (Reynolds *et al*. 2016). Given the desiccated state of much of the Bryophyte material, expert identification verification of each moss in the reference collection, and any ambiguous samples, was provided by Bryophyte expert, Dr Oliver Moore. Hair was identified to species level using high-power microscopy. Tree material, including leaves and bark, was also recorded, as was sheep wool and any inorganic material. Because COVID-19 lockdowns and work-from-home mandates prohibited nest analysis being undertaken in a laboratory and thus access to a high-precision balance, the relative abundance of each material in each nest was scored using a 5-point numerical version of the DAFOR scale (5 = Dominant, 4 = Abundant, 3 = Frequent, 2 = Occasional and 1 = Rare).

Data analysis

To test for any differences in choice between the different nest box designs within the dyad experiment, two chi-square goodness of fit tests were used. The first test used data from new-old nest box dyad where either a new nest box had been chosen rather than an old nest box or vice versa (n = 27) and the second used data from the

deep-old nest box dyad where either a deep nest box had been rather than an old nest box of standard dimensions or vice versa (n = 34). It should be noted that 17 nests from old nest boxes at the same site, but which were not part of the dyad experiment, were excluded from these analyses as no direct choice could be inferred. Given the small sample size, analysis was not further divided to consider patterns for individual species.

To test whether there were any differences in nest mass between study species, and then between nest box designs, non-parametric Kruskal-Wallis and Mann-Whitney U tests were carried out. Non-parametric tests were appropriate because nest mass was not normally distributed. Then, to establish what factors influenced the load of Hen Fleas (dependent variable; parametric count data), a series of candidate Generalized Linear Models (GLMs) were conducted using a Poisson distribution with a loglinear link function. Several candidate models were created. These contained different combinations of fixed factors for discrete categorical independent variables (bird species, nest box design) and covariates for continuous independent variables (nest mass; relative abundance of each nest material); an interaction term for the fixed factors (bird*nest box) was also created. The optimal model was selected using lower-is-better Akaike's Information Criterion (AIC) scores to compare competing candidate models based on a combination of model fit balanced against parsimony (Burnham & Anderson, 2004). This avoided overparameterization of the model caused by entering more factors/ covariates than were needed on the one hand, while also guarding against missing important information by creating a Minimum Adequate Model (Goodenough *et al*. 2012). The factors in the final optimal model are reported in Tables 2; P values were used to identify any significant variables against a critical significance (α) of 0.05 for factors/covariates and 0.10 for interaction terms (Burnham *et al.* 2011). This approach was repeated to analyse Blowfly data (Table 3). All analysis was undertaken in SPSS version 28, IBM.

Results

Nest box design choice

In nest box dyads containing two nest boxes of standard dimensions, there was no significant difference between the number of new nest boxes selected compared to the number of old nest boxes selected (new n = 11, old n = 16, chi-square goodness of fit χ^2 = 0.926, d.f. = 1, P = 0.336). However, in nest box dyads containing deep nest boxes and old nest boxes of standard dimensions, there was significant avoidance of deep nest boxes (deep n = 6, old = 34, chi-square goodness of fit χ^2 = 14.235, d.f. = 1, P < 0.001). Given that both new nest boxes of standard dimensions and deep nest boxes were added immediately before the 2019 breeding season, this suggests that the dimensions of the nest boxes rather than their age were driving avian choice.

Nest composition

Nests comprised numerous materials, including nine different moss species (which would have been fresh at the time of nest construction), as well as dead leaves, roots, twigs, and bark. Animal hair/wool, feathers, mud, stones, lichens, and anthropogenic material (including string and material from tennis balls) were also represented. There were species-specific differences in presence and relative abundance of these materials: the mosses *Kindbergia praelonga* and *Hypnum andoi* dominated Blue Tits nests, Great Tit nests were dominated by wool, *Kindbergia praelonga* and animal hair, while bark dominated Nuthatch and Pied Flycatcher nests (Table 1).

Nest mass

The mean nest mass across all species and nest box designs was 45.70g but this varied considerably (min = 18.23g; max = 230.68g). There was a significant difference in nest mass between the four species (Kruskal-Wallis test: χ^2 = 22.891, df = 3, P < 0.001; Figure 1a). Subsequent Mann-Whitney tests, conducted as a non-parametric post-hoc analysis, showed that Nuthatch nests were significantly heavier than Blue or Great Tit nests (Mann-Whitney U test: U = 27, n_1 = 24, n_2 = 12, P < 0.001 and U = 24, n_1 = 26, n_2 = 12, P < 0.001, respectively). Pied Flycatcher nests had, on average, a similar mass to nests constructed by the tit species, but there was larger variance around the mean (Figure 1a). Across the whole dataset, regardless of the bird species involved, nest mass differed significantly relative to nest box design (Figure 1b). Nests constructed in new deep nest boxes (n = 6) were larger than either nests constructed in new nest boxes of standard dimensions (n = 11) or nests constructed in old nest boxes of standard dimensions (n = 61) (Mann-Whitney U tests: U = 7, P = 0.009 and U = 50, P = 0.003, respectively). There was no difference in mass between nests constructed in old nest boxes or new nest boxes of the same dimensions (U = 293, P = 0.511).

Ectoparasites

Arthropods were identified from several Orders including Coleoptera (the rare *Gnathoncus buyssoni*, which had been found previously in Great Tit nests from the same site (Goodenough, 2007) was especially notable), Lepidoptera, Hymenoptera, Isopoda and Arachnida. The main ectoparasites were adult Hen Fleas and larval/pupal parasitic Blowfly, as well as ticks in low numbers (two individuals in one nest were noted but excluded from further analysis). As detailed in the Methods, we created multiple candidate models with different combinations of independent variables, with AIC being used to determine the optimum model for each parasite. The optimal GLM for Hen Fleas – determined using AIC and separated from the next most supported candidate model by an AIC delta of >10 – was highly significant (likelihood ratio χ^2 = 2773.953, df = 16; P < 0.001) and

included numerous significant variables (Table 2). It included nest mass (positive relationship; heavier nests had greater numbers of Hen Fleas) with positive relationships also being found for two species of moss, animal hair (Deer, Badger), and mud. Dead leaves and roots were negatively associated with Hen Flea abundance. Bird species and nest box design, both entered as fixed factors, were also significant. Subsequent modelling using estimated marginal means and post-hoc analysis showed that Hen Flea load was significantly ($P < 0.01$) higher in Nuthatch nests than each of the other avian species, and that Hen Flea load of Blue Tit nests was also significantly higher than that of Great Tit and Pied Flycatcher nests ($P < 0.01$), which did not themselves differ (Figure 2a). Deep nest boxes were associated with significantly higher Hen Flea loads than either old or new nest boxes; old nest boxes had a higher flea abundance than new nest boxes ($P < 0.01$ in all cases; Figure 2b).

The optimal GLM for Blowfly – separated from the next most supported candidate model by an AIC delta of >10 – was also highly significant (likelihood ratio χ^2 = 702.250, df = 18; $P < 0.001$) and included numerous significant variables (Table 3). It included nest mass (again a positive relationship, with heavier nests containing greater numbers of Blowfly). Positive relationships were also found for three species of moss, Badger hair, feathers, and mud, while dead leaves, bark and roots were negatively related to Blowfly abundance. Bird species and nest box design, entered as fixed factors, were also significant. Blowfly load was significantly higher in Pied Flycatcher nests than in Great Tit or Nuthatch nests ($P < 0.01$) or Blue Tit nests ($P < 0.05$). Blowfly loads in Blue Tit nests were significantly higher than those found for Great Tit or Nuthatch ($P < 0.01$), where Blowfly occurred at very low prevalence (Nuthatch = two nests; Great Tit = one nest) giving a low overall mean, but they did occur at relatively high abundance when they were present (mean = 20 individuals per infested nest). Deep nest boxes were associated with significantly higher Blowfly loads than either old ($P < 0.05$) or new ($P < 0.01$) nest boxes; old nest boxes were significantly ($P < 0.01$) higher than new boxes (Figure 3b).

Discussion

The abundance of Hen Fleas and Blowfly in the nests of cavity-nesting passerines was influenced by bird species, nest box design, amount of nesting material, and nest composition. Both ectoparasites were found at their highest loads in nest structures from deep nest boxes, nests from old nest boxes had intermediate loads, and nests from new nest boxes had the lowest loads; greater amounts of nest material increased the abundance of both ectoparasite species. However, patterns in regard to bird species were more complex: Nuthatch nests contained the highest abundance of Hen Flea, followed by Blue Tit nests, and then Great Tit and Pied Flycatcher (which were about equal), while Pied Flycatcher nests had the highest abundance of

Blowfly, followed by Blue Tit still at reasonably high levels. Birds were more likely to select old nest boxes of standard dimensions over new deep boxes when presented with a choice, but there was no preference between a direct choice between new and old nest boxes of standard dimensions.

The fact that nests in deep nest boxes had high ectoparasite loads, and the positive correlation between nest mass and both Hen Flea and Blowfly abundance, are likely to be partly connected. During the fieldwork for this study, birds nesting in deep nest boxes were observed to continue adding nesting material until the top of the (enlarged) nest structure was a similar distance to the entrance hole as would be found for a smaller "normal" nest in a wooden nest box of standard dimensions. Although this distance was not measured in the field (and would also change over the course of the breeding season as the nest structure becomes ever-more flattened by increasing chick weight and activity), comparisons of precisely-measured nest weight showed that the addition of more nest material did result in significantly larger nests. These findings mirror previous work for Blue and Great Tits nesting in deep nest boxes in Poland (Kaliński et al. 2014). A greater amount of nest material increases the ecosystem for the ectoparasite community, and the relationship between amount of nesting material and parasite loads found here agrees with previous research by Rendell & Verbeek (1996) on the number of Hen Fleas and volume of nest material in Great Tit nests, as well as that of Tomás et al. (2020), which found the same link for *Carnus hemapterus* flies in Common Starling nests. Tomás et al. (2020) also showed experimentally that a smaller gap between the nest box entrance hole and the top of the nest structure increased parasitism risk by Blowfly by making the nest easier to find by free-living adults. Thus whilst deep nest boxes could have, in theory, reduced risk of both parasitism and predation in our study system, avian nest construction behaviour appears to negate this potential. The nest box dyad work also shows that deep nest boxes are significantly less preferred compared to standard size boxes.

The difference between old and new wooden nest boxes showed that the former had a significantly higher abundance of both ectoparasites compared to the latter. Although this was a binary variable, it could potentially mean that the ectoparasite load increases with nest box age. Previous studies have found a link between repeated use of the same nest in different breeding seasons and increased parasite abundance (e.g. Rendell & Verbeek 1996, Tomás et al. 2007) but these studies have tended to focus on repeated use of the nest structure itself rather than repeated use of the nest box, with an assumption that removing nesting material at the end of the breeding season in one year will "reset" a nest box by removing any parasites such as Hen Fleas that are dormant or that have pupated (e.g. Mazgajski 2007). Our results, however, suggest either

that cleaning is ineffective (possibly because pupating Hen Fleas are hard to remove if they are within the feather dust at the bottom of the nest box rather than within the nest *per se*) or that old nest boxes are used by overwintering parasites in the absence of nest material. An alternative explanation is that they are more quickly colonised at the start of the breeding season by ectoparasites with a free-living adult stage, such as parasitic blowflies, detecting nests and their avian occupants using olfactory receptors in their antenna (this detection might be masked by the volatile organic compounds associated with new wooden nest boxes). Interestingly, there was no significant difference between avian choice between old versus new nest boxes of the same size. Although it might be considered surprising that birds did not select new nest boxes given the difference in eventual nest parasite load, this would presuppose that birds would have perfect information, be able to make a perfect prediction, and thus be able to make a perfect choice. It is possible that old nest boxes are an ecological trap for birds or there might be a difference in choice relative to bird age and experience, which is not something we were able to consider within the scope of the current project.

Nest composition was linked to bird species and amount of nesting material, but, in models where both these variables were already entered, adding the *relative* abundance of specific nest materials substantially improved model performance (it should be noted that relative abundance was quantified semi-quantitatively on a simple 1-5 scale rather than using precise weights). For both Hen Fleas and Blowfly, higher relative abundance of badger hair in the nest was associated with higher parasite load; for Hen Fleas the same relationship occurred with deer hair also. As both parasite species are obligate avian parasites, this cannot be a direct effect (e.g. eggs could not be present on mammalian-origin nest material). It is possible that it could relate, especially for Blowfly that have a free-living adult stage, to nests with greater animal content being easier for parasites to detect using olfactory receptors, and therefore easier to parasitise, but this is speculative and would not explain why the same relationship did not exist with Wild Boar hair (the inclusion of this variable did not improve the model either for Hen Fleas or Blowfly).

The fact that the leaf material used in the study nests was dead leaf litter rather than fresh plant material means that the negative correlation between relative amount of leaves and parasite loads is unlikely to be explained by birds utilising aromatic compounds within the plant material (the nest protection hypothesis: Gwinner 1997, Mennerat *et al*. (2009), Dubiec *et al.* (2013), López-Rull & Macías Garcia (2015). However, the negative relationships between parasite load and both bark and roots, both of which can release aromatic compounds depending on the plant species involved, might link to this hypothesis by acting as repellents or

natural fumigants (Scott-Baumann & Morgan 2015), or which might reduce the effects of parasitism, perhaps by acting as immunostimulants (Gwinner & Berger, 2005). Previous research that has quantified nest materials has shown that bark used in bird nests can vary both between and within avian species. For example, Nuthatch use Common Hazel *Corylus avellana*, Yew *Taxus baccata* or Scots Pine *Pinus sylvestris* depending on availability in the immediate area around the nest box (Briggs and Deeming 2022), all of which are present at our study site. As bark from different trees might differ considerably in aromatic compounds, and thus potentially have different mediating effects on ectoparasite presence and abundance, we recommend that more direct research is necessary to better understand nest-parasite relationships. This should also extend into considering potential antiparasitic roles of the specific moss species identified here.

Overall, we have shown that the factors influencing parasite loads in cavity-nesting passerine nests are complex and interrelated, but that pattens of ectoparasite load are not always reflected in similar patterns of next box choice. We recommend that next boxes are regularly replaced to keep ectoparasite loads down and that deep nest boxes are not used in the future, as they result in larger nests (presumably increasing parental effort during nest building (Collias, 1964) and are associated with increased loads of two key haematophagous ectoparasites. Moreover, the behaviour of birds to build up the nest structure in deep nest boxes also removes their raison d'être of reducing predation risk as the nest contents are within reach of mammalian predators such as Pine Martens (Kaliński et al. 2014). We also recommend experimental work be conducted to further investigate the possible link between use of non-green plant material that might have aromatic compounds (especially tree bark) in nests and parasite numbers to complement and extend the current research, which has typically been focused on green plant material (Banbura *et al*. 1995, Mennerat *et al.* 2009, Dubiec *et al.* 2013, Scott-Baumann & Morgan 2015).

References

Atherton, I., Bosanquet, S.D. & Lawley, M. 2010. *Mosses And Liverworts Of Britain And Ireland: A Field Guide*. British Bryological Society, Plymouth.

Atkinson, C.T., Thomas, N.J. & Hunter, D.B. 2009. *Parasitic Diseases Of Wild Birds*. John Wiley & Sons, Chichester.

Banbura, J., Blondel, J., de Wilde-Lambrechts, H. & Perret, P. 1995. Why do female Blue Tits (*Parus careuleus*) bring fresh plants to their nests? *J. Ornithol.* **136:** 217–221.

Benedict, L., Covy, N.E., Love, P.A. & Stier, S.T. 2021. Human presence outweighs non-anthropogenic factors as a driver of avian nest parasite loads. *J. Ornithol.* **162:** 155-164.

Bennett, G.F. & Whitworth, T.L. 1991. Studies on the life history of some species of *Protocalliphora* (Diptera: Calliphoridae). *Can. J. Zool.* **69:** 2048-2058.

Biddle, L.E., Broughton, R.E., Goodman, A.M. & Deeming, D.C. 2018. Composition of bird nests is a species-specific characteristic. *Avian Biol. Res.* **11:** 132-153.

Briggs, K.B. & Deeming, D.C. 2016. Use of materials in nest construction by Pied Flycatchers *Ficedula hypoleuca* reflects localized habitat and geographical location. *Bird Study* **63:** 516-524.

Briggs, K.B. & Deeming, D.C. 2021. Localized habitat affects size and materials used in the construction of Common Redstart *Phoenicurus phoenicurus* nests. Bird Study, **68:** 9-20**.**

Briggs, K.B. & Deeming, D.C. 2022. Effects of year and box size on construction of Eurasian Nuthatch *Sitta europaea* nests. *Ardea* **110:** 61-74.

Britt, J. & Deeming, D.C. 2011. First-egg date and air temperature affect nest construction in Blue Tits *Cyanistes caeruleus*, but not in Great Tits *Parus major*. *Bird Study* **.** 78-89.

Burnham, K.P. & Anderson, D.R. 2004. Multimodel inference: understanding AIC and BIC in model selection. *Sociol. Methods Res.* **33:** 261-304.

Burnham, K.P., Anderson, D.R. & Huyvaert, K.P. 2011. AIC model selection and multimodel inference in behavioral ecology: some background, observations, and comparisons. *Behav. Ecol. Sociobiol.* **65:** 23-35.

Bush, S.E. & Clayton, D.H. 2018. Anti-parasite behaviour of birds. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* **373:** 20170196.

Campbell, B. 1968. The Dean nest box study, 1942–1964. *Forestry* **41:** 27-46.

Cantarero, A., López-Arrabé, J., Saavedra-Garcés, I., Rodríguez-García, V., Palma, A. & Moreno, J. 2014. The significance of nest structure and nesting material for hole-nesting passerines: an experimental study with Nuthatches *Sitta europaea*. *Acta Ornithol.* **49:** 143-155.

Castaño-Vázquez, F. & Merino, S. 2021. Differential effects of environmental climatic variables on parasite abundances in blue tit nests during a decade. *Integr. Zool*. **17:** 511-529

Collias, N.E. 1964. The evolution of nests and nest-building in birds. *Am Zool*. 4: 175-190.

Collias, N.E. & Collias, E.C. 2014. Nest Building And Bird Behavior. Princeton University Press, Princeton.

Dickinson, A.M., Locke, E., Gray, L.A., Bennett, S.L., Biddle, L.E., Goodman, A.M. & Deeming, D.C. 2022. Composition of nests constructed by species in the Motacillidae, Sylviidae and Prunellidae. *Avian Biol. Res*. **15:** 21-33.

Dubiec, A., Góźdź, I. & Mazgajski, T.D. 2013. Green plant material in avian nests. *Avian Biol. Res.* **6:** 133-146.

Common, L.K., Sumasgutner, P., Dudaniec, R.Y., Colombelli-Négrel, D. & Kleindorfer, S. 2021. Avian vampire fly (*Philornis downsi*) mortality differs across Darwin's finch host species. *Sci. Rep.* **11:** 1-12.

Deeming, D.C., Mainwaring, M.C., Hartley, I.R. & Reynolds, S.J. 2012. Local temperature and not latitude determines the design of Blue Tit and Great Tit nests. *Avian Biol. Res.* **5:** 203-208.

Dudek, B.M., Henderson, M.T., Hudon, S.F., Hayden, E.J. & Heath, J.A. 2021. Haematophagous ectoparasites lower survival of and have detrimental physiological effects on golden eagle nestlings. *Conserv. Physiol.* **9:** coab060.

Dugas, M.B. & Border, S.E. 2022. Experimental reduction of a nest ectoparasite affects mouth coloration of nestling Cliff Swallows *Petrochelidon pyrrhonota*. *J. Ornithol.* In press.

Falk, S. 2016. British Blowflies (Calliphoridae) and Woodlouse Flies (Rhinophoridae): Draft Key, available at http://www.stevenfalk.co.uk/files/21577/testkeytobritishblowflies132016.pdf

Goodenough, A.E. 2007. The diversity and abundance of nest-dwelling arthropods, particularly ectoparasites, in great tit nests at Nagshead RSPB Nature Reserve. *The Gloucestershire Naturalist* **18:** 23-31

Goodenough, A.E., Elliot, S.L. & Hart, A.G. 2011. Do orientation-based differences in nest box temperature cause differential ectoparasite load and explain patterns of nest-site selection and offspring condition in great tits? *Int. J. Zool.* 514913.

Goodenough, A.E., Hart, A.G. & Stafford, R. 2012. Regression with empirical variable selection: description of a new method and application to ecological datasets. *PloS One* **7:** e34338.

Griebel, I.A., Dawson, R.D. & Clark, R.G. 2020. Cavity type influences abundance of nest-dwelling avian blow flies: an experiment with tree swallows. *Ecol. Entomol.* **45:** 434-443.

Gwinner, H. 1997. The function of green plants in nests of European starlings (*Sturnus vulgaris*). *Behav.* **134:** 337-351.

Gwinner, H. & Berger, S. 2005. European starlings: nestling condition, parasites and green nest material during the breeding season. *J Ornithol.* **146:** 365-371.

Hilton, G.M., Hansell, M.H., Ruxton, G.D., Reid, J.M. & Monaghan, P. 2004. Using artificial nests to test importance of nesting material and nest shelter for incubation energetics. *Auk* **121:** 777-787.

Kaliński, A., Wawrzyniak, J., Bańbura, M., Skwarska, J., Zieliński, P., Glądalski, M. & Bańbura, J. 2014. Does the threat of European Pine Marten (*Martes martes*) predation influence the height of nests built by Blue Tits (*Cyanistes caeruleus*) and Great Tits (*Parus major*)?. *Avian Biol. Res.* **7:** 83-90.

Lamarre, V., Legagneux, P., Franke, A., Casajus, N., Currie, D.C., Berteaux, D. & Bêty, J. 2018. Precipitation and ectoparasitism reduce reproductive success in an arctic-nesting top-predator. *Sci. Rep.* **8:** 1-7.

López-Arrabé, J., Cantarero, A., Pérez-Rodríguez, L., Palma, A. & Moreno, J. 2014. Experimental pyrethroid treatment underestimates the effects of ectoparasites in cavity-nesting birds due to toxicity. *Ibis* **156:** 606-614.

López-Rull, I & Macías Garcia, C. 2015. Control of invertebrate occupants of nests. In: Deeming, C.D & Reynolds, S.J. *Nests, Eggs, and Incubation: New Ideas about Avian Reproduction*. Oxford University Press, Oxford, Pp 82-96.

Mansouri, I., Ousaaid, D., Squalli, W., Douini, I., Mounir, M., El Agy, A., Assouguem, A., Achiban, H., El Ghadraoui, L. & Dakki, M. 2021. Nest building, dimension, and selection of aromatic and medicinal twigs to repel ectoparasites in the European turtle dove. *J. Anim. Behav. Biometeorol.* **9:** 2133-2133.

Mazgajski, T.D. 2007. Effect of old nest material in nest boxes on ectoparasite abundance and reproductive output in the European Starling *Sturnus vulgaris*(L.). *Pol. J. Ecol.* **55:** 377-385.

Mennerat, A., Perret, P. & Lambrechts, M.M. 2009. Local individual preferences for nest materials in a passerine bird. *PLoS One*, **4: e5104.**

Merino, S. & Potti, J. 1995. Mites and blowflies decrease growth and survival in nestling pied flycatchers. *Oikos* **73:** 95-103.

Moore, J. 2002. *Parasites and the Behaviour of Animals*, Oxford University Press, Oxford, UK.

Oppliger, A., Richner, H. & Christe, P. 1994. Effect of an ectoparasite on lay date, nest-site choice, desertion, and hatching success in the great tit (*Parus major*). *Behav. Ecol.* **5:** 130-134.

Podofillini, S., Cecere, J.G., Griggio, M., Curcio, A., De Capua, E.L., Fulco, E., Pirrello, S., Saino, N., Serra, L., Visceglia, M. & Rubolini, D. 2018. Home, dirty home: effect of old nest material on nest-site selection and breeding performance in a cavity-nesting raptor. *Curr. Zool.* **64:** 693-702.

Rendell, W.B. & Verbeek, N.A. 1996. Are avian ectoparasites more numerous in nest boxes with old nest material?. *Can. J. Zool.* **74:** 1819-1825.

Reynolds, S.J., Davies, C.S., Elwell, E., Tasker, P.J., Williams, A., Sadler, J.P. & Hunt, D. 2016. Does the urban gradient influence the composition and ectoparasite load of nests of an urban bird species? *Avian Biol. Res.* **9:** 224-234.

Richner, H., Oppliger, A. & Christe, P. 1993. Effect of an ectoparasite on reproduction in great tits. *J. Anim. Ecol.* **62:** 703-710.

Rogers, C.A., Robertson, R.J. & Stutchbury, B.J. 1991. *Patterns and effects of parasitism by Protocalliphora sialia on tree swallow nestlings* in Bird-Parasite Interactions: Ecology, Evolution and Behaviour (Loye, J.E. & Zuk, M.) Oxford University Press, Oxford, UK. pp. 123–139.

Scott-Baumann, J.F. & Morgan, E.R. 2015. A review of the nest protection hypothesis: does inclusion of fresh green plant material in birds' nests reduce parasite infestation?. *Parasitology* **142:** 1016-1023.

Tomás, G., Merino, S., Martinez-de la Puente, J., Moreno, J., Morales, J., Lobato, E. & del Cerro, S. 2012. Interacting effects of aromatic plants and female age on nest-dwelling ectoparasites and bloodsucking flies in avian nests. *Behav. Processes* **90:** 246–253.

Tomás, G., Merino, S., Moreno, J. & Morales, J. 2007. Consequences of nest reuse for parasite burden and female health and condition in blue tits, *Cyanistes caeruleus*. *Anim. Behav.* **73:** 805-814.

Tomás, G., Ruiz-Castellano, C., Ruiz-Rodríguez, M. & Soler, J.J. 2020. Smaller distance between nest contents and cavity entrance increases risk of ectoparasitism in cavity-nesting birds. *J. Avian Biol.* **51:** 1-11.

Tripet, F. & Richner, H. 1999. Density-dependent processes in the population dynamics of a bird ectoparasite *Ceratophyllus gallinae*. *Ecology* **80:** 1267-1277.

Tripet, F. & Richner, H. 1997. The coevolutionary potential of a 'generalist' parasite, the hen flea *Ceratophyllus gallinae*. *Parasitology* **115:** 419-427.

Warren, Y. 1994. *Protocalliphora braueri* (Diptera: Calliphoridae) induced pathogenesis in a brood of marsh wren (*Cistothorus palustris*) young. *J. Wildl. Dis.* **30:** 107-109.

Wesołowski, T. 2001. Host–parasite interactions in natural holes: Marsh Tits (*Parus palustris*) and blow flies (*Protocalliphora falcozi*). *J. Zool.* **255:** 495-503.

Whitaker, A.P. 2007. Handbook of British Fleas. UK, Field Studies Council and the Royal Entomological Society.

Table 1. Mean (± standard error) occurrence of materials found in the nests of four passerine species (5 = Dominant, 4 = Abundant, 3 = Frequent, 2 = Occasional, 1 = Rare),. Materials with an asterisk occurred in a sufficient number of nests (n ≥ 5) to be included within candidate Generalised Linear Models; optimal GLMs were identified from the candidate models using Akaike's Information Criterion (see Methods) and are reported in Tables 2 and 3.

Nest Material	Blue Tit	Great Tit	Nuthatch	Pied Flycatcher
*Brachythecium rutabulum**	1.33 (± 0.30)	0.88 (± 0.24)	0.17 (± 0.11)	
*Hypnum andoi**	2.42 (± 0.35)	0.58 (± 0.20)	0.08 (± 0.08)	
*Isothecium myosuroides**	0.58 (± 0.24)	0.19 (± 0.11)		
*Kindbergia praelonga**	3.29 (± 0.24)	2.85 (± 0.27)	0.15 (± 0.08)	1.88 (± 0.33)
*Polytrichastrum formosum**		0.74 (± 0.25)		
Mnium hornum		0.15 (± 0.11)		
*Pseudoscleropodium purum**	0.25 (± 0.18)	0.62 (± 0.25)		
*Rhytidiadelphus squarrosus**	0.83 (± 0.27)	0.12 (± 0.12)		
*Thuidium tamariscinum**		2.00 (± 0.30)		
Dead leaves*		0.19 (± 0.11)	1.62 (± 0.60)	1.63 (± 0.35)
Roots*		1.00 (± 0.31)		1.06 (± 0.30)
Twigs*	0.04 (± 0.04)	0.65 (± 0.23)	2.23 (± 0.32)	
Bark*	2.13 (± 0.30)	0.08 (± 0.05)	4.77 (± 0.18)	3.19 (± 0.42)
Fallow Deer *Dama dama* hair*	0.38 (± 0.13)	1.27 (± 0.28)		0.25 (± 0.11)
Badger *Meles meles* hair*	0.66 (± 0.19)	1.23 (± 0.21)		0.25 (± 0.11)
Wild Boar *Sus scrofa* hair*	1.33 (± 0.23)	2.08 (± 0.22)	0.08 (± 0.08)	0.31 (± 0.12)
Sheep wool*	0.21 (± 0.15)	2.96 (± 0.34)		
Feathers*	1.62 (± 0.29)	0.46 (± 0.14)		0.06 (± 0.06)
Mud*			0.85 (± 0.34)	
Stones*			0.31 (± 0.22)	
Lichen				0.13 (± 0.07)
Anthropogenic material	0.04 (± 0.04)	0.27 (± 0.14)		0.13 (± 0.09)

Table 2. Factors, covariates and interactions included in the optimum Generalised Linear Model for Hen Flea *Ceratophyllus gallinae* abundance.

		Wald χ^2	d.f.	P	Details
Factors:	Bird Species	649.5	3	<0.001	See Fig 2a
	Nest Box Design	150.8	2	<0.001	See Fig 2b
Interactions:	Bird Species*Nest Box Design	184.9	3	<0.001	
Covariates:	Nest Mass	111.2	1	<0.001	Positive relationship
	Kindbergia praelonga	119.8	1	<0.001	Positive relationship
	Rhytidiadelphus squarrosus	20.0	1	<0.001	Positive relationship
	Deer Hair	68.6	1	<0.001	Positive relationship
	Badger Hair	75.4	1	<0.001	Positive relationship
	Dead Leaves	335.3	1	<0.001	Negative relationship
	Mud	417.5	1	<0.001	Positive relationship
	Roots	211.0	1	<0.001	Negative relationship

Table 3. Factors, covariates and interactions included in the optimum Generalised linear model for parasitic Blowfly *Protocalliphora* spp. abundance

		Wald χ^2	d.f.	P	Details
Factors:	Bird Species	43.3	3	<0.001	See Fig 3a
	Nest Box Design	83.2	2	<0.001	See Fig 3b
Interactions:	Bird Species*Nest Box Design	10.2	1	<0.001	
Covariates:	Nest Mass	39.4	1	<0.001	Positive relationship
	Kindbergia praelonga	10.1	1	<0.001	Positive relationship
	Hypnum andoi	18.1	1	<0.001	Positive relationship
	Brachythecium rutabulum	18.7	1	<0.001	Positive relationship
	Badger Hair	20.8	1	<0.001	Positive relationship
	Feathers	31.0	1	<0.001	Positive relationship
	Dead Leaves	9.3	1	0.002	Negative relationship
	Bark	8.7	1	0.003	Negative relationship
	Mud	9.9	1	0.002	Positive relationship
	Roots	26.0	1	<0.001	Negative relationship

Figure 1: Amount of nesting material showing: (a) differences between avian species; and (b) differences between nest box design. Error bars show standard error; solid black lines between bars indicate statistically significant difference at α = 0.01.

Figure 2: Abundance of Hen Flea *Ceratophyllus gallinae* in the nests of secondary cavity-nesting passerines showing: (a) differences between avian species; and (b) differences between nest box design. Error bars show standard error; solid black lines between bars indicate statistically significant difference at α = 0.01.

Figure 3: Abundance of Blowfly *Protocalliphoria* in the nests of secondary cavity-nesting passerines showing: (a) differences between avian species; and (b) differences between nest box design. Error bars show standard error; solid black lines between bars indicate statistically significant difference at α = 0.01 while dashed black lines between bars indicate statistically significant difference at α = 0.05.