

**Does nestbox type significantly alter the  
ectoparasitic load and breeding success in the  
nests of secondary-cavity-nesting passerines,  
at Nagshead, Forest of Dean?**

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“DECLARATION: This dissertation is the product of my own work and does not infringe the ethical principles set out in the University’s Handbook for Research Ethics. I agree that it may be made available for reference and photocopying at the discretion of the University”.

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## Abstract

This research aims to find if nestbox type causes a significant difference in the ectoparasite load and breeding success rate in the nests for four co-occurring secondary cavity nesting passerines, breeding in nestboxes at Nagshead Nature Reserve, Forest of Dean. The different nestbox types used in this study are old and new standard wooden nestboxes and deep nestboxes, which are designed to reduced predation. Nests were taken from nestboxes used by Blue Tit (*Cyanistes caeruleus*), Great Tit (*Parus major*), Nuthatch (*Sitta Europaea*) and Pied Flycatcher (*Ficedula hypoleuca*) by Nestbox Species Surveyors at the end of the 2019 breeding season, and later dissected to determine ectoparasite abundance and nest composition. Nest data collected by Nestbox Species Surveyors was used to calculate the breeding success rate. Population of woodland birds in 2018 was found to be overall 29% than in 1970. Deforestation and habitat management are causes in the reduction of nesting sites available for woodland bird species. Pine Martens (*Martes martes*), a known predator to secondary cavity nesting birds, were reintroduced into the Forest of Dean in September 2019. Individuals using nestboxes are at a potential increased risk of predation as nestboxes are accessible to Pine Martens. The abundance of adult Hen Fleas and Blowfly pupae in deep nestboxes was found to be significantly higher compared to the nestbox types. Nuthatch nests were found to have the highest mean abundance of adult Hen Fleas, while Pied Flycatcher nests had the highest mean abundance of Blowfly pupae, compared to the other species. Leaves as a nest material were found to have a significant influence and negative correlation with both ectoparasites mentioned. Breeding success rate in standard new boxes was significantly lower than the other nestbox types, with no significant difference occurring between deep and standard old nestboxes. Breeding success in Nuthatch nests were found to be significantly lower than the other avian study species potential linking the increased abundance of adult Hen Fleas previously found. Leaves were found to be significantly influencing and positively correlating with breeding success. These findings add to the data and knowledge about the difference in ectoparasite load and breeding success between nestbox types and the secondary cavity nest passerines, which use these nestboxes. This study also opens up potential for recommended future studies to be carried out at Nagshead.

Glossary of terms

Altricial - hatched or born in an undeveloped state and requiring care and feeding by the parents.

Biodiversity - every living organism within a single ecosystem or habitat, including numbers and diversity of species and all environmental aspects such as temperature, oxygen and carbon dioxide levels and climate.

Competition - relationship between organisms in which one is harmed when both are trying to use the same resource related to growth, reproduction, or survivability.

Deforestation- the cutting down of trees in a large area, or the destruction of forests by people.

ecosystem engineers - any animal that creates, significantly modifies, maintains or destroys a habitat.

Ecosystems - a biological community of interacting organisms and their physical environment.

Ectoparasite - parasites that live on the outside of the host's body.

Extirpation - the act of removing or destroying something completely.

Fledge - to grow feathers and learn to fly.

Fledgling - a young bird that has grown feathers and is learning to fly.

Forage - to go from place to place searching for things that you can eat or use

Habitat - the natural environment in which an animal or plant usually lives

Host - a plant or animal that another plant or animal lives on as a parasite

Imago - the final and fully developed adult stage of an insect.

Incubation - the process in which a bird keeps its eggs warm until the young come out, or the process in which an egg develops until the stage at which the young come out.

Interspecific competition - competition is between individuals which are different species.

Invasive species - organism that causes ecological or economic harm in a new environment where it is not native.

Larvae - a form of an insect or an animal that has left its egg but has not yet developed into a pupa or adult insect or animal

Life cycle - the series of changes that a living thing goes through from the beginning of its life until death.

Microclimate - an area in which the environmental conditions is usually different from the areas around it.

Nestlings - a young bird that has not yet learned to fly and still lives in the nest built by its parents.

Parasite - an animal or plant that lives on or in another animal or plant of a different type and feeds from it

Parasitism - type of symbiotic relationship, or long-term relationship between two species, where one member, the parasite, gains benefit that come at the expense of the host member.

Passerine - relating to or denoting birds of a large order distinguished by having feet that are adapted for perching.

Predation - flow of energy between two organisms, predator and prey. In this interaction, the prey loses energy, and the predator gains energy.

Provisioning - the activity of obtaining the equipment and resources you need for a particular activity

Pupa/Pupae - an insect in the stage of development after it has been a larva and before it becomes an adult, during which it is contained in and protected by a hard covering and does not move

Pupate - become a pupa.

Reintroduced - to put something into use, operation, or a place after it has not been used or in a place for some time

Secondary-cavity nesting bird – a species of bird which uses a pre-existing cavity to nest in.

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# Chapter 1

## Introduction

### *Summary*

*This chapter introduces the woodland passerines, the threats which are currently occurring to them and the population trends of woodland birds in Europe and the UK. The different aspects of woodland passerine nesting are discussed. Specific threats from predation and parasites that passerines face while nesting are discussed. The different nestbox types are introduced. The overall aim of this thesis is outlined as well as what each chapter contains.*

### 1.1 Birds and their roles in ecosystems

Birds are found all over the world in various habitats from tropical rainforests to temperate woodlands, from wetlands to deserts, and from the arctic tundra to temperate grassland. Birds play important ecological roles within ecosystems, including the dispersal of seeds (Whelan *et al.*, 2015). Seeds are ingested then excreted at a later point, allowing the dispersal of seeds to new locations. Faeces then provides additional fertilisation to the seeds (Tabur and Ayvaz, 2010). In some environments, birds also play a role as pollinators. Nectivorous birds including Sunbirds (Nectariniidae) pollinated approximately 5% of the plant species in Cape Flora of South Africa (Whitehead, 2018). Birds can be both predators and prey within ecosystems, with some species of birds preying on other birds such as the Sparrow Hawk (*Accipiter nisus*) which preys on small bird species (Bujoczek and Ciach, 2009). Some bird species can also be classed as ecosystem engineers: for example, colonial seabirds act as ecosystem engineers by improving the soil with faecal matter. Penguin (Spheniscidae) faeces from colonies on Marion Island represent about 85% of all organic debris deposited on the substrate (Otero *et al.*, 2018). Faecal matter adds nutrients to the soil which have an important effect on the development of Arctic plant communities (Otero *et al.*, 2018).

### 1.1.1 Passerines.

Passeriformes is the largest order of birds comprising around 5,700 different species and equating to over half of all known species of birds (Ricklefs, 2012). Passerines can be found on almost all terrestrial habitats (Ames, 1971) and are true perching birds with an anisodactyl foot arrangement, meaning they have three toes in front and one behind (Bochenski *et al.*, 2014).

Passerines range from 6.5cm (Short-tailed Pygmy tyrant (*myiornis ecaudatus*)) to 70cm (Thick-billed Raven (*Corvus crassirostris*)) overall length, however most range from 12.5cm to 20cm. Diet differs within passerines with some species being insectivorous, such as Buff-throated Woodcreeper (*Xiphorhynchus guttatus*), others being granivorous, such as the Dark-eyed Junco (*Junco hyemalis*). Other species are omnivorous, and use food sources including fish, leaves, nectar and small vertebrates (Ricklefs, 2012).

## 1.2 Woodland birds

With woodland habitat occurring in tropical rainforest biomes, temperate forest biomes and boreal forest biomes, and being complex habitats in terms of structure, it is no surprise that woodlands support a wide diversity of birds. Birds have speciated to fill different niches within woodland habitats, due to morphological and behavioural differences in both feeding and nesting (Moreno, 1981). For example, Clark's Nutcracker (*Nucifraga columbiana*) in coniferous woodland specialises in eating conifer seeds and builds open nests (Tomback, 1982), while Tawny Owls (*Strix aluco*) in deciduous woodlands feed on small mammals, frogs, fish and small birds and is a cavity-nesting species (Galeotti *et al* 1991).

### 1.2.1 Woodland passerines

As previously mentioned, passerines account for over half the bird species in the world (1.1.1). Woodland passerines are found in all types of woodlands, including ancient, broadleaf and coniferous woodland. Some woodland passerines are migratory such as Willow Warbler (*Phylloscopus trochilus*),

which migrates to the UK from southern Africa (Remisiewicz and Underhill, 2020). Others are non-migratory such as the Tunki (*Rupicola peruvianus*), which has a large distribution in the cloud forest of the Andes.

#### 1.2.1.1 Threats

Woodland birds including many passerines are subject to threats throughout their distributions including habitat loss, invasive species and climate change (Ford, 2011; Clavero *et al.*, 2009; Mackay and Gross, 2019). Loss of woodland habitat due to deforestation and poor habitat management is a major threat to birds. This causes a cascade effect to key ecological processes including increasing interspecific competition, increased nest predation and a decline in resources such as food (Ford, 2011). Brook *et al.*, (2003) examined the extinctions of different taxa over 183 years (1819-2002) in Singapore. During this period, over 95% of original vegetation cover was cleared. Extirpations were estimated using historical and modern checklists of species, with birds estimated at 34-59%. This is a significant local loss of bird species diversity due to habitat loss. Kupsch *et al.*, (2019) investigated the deforestation impact on native birds in southwest Cameroon. When forest cover was below 74%, richness of Guinea-Congo biome-restricted, large-bodied arboreal foliage gleaning, tree nesting and frugivorous bird species declined. The results supported that in areas of high deforestation there can be considerable change in the avian species community.

Invasive species are another threat to woodland passerine species diversity and survival. They can cause a negative impact on isolated populations including on islands where species are often sedentary (Clavero *et al.*, 2009). The smaller the distribution range of a species, the higher the impact invasive species is likely to have. For example, the primary cause of nest failure for the New Zealand Rockwren (*Xenicus gilviventris*) was found to be predation from invasive species,

Stoats (*Mustela erminea*) and House Mice (*Mus musculus*) (Weston *et al.*, 2018). Trapping of these predator species improved the daily survival, hatching and fledgling rates. The impact of an introduced species on a native woodland passerine species, can also be exacerbated due to the ecological sensitivity of the ecosystem.

Climate change is a major threat to the biodiversity and abundance of woodland bird species. Current changes and those projected from climate models are predicted to occur at a rate higher than that during the last 10,000 years (Mackay and Gross, 2019). Predictions of a 3.5°C surface warming by 2100 could result in extinctions of 600-900 land bird species (Şekercioğlu *et al.*, 2012). The extinctions will likely be driven by a combination of habitat becoming less suitable, inability of some species to move distribution in accordance with the changing location of their climate envelope, changes in migration or breeding phenology that are asynchronous with species upon which they rely, exposure to more extreme weather, or altered competition. Tropical woodland bird species on islands, including the endangered Mangrove Finch (*Camarhynchus helio*), are vulnerable to climate change as these ecosystems are very sensitive to environmental change (Şekercioğlu *et al.*, 2012). Species in extensive lowland woodlands such as the Amazon basin may need to move large distances to continue to stay in their preferred climate. Birds of the woodland interior which are poor at dispersing may not be able to make this range shift (Şekercioğlu *et al.*, 2012). Birds in boreal woodlands are being impacted by climate change, with some species populations being driven upwards and towards the pole (Virkkala, 2016; Virkkala *et al.*, 2018).

Woodland passerine species face multiple threats, which can be influenced by human populations, it is important to keep track of species populations. Population trends can inform on the species which are in decline and action can be taken to help these species.

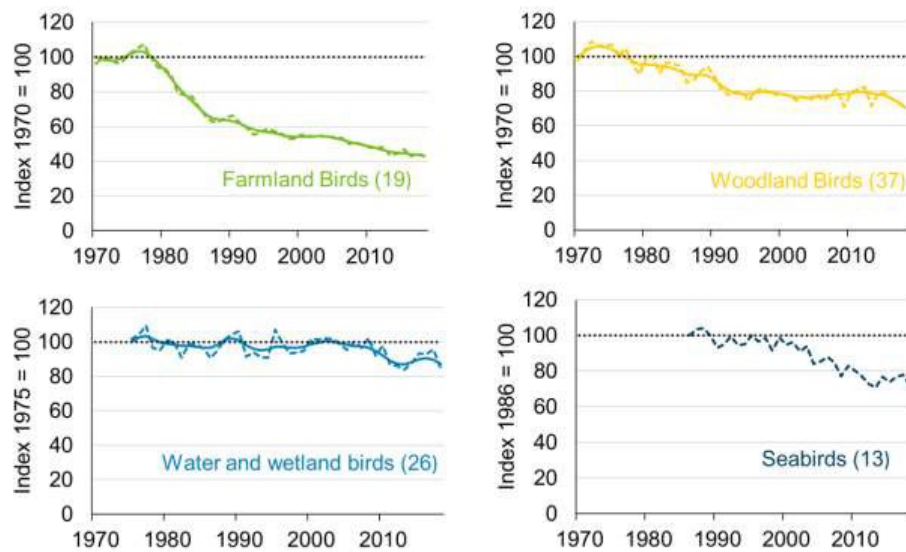
### 1.2.1.2 Population trends

Population trends of woodland birds have been assessed in different areas and at different scales. In Europe, population trends of birds are measured using population indices, with the total number of individuals from the initial year of data collection usually being standardised at 100. Gregory *et al.*, (2007) looked at the trends of widespread and common woodland birds in Europe, using data from an extensive European network of ornithologists between 1980–2003. Population trends were collected from a total of 77 different European species considered to be widespread and abundant, with 33 of these species having a close association with woodland.

Common woodland birds were found to have declined by 13%, while common woodland specialist declined by 18% from 1980-2003. Trends specific to woodland passerines found that there was a decline in Willow Tit (*Poecile montanus*), Great Tit (*Parus major*) and Common Nightingale (*Luscinia megarhynchos*). Other passerines including Blue Tit (*Cyanistes caeruleus*) and Long-tailed Tit (*Aegithalos caudatus*) have seen to be maintaining or increasing on the population index.

In 2019, the UK Department for Environment Food and Rural Affairs published the Wild Bird Populations in the UK, 1970 to 2018. In total, 130 species of birds were included in the index, of which 37 were woodland species. All these species were included on the basis they had a population size of at least 500 breeding pairs, and had sufficient data to

calculate a trend. Figure 1 shows the populations of wild birds in the UK by habitat, between 1970 and 2018.



**Figure 1.** Populations of wild birds in the UK by habitat, 1970 to 2018. (Gov.uk (2019) [online] Department for Environment Food and Rural Affairs. Wild Bird Populations in the UK, 1970 to 2018).

Organisations including British Trust for Ornithology (BTO), the Joint Nature Conservation Committee (JNCC), and the Royal Society for the Protection of Birds (RSPB) worked in conjunction to compile the bird population indices. Surveys upon which these indices were based were carefully designed and included surveys such as the BTO/JNCC/RSPB Breeding Bird Survey (from 1994 to 2019) and the Common Birds Census (from 1966 to 2000) (Gov.uk, 2019). Woodland birds overall were found to be 29% below the original value in 2018 that in 1970. More specifically, woodland generalists were found to be 3% higher in 2018 than in 1970, while woodland specialists were found to be 47% lower in 2018 than 1970.

Another method of categorising UK bird's conservation status uses the Birds of Conservation Concern list (BoCC) from the British Trust of Ornithology. This uses a traffic light system where, red is the highest area of conservation concern where species needing urgent conservation action, amber is an intermediate rating where there is some conservation concern or necessary action, while green-listed species are stable or

increasing in the UK. Table 1 shows the different UK generalist woodland passerine species with their long-term (1970-2017) and short-term (2012-2017) change, as well as their status on the BoCC list.

**Table 1.** Generalist woodland passerine species with their long-term (1970-2017) and short-term (2012-2017) change, as well as their status on the BoCC list. (UK Department for Environment Food and Rural Affairs (2019) The Wild Bird Populations in the UK, 1970 to 2018).

Generalist Woodland Passerines	Long-term change (1970-2017)	Short-term change (2012-2017)	Status from BoCC list
Blackbird ( <i>Turdus merula</i> )	Little change	Little change	Green
Blue Tit ( <i>Cyanistes caeruleus</i> )	Little change	Weak decline	Green
Bullfinch ( <i>Pyrrhula pyrrhula</i> )	Little change	Little change	Amber
Chaffinch ( <i>Fringilla coelebs</i> )	Little change	Strong decline	Green
Dunnock ( <i>Prunella modularis</i> )	Little change	Little change	Amber
Great Tit ( <i>Parus major</i> )	Weak increase	Weak decline	Green
Lesser Whitethroat ( <i>Sylvia curruca</i> )	Little change	Little change	Green
Long-tailed Tit ( <i>Aegithalos caudatus</i> )	Weak increase	Little change	Green
Robin ( <i>Erithacus rubecula</i> )	Little change	Strong increase	Green
Song Thrush ( <i>Turdus philomelos</i> )	Weak decline	Strong increase	Red
Wren ( <i>Troglodytes troglodytes</i> )	Little change	Strong increase	Green

Majority of generalist woodland passerines were found to have little change when analysed long term (1970-2018), with Great Tit and Long-



tailed Tit having a weak increase and Song Thrush (*Turdus philomelos*) having a weak decrease. Trends for some species change when looking at change in the short-term (2012-2017) rather than the long term: for example, Blue Tit and Great Tit have a weak decline over short timescales instead of little change and weak increase, respectively, over longer timescales. Song Thrush is the only generalist species with red status from the BoCC list, two species are at amber status with the rest are green status. Table 2 shows the different UK specialist woodland passerine species with their long-term (1970-2017) and short-term (2012-2017) change, as well as their status on the BoCC list.

**Table 2.** Specialist woodland passerine species with their long-term (1970-2017) and short-term (2012-2017) change, as well as their status on the BoCC list. (UK Department for Environment Food and Rural Affairs (2019) The Wild Bird Populations in the UK, 1970 to 2018).

Specialist Woodland Passerines	Long-term change (1970-2017)	Short-term change (2012-2017)	Status from BoCC
Blackcap ( <i>Sylvia atricapilla</i> )	Strong increase	Little change	Green
Chiffchaff ( <i>Phylloscopus collybita</i> )	Weak increase	Weak increase	Green
Goldcrest ( <i>Regulus regulus</i> )	Little change	Weak increase	Green
Marsh Tit ( <i>Poecile palustris</i> )	Strong decline	Strong decline	Red
Nightingale ( <i>Luscinia megarhynchos</i> )	Strong decline	Strong decline	Red
Nuthatch ( <i>Sitta europaea</i> )	Weak increase	Little change	Green
Lesser Redpoll ( <i>Carduelis cabaret</i> )	Strong decline	Weak decline	Red
Redstart ( <i>Phoenicurus phoenicurus</i> )	Little change	Strong decline	Amber

Spotted Flycatcher ( <i>Muscicapa striata</i> )	Strong decline	Little change	
Tree Pipit ( <i>Anthus trivialis</i> )	Strong decline	Weak decline	Red
Treecreeper ( <i>Certhia familiaris</i> )	Little change	Little change	Green
Willow Tit ( <i>Poecile montana</i> )	Strong decline	Strong decline	Red
Willow Warbler ( <i>Phylloscopus trochilus</i> )	Weak decline	Weak decline	Amber
Pied Flycatcher ( <i>Ficedula hypoleuca</i> )	Weak decline	Weak increase	Red
Wood Warbler ( <i>Phylloscopus sibilatrix</i> )	Strong decline	Little change	Red
Common Crossbill ( <i>Loxia curvirosta</i> )	Little change	Strong decline	Green
Siskin ( <i>Carduelis spinus</i> )	Weak increase	Strong decline	Green

Table 2 shows that there are eight specialist woodland passerines which have currently in the category of strong decline for long-term change, with only Blackcaps (*Sylvia atricapilla*) being classed in the category of strong increase. Similarly, to the generalist woodland passerine species there are differences in the long-term and short-term change of some specialist woodland passerines. Siskin (*Carduelis spinus*) is categorised as weak increase for long-term but strong decline in the short-term, while Wood Warbler (*Phylloscopus sibilatrix*) is categorised little change in the short-term but strong decline in the long-term. The specialist woodland passerine list (table 2) has eight species with red status from the BoCC list compared to one generalist species (table 1).

Both the woodland bird population studies show that, overall, there is a decrease in the combined population of woodland bird species. Specialist woodland bird species are decreasing more with some generalist species maintaining and increasing in population compared to the original indices.

If a species is slightly decreasing in the short term but long term is maintaining its population, routine monitoring might be appropriate. However, if a species is declining substantially in either the short or the long term, intensive monitoring system likely to be necessary. To understand how threats are influencing population trends, the ecology of avian species in woodland needs to be understood. The threat of habitat loss causes a reduction in the breed sites available to avian species. Reviewing the nesting ecology of woodland passerines will help in mitigating this threat.

### 1.3 Woodland passerine nesting

Passerines lay their eggs and raise their young in constructed nest structures as these provide safety and create a suitable microhabitat. Nest placement and construction differs between species, as well as in terms of timing within the breeding season, geographical location and sex of the bird carrying out the construction (Briggs and Deeming, 2016).

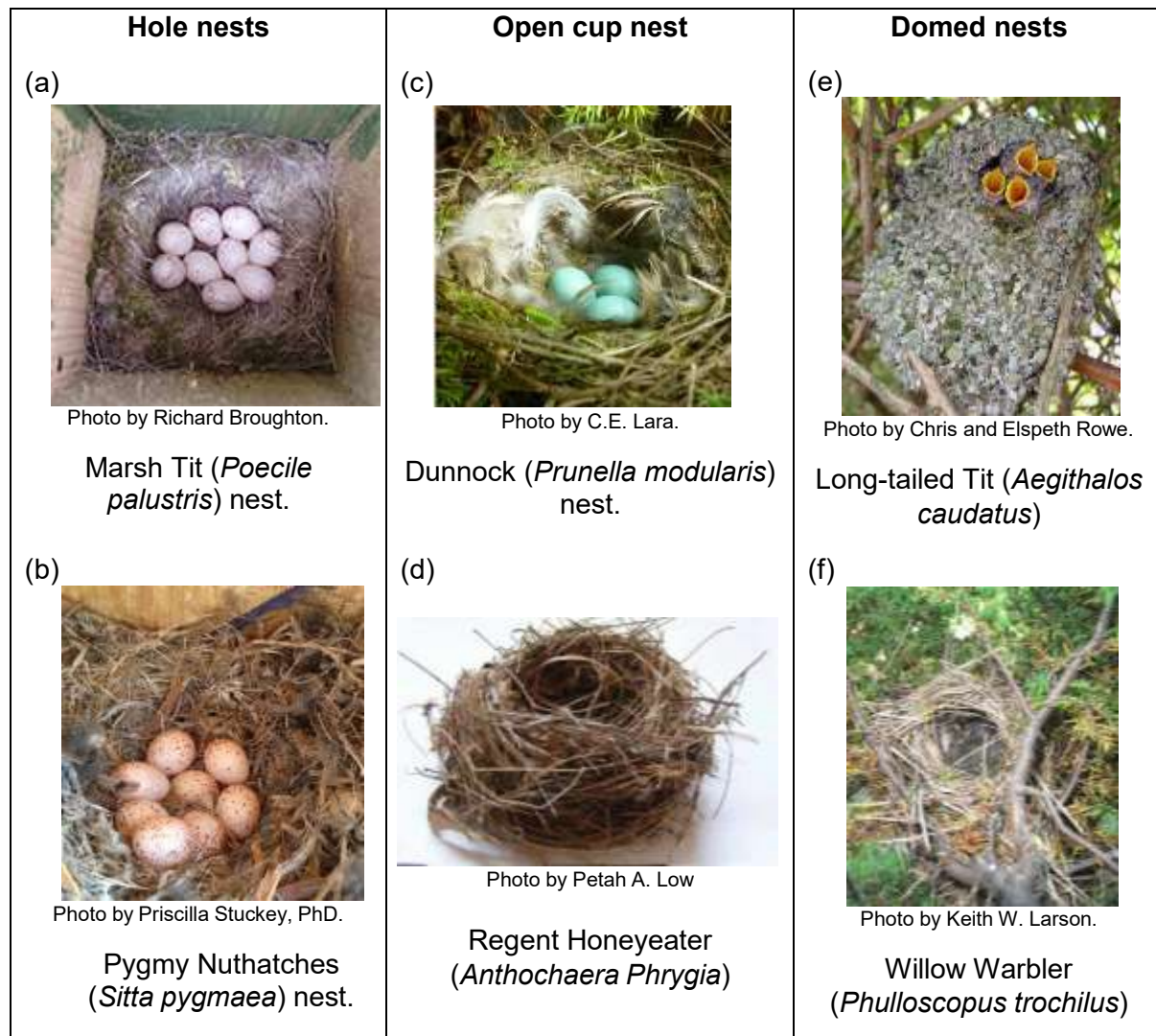
#### 1.3.1 Nest site selection

Selection of nesting site is important as it can determine of success and survival of adults and nestlings (Citta and Lindberg, 2007). The choice of nest site is considered a hierarchical process, with individuals making choices at varying spatial scales from region, to macro habitat type to specific nest site. The physical structure of a habitat has been assumed as a cue used by individuals for site selection (Citta and Lindberg, 2007), together with food availability and predation rate (Mezquida, 2004). Past reproductive success can also influence whether specific individuals will nest in an area in future breeding seasons (Citta and Lindberg, 2007).

#### 1.3.2 Nest types

The basic nest type among woodland passerine birds are hole nests, open cup nests and domed nests with a constructed roof (Collias, 1997). Hole nests

and domed nests allow a restricted access to the nest with the entrance being the only area which is exposed, while open cup nest can be accessed from above (Martin *et al.*, 2017). Figure 2 shows two examples for each of the different nest type mentioned.



**Figure 2.** Three different nests types from six bird species. (a) Hole nest from a Marsh Tit (*Poecile palustris*) (sourced: Broughton, 2017). (b) Hole nest from a Pygmy Nuthatches (*Sitta pygmaea*) (sourced: Stuckey, 2012). (c) Open cup nest from a Dunnock (*Prunella modularis*) (sourced: Tomkins *et al.*, 2015). (d) Open cup nest from a Regent Honeyeater (*Anthochaera Phrygia*) (sourced: Low *et al.*, 2013). (e) Domed nest from a Long-tailed Tit (*Aegithalos caudatus*) (sourced: Robinson *et al.*, 2018). (f) Domed nest from a Willow Warbler (*Phylloscopus trochilus*) (sourced: Larson and Kundisch, 2012).

### 1.3.2.1 Natural nest sites

Natural nests sites for woodland passerine species are either open, usually elevated within vegetation (e.g. Goldfinch (*Carduelis carduelis*))

but occasionally on the floor (e.g. Tree Pipit (*Anthus trivialis*)) or in cavities, usually cavities (e.g. Willow Tit (*Poecile montanus*)).

Ground nesting woodland passerines are rare, but do occur, one being the Tree Pipit (*Anthus trivialis*). Passerines can also nest in ground cavities on example being the Coal tit (*Periparus ater*). Nests that are built in trees require a solid and stable base, however in ground nesting species this constraint is reduced allowing for wider nest-cups (Herranz *et al.*, 2004). Predation in ground nesting passerines has previously been assumed to be higher than in species that nest above ground. This has been argued against with studies suggesting that habitat can also play a factor in nest construction (Yanes and Suarez, 1995).

Cavity nesting passerines are more common than ground nesting passerines. There are two types of cavity nesting birds. Primary cavity nesting birds are species which excavate their nest holes themselves, usually in decaying trees (Keisker, 1986). Secondary cavity nesting birds, such as Eastern Bluebirds (*Sialia sialis*), are unable excavate cavities themselves therefore rely on existing cavities to nest in, which can often be in short supply (Newton, 1998; Löhmus and Remm, 2005). Choice of a natural cavity nest site is important as many different factors can contribute to the microclimate of the nest including levels of solar radiation, thickness of tree walls, density of surrounding vegetation, orientation, cavity size, bark type and exposure to rain (Rhodes *et al.*, 2009). A study found that temperature of tree-cavities with a nest varied less than those of the ambient air and unused tree cavities, providing a more stable microclimate for nestlings (Rhodes *et al.*, 2009). Unused nests were found to have deeper cavities, which were lower to the ground and in trees with significantly smaller diameters (Rhodes *et al.*, 2009).

### 1.3.3 Clutch size and egg laying

Woodland passerine nest contains a cup which differs in size due to a difference clutch size. Clutch size differs between species for example the

European Goldfinch (*Carduelis carduelis*) lays 5-6 eggs per brood while Coal Tit (*Parus ater*) lays 9-10 eggs per brood (Klomp, 1970). Eggs are produced one per day, once laying starts, until a clutch size is reached. A number of different factors can influence clutch size including food availability, weather and age of female (Klomp, 1970).

#### 1.3.4 Incubation

Nests provide an area to incubate eggs and care for young in the different stages of chick development. Incubation facilitates embryonic development, albeit being costly to the adults as they cannot forage for food while incubating (Jonsomjit *et al.*, 2007).

#### 1.3.5 Chick care and fledging

Woodland passerine nestlings are altricial and remain in the nest for some time before fledging. Development of nestlings lasts around 10-14 days: it is a crucial stage and will impact overall reproductive success as measured by number of young to fledge (Jonsomjit *et al.*, 2007). When hatched, altricial young are featherless, blind and immobile being completely dependent on adults for survival. Nestlings initially grow down feathers as these are good for insulation, providing thermoregulation (Jonsomjit *et al.*, 2007). As the nestlings develop, contour feathers emerge in pin. During this feather development eyes open and they increase in size.

Provisioning rates and parental care both play key roles in the size and development rate of nestlings. Mariette and Griffith, (2015) studied biparental care in Zebra Finches (*Taeniopygia guttata*) researching whether there is an adaptive significance for coordinating provisioning and foraging between breeding pairs. Zebra Finch pairs are known to breed for life having low levels of extra-pair paternity in the wild, both sexes also contribute to all stages of breeding (Zann, 1996). Mariette and Griffith (2015), carried out a brood manipulation experiment to test whether nest visit synchrony was flexible and beneficial for nestlings' growth. It was found that partners synchrony and work rate increased with experimental brood size, suggesting that partners coped

with addition offspring demand by increasing cooperation rather than sexual conflict.

Figure 3 shows the development of the Tawny-crowned Tunchiornis (*Tunchiornis ochraceiceps*) from hatching blind with no feathers to abandoning the nest over an 11-day period (Loaiza-Muñoz *et al.*, 2017).



**Figure 3.** Photographic sequence of key nestling development features of Tawny-crowned Tunchiornis (*Tunchiornis ochraceiceps*) throughout the nestling period. (A) Three-day-old nestlings. (B) Eight-day-old nestlings. (C) Ten-day-old nestlings, this is the day before the nestlings abandoned the nest. (D) First night after the nestlings left the nest. These photos are from the nest found at Pantiacolla Lodge in 2013. Photos by W. Valencia (sourced: Loaiza-Muñoz *et al.*, 2017).

### 1.3.6 Nest material

The choice of nest material which species use is important for the survival for nestlings. Incubation has already been mentioned as a costly undertaking for adults (1.3.4), due to them being unable to forage for food. Energy exchange from the adults to the eggs is a costly process. If the nest material can reduce that heat loss, then the adult can incubate for longer (Hilton *et al.*, 2004).

Materials used to construct a nest varies between species, with the prevailing temperature, geographic location, nature and abundance of material available also having an impact on the choice of material (Mennerat *et al.*, 2009; Biddle *et al.*, 2018). Different materials have been thought to have different uses.

Materials such as moss and sticks are used to form a structural layer providing the shape of the nest (Biddle *et al.*, 2018). Down feathers are referred to by Collias *et al.*, (2014) as the “ideal nest lining material” due to the lack of barbules creating a feather structure that traps air and thus provides excellent insulation. While materials such as fresh plant matter have been suggested reduce the ectoparasite load in nest (Mennerat *et al.*, 2009). Species choose specific nest material due to specific properties: Common Blackbirds (*Turdus merula*) use dry grass to insulate nest while fresh plant material is used by some species, including Great Tits, for presumed antimicrobial or antiparasitic properties (Briggs and Deeming, 2016).

### 1.3.7 Threats

While carrying out nesting behaviours secondary nesting passerines are faced with threats from other organisms. Key threats that are both ecologically driven and linked by ecological interactions to woodland birds are predation and parasites.

#### 1.3.7.1 Nest predation

Nest predation is often the primary source of nest losses and individuals that choose habitats with low predation risk often have higher reproductive success (Martin, 1993). Nest predators are mainly mammals such as Martens (*Martes martes/foina*), Weasels (*Mustela*), Squirrels (Sciuridae) and Mice (*Mus musculus*). Birds including Jays (*Garrulus glandarius*), Buzzards (*Buteo buteo*), Crow (*Corvus*) and Woodpeckers (Picidae) can also be nest predators. Weidinger, (2010) observed both mammalian and avian nest predation on 13 different passerine species in woodlands in the Czech Republic, with results indicating that 32% of predation events were nocturnal and that mammals accounted for 95% of the nocturnal visits. Overall, Martens and Jays were the two most common predators in this study, with Martens taking all nestlings in one predation event whereas Jays would revisit and depredate over 1-4 days. Weidinger, (2010) also found that 5% of the predated nest were visited by multiple predators. Know that nest predation in common occurrence any reintroduction of a predator species should be closely monitored.



*Pine Marten reintroduction in the Forest of Dean*

In September 2019, Pine Martens were reintroduced into the Forest of Dean. Pine Marten diet has included nesting birds (Caryl *et al.*, 2012) suggesting the reintroduction could have negative implications on the populations of secondary nesting cavity birds. Research into the impacts of Pine Marten reintroduction found that birds nesting in nestboxes were at a higher risk of having their nest destroyed than individuals that nest in tree cavities (Kalinski *et al.*, 2014). This could be due to nestboxes being more visible to predators than cavities within trees (Huhta *et al.*, 1998), or the boxes themselves being more vulnerable to predatory action. The populations of birds that use the nestboxes at Nagshead Nature Reserve are, theoretically, now at an increased risk of nest predation than before September 2019 (although this does depend on the exact distribution of the released Martens and the diet of individuals in this new location). Erecting nestboxes that would reduce this risk of predation is a potential mitigation option, but, crucially, only as long as a predator-proof design does not adversely affect other aspects of nesting success, such as increasing ectoparasite load or decreasing fledgling success.



**Figure 4.** Pine Marten in Waterford by Maurice Flynn (sourced: O'Meara, 2014).

#### 1.3.7.2 Nest parasites

Nest parasites are another threat to the overall fledging success of broods within a nest. Parasites often have negative impacts on infected individuals causing a reduction in host fitness (Bush and Clayton, 2018). Both ectoparasites and endoparasites infect woodland passerines, but nest parasites are mostly ectoparasites and include Hen Fleas (*Ceratophyllus gallinae*), Louse Fly (Hippoboscidae), parasitic Blowfly (*Protocalliphora*), Ticks (Ixodida), Lice (Phthiraptera), True Bugs (Hemiptera) and Mites (Arachnida) (Bush and Clayton, 2018; Atkinson *et al.*, 2009). These parasites carry out important parts of their life cycle in the nest materials after reproducing alongside the birds.

Feeding on the birds causes a direct loss of nutrients to the nestlings and reduces the metabolic capacity (López-Arrabé *et al.*, 1996). Species with large group sizes provide a more suitable living condition for parasites to thrive, with studies finding that there is a positive association with group size and parasite risk in birds (Rifkin *et al.*, 2012). Birds do have methods to avoid these parasites including the removal of old nest material before reproduction takes place, a behaviour shown by Male House Wrens (*Troglodytes aedon*) (Pacejka *et al.*, 1996).

#### *Hen Flea life cycle*

Hen Fleas are a very common species of ectoparasite in wild birds and has been found in the nests of 72 wild bird species (Tripet and Richner, 1999). The life cycle of Hen Fleas is mainly confined to the host's nests except when dispersing. Overwintering as imagos in the pupal cocoon, in or near the nest, Hen Fleas breed during the hosts nesting period (Tripet and Richner, 1999; Harper *et al.*, 1992). Adults take bloodmeals from the adult birds and nestlings, and then lay eggs. Larvae then hatch from the eggs and feed on detritus and undigested blood excreted by the parents (Tripet and Richner, 1999). The larvae then pupate and given adequate food and humidity emerge from the cocoon (Harper *et al.*, 1992). These new adults can then breed again in the nest, disperse on adults or fledgelings or if at the end of the breeding season overwinter as imagos.

### *Blowfly life cycle*

Blowfly species which parasitise bird species are found in the genus *Protocalliphora* but it is only the larval stage that is parasitic in their lifecycle. Adult females lay around 100 eggs in the nests of the host species (Gussman, 2018). Once the blowfly larvae hatch from the eggs they actively feed attaching themselves to the host and feeding as much as possible before exhaustion of the resources (Gomes *et al.*, 2006; Gussman, 2018). Once they have sufficient fat reserves the larvae then enter a post-feeding larval dispersal phase. This is where they search for a pupation site in the habitat (Gomes *et al.*, 2006). Larvae then pupate usually within the nest of host species eventually emerging as adults, and the life cycle repeats.

### 1.3.8 Research on nest types

The basic types of nests and examples from different species can be seen in figure 2 (1.3.2). There are different trade-offs which occur when birds construct these nests. When in an enclosed elevated cavity, they reduce predation risk but increase parasite risk, whereas open-nesting species have increased predation risk but lower parasite risk. Previous studies have compared these two types of nests on the benefits they may have for nesting survival. Martin *et al.*, (2017) evaluated whether hole and dome nests provided any benefits in terms of reducing predation and improving thermal regulation for passerines, in tropical and southern hemisphere regions. From 319 species reviewed, enclosed nests did not show any consistent benefits of reduced predation. It was suggested that enclosed nests provide more consistent thermal insulation than open cup nests as it was noted that growth of mass and wings was enhanced in species which built enclosed nests (Martin *et al.*, 2017). Nests can be seen to be complex habitats on their own, with different ecological processes carried out within them. The impact of adding artificial nesting site to an area must be assessed to ensure the best outcome.

### 1.3.9 Nestboxes

With the removal of older or decaying trees a common management practice in woodlands cavities as a nesting resource decreases, limiting the number of potential nest locations available for cavity nesting species (Maziarz *et al.*, 2017). Nestboxes are a popular method to increase the nest site availability within a habitat for cavity nesting birds, particularly secondary cavity nesting birds (Mänd *et al.*, 2005). Nestbox selection by birds can be influenced by the characteristics including size, age and orientation of a nestbox (Mariarz *et al.*, 2017). Nesting success in nest boxes is often higher than in the natural cavities (Robertson & Rendell, 1990; Eaton *et al.*, 2015). Figure 5 shows different types of nestboxes, including three options designed to reduce predation risk.



**Figure 5.** Types of nest boxes involved in the study. (a) Wooden unmodified nestbox (sourced: NHBS, 2016). (b) Deep nestbox (sourced: Green-tech, 2018). (c) Woodcrete nestbox (sourced: Ark Wildlife, 2019). (d) Guardian nestbox (sourced: Gardenbird, 2019).

#### 1.3.9.1 Standard wooden nestboxes

Standard wooden nestboxes (fig. 4a) have been effective at providing sites for birds to establish nests where the abundance of tree cavities has decreased. However, they have not provided any specific protection from nest predators which prey on nestlings and adult birds. Nest predation has been previously stated to be the primary source of nest losses (Martin, 1993). Age of the nestbox was a potential factor

when looking at ectoparasite load and breeding success. New standard wooden nestboxes were useful in evaluating age as a factor when comparing them to standard old nestboxes. Vilka (2003) looked into the importance of nestbox age in monitoring populations of Pied Flycatchers and Great Tits in Latvia pine forest. A type of predator-proof nestbox was used in the study to protect against Pine Martens, although the specific type of nestbox was not stated. A decrease in numbers of both species viewed over 20 years (1981-2000) was seen. This could have been due to a number of other factors rather than nestbox age. Additionally, the age of the nestbox was suggested to affect brood numbers in Pied Flycatchers, with the clutch size increasing in older nestboxes.

#### 1.3.9.2 Deep nestboxes

Deep nestboxes (fig. 4b) are designed to reduce the predation rate by having nestlings beyond reach of predators. With the bottom of the box extended nests will be built lower, meaning nestlings are further away from the entrance and predators that reach into nestboxes to remove eggs, young, or incubating/brooding adults' will be unable to do so. However, there is potential that birds nesting in such boxes will just collect more nest material to build the nest up higher, potentially increasing habitat available for ectoparasites and negating effectiveness as a predator-proof design.

#### 1.3.9.3 Woodcrete nestboxes

Woodcrete nestbox (fig. 4c) is made of a mixture of wood and concrete providing a safer casing as predators that would normally break wooden nestboxes are unable to do so (García-Navas *et al.*, 2008). It has also been suggested that woodcrete boxes provide more insulation to nesting birds, creating higher internal temperature and more favourable microclimate (García-Navas *et al.*, 2008), however, this might also increase parasite development and load, thereby creating a trade-off.

#### 1.3.9.4 Guardian nestboxes

Guardian nestboxes (fig. 4d) involve a tube being attached to the outside of a nestbox extending the entrance. This can prevent predators from accessing the nest by climbing into the hole, while also stopping larger predators from reaching into the nest to grab the developing young or enlarging the hole (Eaton *et al.*, 2015). However, there could be trade-offs with provisioning rates.

Table 3 shows a summary of the positive and negatives of the different nestbox types, taking into account predator prevention, parasites and microclimate in the nestbox.

**Table 3. Summary of the positives and negatives of different nestbox types for secondary cavity nesting passerines.**

	Nestbox Type			
	Standard	Deep	Woodcrete	Guardian
Positives	Provides a nesting site for secondary cavity nesting birds where tree cavities are less available (Mänd <i>et al.</i> , 2005).	Provides protection against predation by preventing predators such as Pine Martens from reaching nestlings (Kaliński <i>et al.</i> , 2014).	Provides protection against predation as this nestbox type cannot be broken like standard boxes can (Enciso <i>et al.</i> , 2016).	Provides protection against predation by preventing predators from reaching into nestboxes (Eaton <i>et al.</i> , 2015).
Negatives	Nestlings are not provided extra protection against predators.	Parasitic load increases as parental birds fill up nestbox to that the nest is closer to entrance. This can also negate the intended	Increased fluctuation in temperature can cause an unstable microclimate for nestlings (Enciso <i>et al.</i> , 2016) and	Increases the amount of time taken for an adult to enter and leave the nest potentially reducing the amount of

protection against predation. (Kaliński <i>et al.</i> , 2014; Rendell and Verbeek, 1996)	could increase parasite larval development, thus increasing the parasite burden on chicks.	provisioning that can occur.
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#### 1.4 My research

This research looked to answer the question does nestbox type significantly alter the ectoparasitic load and breeding success in the nests of secondary-cavity-nesting passerines, at Nagshead, Forest of Dean? My research aimed to identify the most suitable nestbox type for secondary cavity nesting woodland passerines species at Nagshead, Forest of Dean, especially in terms of the trade-off between predator proofing and parasite load. Achieving this aim assists in improving breeding conditions for declining species, at sites in the UK and the world. Providing the optimum nesting site in areas, which have lost of natural nesting sites due to habitat loss and management, can help reduce the decline of woodland avian populations (1.2.1.2).

The nestbox types considered are those mentioned in 1.3.9.1-3 – standard (old and new), deep and woodcrete; the original plan to use guardians was not possible due to changes related to the Covid19 pandemic. The boxes types that are used differ in their predator resistance but there might also be important differences in terms of their average parasite load and the overall success of the birds nesting within them. The study species will include three resident species, Blue Tit (*Cyanistes caeruleus*), Great tit (*Parus major*) and Nuthatch (*Sitta europaea*), and a migratory species, Pied Flycatcher (*Ficedula hypoleuca*). The study species will be expanded on in Chapter 2: Methods.

After this chapter, this thesis will comprise 4 more chapters:

- Chapter 2 details the main study setup, providing information on Nagshead as a study site, the nestbox scheme, the study species, and the Pine Marten reintroduction in September 2019.

- Chapter 3 examines how nest parameters (nestbox type, nestbox age, nest weight, and nest composition) influence ectoparasite load.
- Chapter 4 analyses the potential impact of nest parameters and ectoparasite load on avian breeding success.
- Chapter 5 will discuss the findings from Chapters 3 and 4, formulate conclusions, and make any recommendations for future work.

Overall, it is hoped that this research will help determine if and when antipredator nestboxes are needed in an area, relative to predation risk, or whether they could be more detrimental to secondary cavity nesting species in areas where predator risk is low.



## Chapter 2

### Methods

#### *Summary*

*This chapter describes the study site as well as the fauna and flora which occur at in. The avian study species which nest in the nestboxes at the site are introduced. Past and present management of the nestboxes, dating back to 1942, is outline. The nestboxes types being used in this study are expanded on in this chapter. The reintroduction of Pine Martens to the area is discussed including the impact it could have on the avian study species.*

#### 2.1 Study site: Nagshead Nature Reserve, Forest of Dean.

Nagshead Nature Reserve (Gloucestershire, UK), near Parkend in the Forest of Dean, covers 308 hectares centred on 2°34'0"W, 51°47'0"N (Campbell, 1968). In the 1970s, Nagshead was originally proposed as a reserve by the Royal Society of Birds (RSPB) due the abundance birdlife, and especially the regionally-important breeding population of Pied Flycatchers (Goodenough, 2007). Since 1972, 40% of the reserve (120.1 hectares) has been designated as a Site of Special Scientific Interest (SSSI) (Goodenough, 2007). The reserve is currently managed by the Forestry Commission and the RSPB.

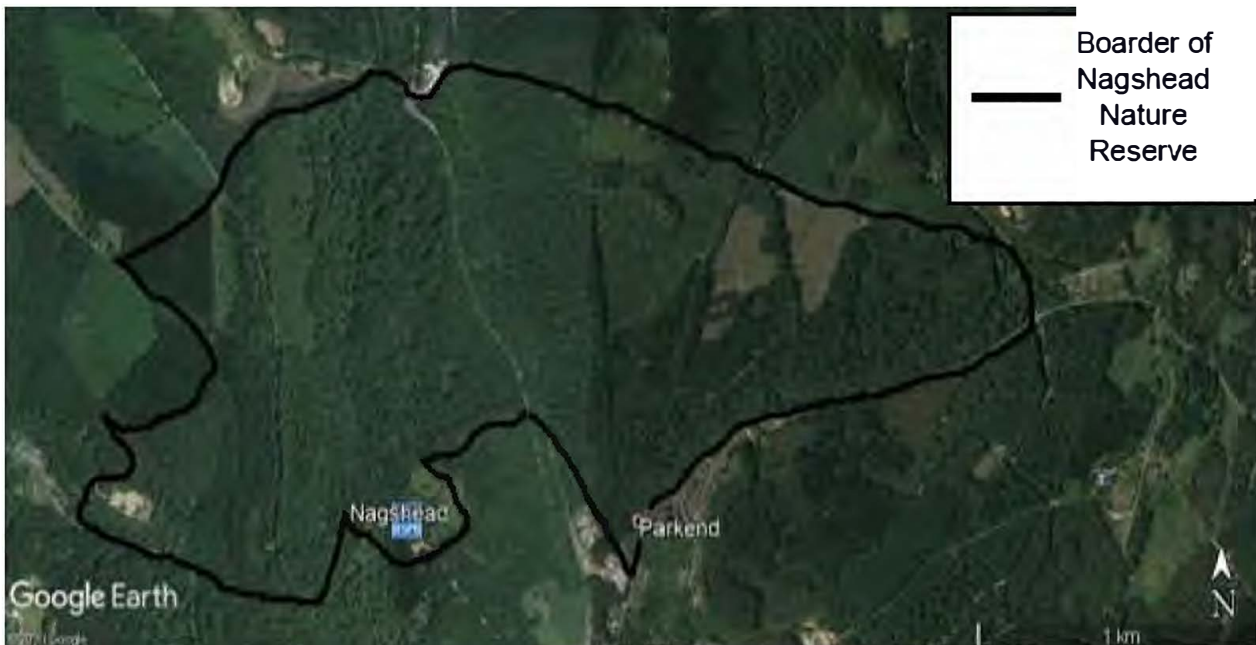
##### 2.1.1 Fauna and Flora

Nagshead is predominately broadleaf woodland, mainly dominated by Pedunculate Oak (*Quercus robur*). Nagshead is now the largest continuous patch of ancient semi-natural broadleaf woodland in Southwest England (Proctor and Pollard, 2000). Overall, the Nagshead Nature Reserve is 308ha. Approximately 200 hectares of the woodland has been enclosed by a fence since 1940s. This has allowed a diverse structure of shrub and field layers of Holly (*Ilex aquifolium*), Bramble (*Rubus* spp.) and regenerating Oak (*Quercus*) and Ash (*Fraxinus excelsior*) to develop (Campbell, 1968). The site also includes some areas of

acidic shrubland and semi-improved grassland. In the reserve 108 hectares has been historically grazed by sheep, while other enclosed areas are subject to light grazing from a population of Fallow Deer (*Dama dama*) (Campbell, 1968). The reserve has also been inhabited by a population of Wild Boar (*Sus scrofa*) which have been in the area since escaping from farms in 1990s. Nagshead has been home to populations of resident and migratory bird species such as Great Spotted Woodpecker (*Dendrocopos major*) and Pied Flycatcher respectively. Figure 6 shows the location of Nagshead Nature Reserve in relation to the UK while Figure 7 is a Satellite map of Nagshead Nature Reserve.



**Figure 6.** Location of Nagshead Nature Reserve (represented with the point of the marker) in reference to UK. Map from Digimaps (2020).



**Figure 7.** Satellite map of Nagshead Nature Reserve, Forest of Dean. Black line outlines the nature reserve boundaries. Map from Google Earth (2020).

## 2.2 Study species

As previously mentioned, the study species comprise four secondary nesting cavity passerines, of which three are resident and one is migratory (1.5).

### 2.2.1 Blue Tit (*Cyanistes caeruleus*)

Blue Tits are a common UK woodland passerine and have green status on the BoCC list (1.2.1.2). Blue Tits have been found all around the UK with the exception of some areas in Scotland. Their diet has consisted mainly of insects, caterpillars, seeds and nuts. During breeding season, the UK population has been around 3.6 million breeding pairs; the wintering population has been around 15 million. Blue Tits have been the model study organism in previous studies in the UK, including a study looking at the effects of feather supplementation to Blue Tit nest. Based in deciduous and mixed woodland within the grounds of Lancaster University, Mainwaring *et al.*, (2016) used 111 nestboxes checking them every fourth day to determine nest development. Individually-marked feathers were used to supplement Blue Tit nests allowing feather use to be tracked. Supplemental feathers were more frequently used during incubation rather than during the nest building process but no evidence was found indicating breeding was improved with

supplemented feather use. It was concluded that the primary function of feather in Blue Tit nests is to serve as a sexual signal from an extra-pair male trying to copulate with the nesting female using nuptial gifts. The original male will remove these feathers, perceived as foreign nuptial gifts, from the nest.

### 2.2.2 Great Tit (*Parus major*)

Another common UK woodland passerine Great Tit also have green status on the BoCC list. Being largest UK Tit, their diet has consisted of insects, seeds and nuts. Great Tits have 2,500,000 breeding pairs spreading the whole of the UK except the Northern and Western Isles of Scotland. Previous research on this species in the UK has investigated whether brown speckles on eggshells is to encouraged males to contribute more to a breeding attempt (Stoddard *et al.*, 2012). This was called the sexually selected eggshell coloration (SSEC) hypothesis. Conducted using 145 woodcrete nestboxes in Burnt Farm Plantation, an adjoining short nursery plantation and Madingley wood. No evidence was found to support to SSEC hypothesis (Stoddard *et al.*, 2012). It was also found that egg speckling did not advertise offspring quality, due to egg pattern attributes being unrelated to the growth rate of nestlings when fostered to other nests (Stoddard *et al.*, 2012).

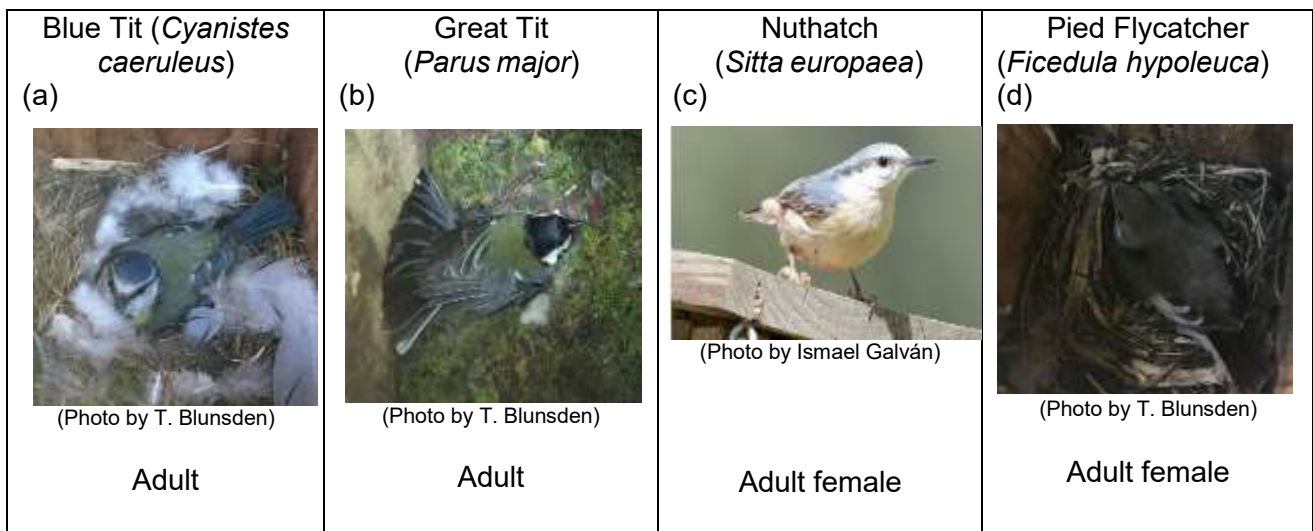
### 2.2.3 Nuthatch (*Sitta europaea*)

Nuthatches have been most often found in mature woods and established parkland throughout England and Wales, with some sightings in southern Scotland. There have been thought to be 220,000 breeding pairs of Nuthatches in the UK. Previous studies on the Nuthatch in the UK have looked into how local distribution patterns could be influenced by habitat, landscape structure and climate. Carried out in west Cambridgeshire, England, 3% of the area was wooded, with 20% of this woodland being suitable for Nuthatches (Bellamy *et al.*, 1998). 80 out of 450 woods in the area were visited, 65 of which were surveyed in detail for breeding birds. The study area was found to have suitable habitat for Nuthatches to breed in, with some woods being large enough to support 10 breeding pairs (Bellamy *et al.*, 1998). However, the population of Nuthatches at the time of sampling was lower, with no more than two breeding pairs observed in one wood each year. It was

suggested that landscape effects including isolation could be the reason (Bellamy *et al.*, 1998).

#### 2.2.4 Pied Flycatcher (*Ficedula hypoleuca*)

Pied Flycatchers winter in West Africa, returning to the UK in April and departing in August/September. They mainly breed in the West of England and Wales, with some populations found in the West Scotland. There have been an estimated 17,00-20,000 breeding pairs in the UK, including a regionally-important population located at Nagshead reserve, Forest of Dean. Migration between the UK and the Netherlands of Pied Flycatchers fledglings has been studied by Both *et al.*, (2012). Between 1970 and 2009 around 250,000 nestlings and 30,000 breeding adults were ringed in the UK and the Netherlands. It was strongly suggested that distance dispersal is more common than thought, with dispersal of young born in the UK to breeding the Netherlands occurring 43 times per year. Dispersal of new individuals also adds genetic variation to distance populations, shown in this study by darker plumage males found in the UK breeding in two populations in the Netherlands (Both *et al.*, 2012).



**Figure 8.** Shows the different bird study species which will be focused on in this study. (a) Adult Blue Tit on a nest (sourced: T. Blunsden). (b) Adult Great Tit on a nest (sourced: T. Blunsden). (c) Adult female Nuthatch (sourced: Galván, 2017) (d) Adult female Pied Flycatcher (sourced: T. Blunsden).

## 2.3 Nagshead nestbox scheme

### 2.3.1 History of management

Nestboxes were first originally put up within the nature reserve before the breeding season in 1942. Eighty-four nestboxes were assembled to encourage insectivorous birds to the area as pest control due to caterpillar epidemics in the 1930s (Goodenough, 2007). Other nestboxes were erected in the Forest of Dean, such as seventy nestboxes in the Perch Enclosure however, these suffered damage. Nestboxes were inspected by researcher J.M.B Brown in 1942, who was surprised to find that Pied Flycatchers were using these nestboxes (Campbell, 1968). Since this time nestboxes have been consistently monitored at Nagshead by the RSPB. More boxes were added and a regular monitoring scheme was started; the Nagshead nestbox scheme is the longest running nestbox scheme in the UK (Campbell, 1968; Goodenough 2007).

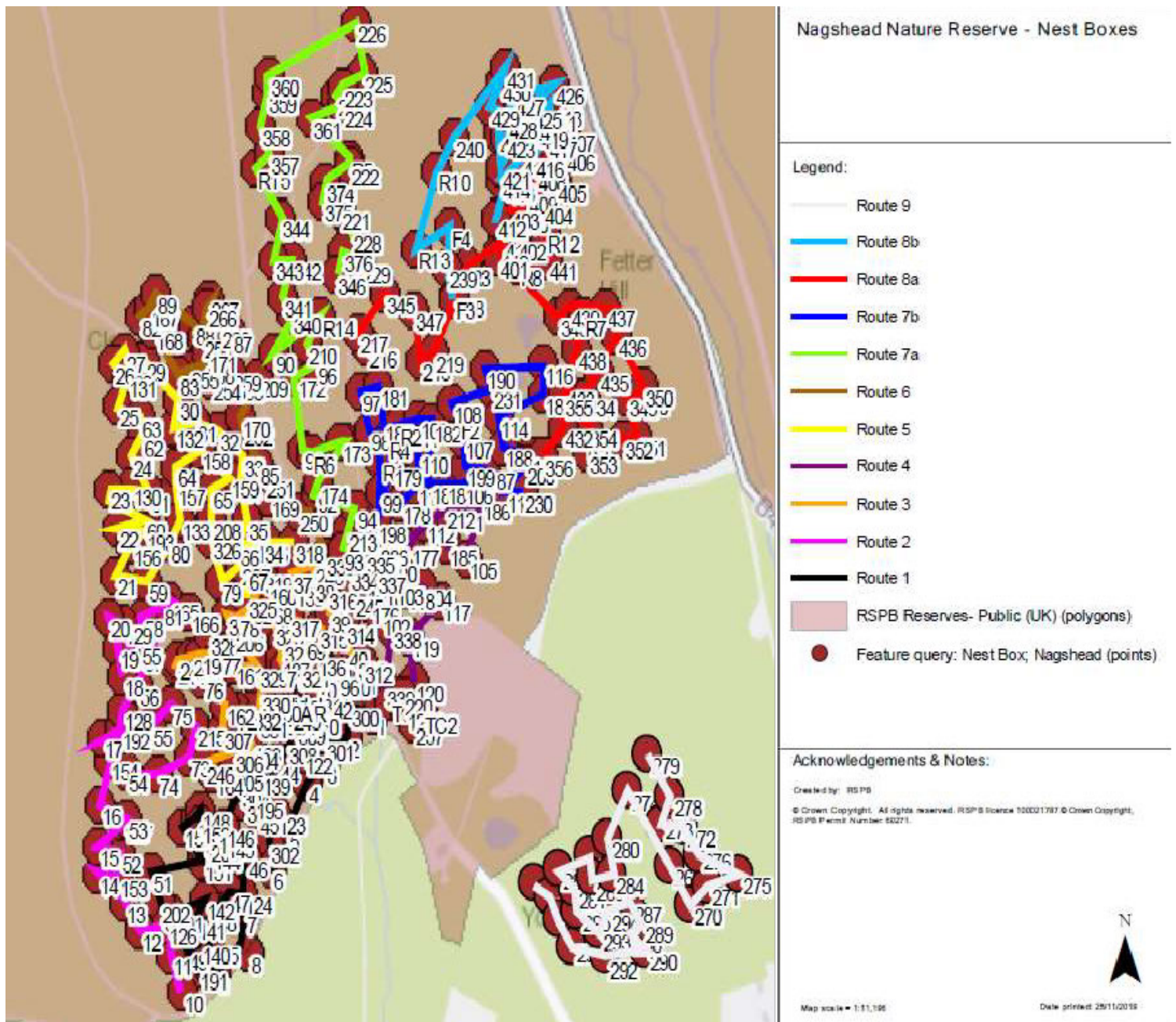
### 2.3.2 Recent management

In 2004, the nature reserve contained 389 nestboxes, with one nestbox per tree, and this number was fairly stable between years. A mean occupation rate of nestboxes of 71.74% between 1990 and 2004 showed there is an abundance in nesting sites for the populations of primary and secondary cavity nesting birds in the area (Goodenough *et al.*, 2010). Nestbox monitoring has been, and still is, organised by the RSPB and carried out by trained volunteers as Nestbox Species Surveyors. From the end of April onwards nestboxes were checked once a week to initially determine if a nestbox is being used. Once nesting has been established monitoring continues until fledging or failure of nestlings. Species, number of eggs/nestlings, nestling feather development, incubation by adults and success of the nest were recorded during the bird breeding season. Figure 9 shows a Nestbox Species Surveyor carrying out a check of a standard new nestbox



**Figure 9.** Nestbox Species Surveyor checking a standard new nestbox at Nagshead (sourced: T. Blunsden).

Figure 10 shows the different nestbox routes which are used to monitor the nestboxes during breeding season by Nestbox Species Surveyors.



**Figure 10.** Different routes of nestboxes at the Nagshead Nature Reserve. Map provided by Lewis Thomson, Site Manager at Nagshead Nature Reserve.



### 2.3.3 Nestbox types

The different types of nestboxes used to create artificial nesting sites for cavity nesting birds have previously been mentioned and have been shown in figure 5 (1.3.9). Nestboxes shown in figure 5 (b-d) are anti-predator nestboxes providing protection using different methods to reduce nest predation. At Nagshead Nature Reserve 100 deep, 100 woodcrete and 100 new standard wooden nestboxes were placed next to pre-existing standard wooden nestboxes (i.e. on the same tree) in a systematic manner: a deep box next to the first existing box on a checking route, a woodcrete box next to the second existing box on a route, a new standard wooden box next to the third existing box on a route. This pattern was repeated across the reserve. The same tree received a secondary nestbox before the breeding season of 2019 to attempt to provide predator proof nestbox option for avian nesting species. The new standard wooden nestboxes have been erected at Nagshead Nature Reserve, have not had any anti-predator properties but act as a control when comparing the woodcrete and deep nestboxes which have been set up.

Together with the original wooden boxes, the new boxes gave four different box types:

- Standard old (SO) wooden nestboxes.
- Standard new (SN) wooden nestboxes.
- Deep (DB) nestboxes.
- Woodcrete (WC) nestboxes.

## Chapter 3

# Do nest parameters influence the parasitic load within nests of secondary cavity nesting passerines at Nagshead?

### *Summary*

*In this chapter the interactions between the parasites and nest parameters are explored. Adult Hen Fleas and Blowfly pupae are the ectoparasites with sufficient abundance to be compared between nests. Generalized linear models (GLM) are used to analyse if there is any difference in the ectoparasite abundance in the nests found in the different nestbox types and avian study species, collected from nests in the 2019 breeding season. Any significant difference in ectoparasite load between the nestbox types and the study species are identified. Any nest materials which may be influencing the abundance of either ectoparasite species are mentioned and later discussed. Findings are discussed and implications on the avian study species explored.*

### 3.1 Introduction

Ectoparasites have previously been mentioned as threat to the fledging success of secondary cavity nesting birds (1.3.7.2). Nest ectoparasites survive in the nest material of birds and on the birds themselves, taking blood meals from adults and/or nestlings. There are a number of different nest parameters which could potentially influence the ectoparasite load found within a nest including nest composition, volume/weight of nest material, nestbox type and nesting species. Multiple studies have been previously carried out on ectoparasites and nest composition in nestboxes, adding to the overall understanding of how these nest parameters interact and influence each other. This study looks to expand on previous research comparing the ectoparasite load in the different types of nestboxes (1.3.9.1-3:) and the avian study species (2.2) which have been previously mentioned.

The effect of Hen Fleas on the lay date, nest-site choice, desertion and hatching success of Great Tits has been previously researched. Interesting differences have been found when presenting Great Tits different nestbox choices. When given the

choice between an infested nestbox and parasite free nestbox Great Tits chose the parasite free option (Oppliger *et al.*, 1994). When only given the option of an infested nestbox the laying date of the clutch was delayed 11 days compared to parasite free nestboxes. It was also stated that the nest desertion between laying and shortly after hatching was significantly higher infested nests and that hatching success was significantly smaller (Oppliger *et al.*, 1994). This indicates that adult Great Tit are aware of the presence of parasites in nestboxes and behaviours are carried out to avoid them when choosing nesting site. Other research has also been conducted on the impact of leaving nests in nestboxes has on ectoparasite load and the health of the adults. Tomás *et al.*, (2007) looked at the consequences of nest reuse for parasite burden and female health and condition in Blue Tits in Segovia province, central Spain. Nestboxes with old nests left unfumigated had the most ectoparasites and individuals who used these nests had reduced reproductive success. Female body mass was also decreased indicating that more energy was used by females to maintain the nest. Tomás *et al.*, (2007) findings support that increased ectoparasite load in nests could reduce reproductive success and female body mass in Blue Tits. If a significantly increased ectoparasite load is found for a specific box type this could cause less suitable conditions for the occupier.

Research has also focused on the significance of nest structure and nesting material for secondary cavity nesting passerines. Cantarero *et al.*, (2014) carried out a study on Nuthatches during 2012/13 in Valsaín, central Spain. Ectoparasite load of Nuthatches did not differ when in unstructured bark nests compared to structured cup moss nests from Great Tits. The study went on to suggest that the choice of bark is not related to reducing ectoparasite load. This indicates that the type of nests which is used by Nuthatch, Great Tit and Blue Tit, which uses a similar nest type to Great Tit, may not be influencing the ectoparasite load. A difference in ectoparasite load between species is then potentially less likely to be due to nest type. Research on Pied Flycatchers investigated how their use of materials in nest construction reflects localized habitat and geographical location (Briggs and Deeming, 2016). They found that the leaves used to build the nests generally reflected the local wooded area and that there is not a conscious decision to use leaves from a certain species of tree. This was backed up when comparing to nests in north Wales and central Spain as the nest composition was significantly different. One species of moss, *Hypnum*

*andoi*, was dominant, being found in all but two of the nests. Use of nest materials which link to a reduced ectoparasite load are restricted to geographical location and thus, may differ between studies.

Kaliński *et al.*, (2014) study focused on whether the threat of European Pine Marten predations influences the height of nest build by Blue Tits and Great Tits. Research was conducted during breeding seasons over three consecutive years (2004-2007) in Łagiewniki Forest, Poland. Nest height was measured before and after two types of anti-predator nestboxes were deployed. Deep nestboxes, referred to as “big” nestboxes by Kaliński *et al.*, (2014), and guardian nestboxes were used. Some “big” nestboxes had guardian tubes fitted on them as well. Nests in “big” nestboxes were found to have reduced predation than nestboxes smaller in size for both species. However, if nests created by the birds within the deep nestboxes were built over 6cm, they were more frequently depredated. Building of a larger nest by the nesting individual has been found as a drawback of deep nestboxes as it can negate the intended effect to reduce predation. Positive correlations have also been found between the volume of nest material and the number of Hen Fleas (*Ceratophyllus gallinae*) and Fowl Mite (*Ornithonyssus sylviarum*) (Kaliński *et al.*, 2014; Rendell and Verbeek, 1996) so there could be an important tradeoff between predator protection and parasite load.

This part of the research will analyse whether nestbox or nest parameters are associated significantly with the abundance of ectoparasites. These nestbox parameters include nest composition, weight of nest material, nestbox type and nesting species.

### 3.2 Study site

All nests were retrieved from Nagshead Nature Reserve (Gloucestershire, UK), near Parkend in the Forest of Dean. Management at Nagshead as well as the overall fauna and flora present had been discussed previously (2.1:2.3.1-2). A total of 78 nests was used in this study. This included 24 Blue Tit nests, 26 Great Tit nest, 12 Nuthatch nest and 16 Pied flycatcher nests. There were 61 nests from standard old boxes, 11 from standard new boxes and 6 from deep boxes. When selecting nests for use all nests from deep and standard new were selected regardless of species

and all Pied Flycatcher and Nuthatch nests were selected due to low sample sizes. Table 4 shows the number of nestboxes used in this study from each of the study species and the different nestbox types.

**Table 4.** Number of nestboxes used for the different species in the different nestbox types.

	Standard Old	Standard New	Deep
Blue Tit	19	5	0
Great Tit	18	4	4
Nuthatch	8	2	2
Pied Flycatcher	16	0	0

All nests were taken from nestboxes after the breeding season of 2019 by Nestbox Species Surveyors throughout August 2019. Nests were checked for eggs and dead chicks before removing and put into a ziplock bag with minimal air left in. They were then taken to the University of Gloucestershire where they were frozen at  $-18^{\circ}\text{C}$  as soon as possible.

### 3.3 Methods

#### 3.3.1 Ectoparasites

Methods for collecting nests for ectoparasite analysis often state that the removal of the nests it should be carried out within 24 hours post-fledging (Goodenough *et al.*, 2011). Removal of nest structure for this study was not carried out 24 hours post-fledging due to disturbance or removal of nest material being only permitted between August and January (under the GOV.UK licence GL12). Loss of ectoparasites in the period after fledging but before nest collection was a concern as ectoparasites could leave nests on the birds themselves or once nestlings fledged. However, adult Hen Fleas were still present in the nest allowing for the population of this ectoparasitic species to be analysed (Tripet and Richner, 1999; 1.3.7.2). Blowfly larvae which had not yet pupated as well as unhatched pupae and pupal cases that

had been left in the nest, were counted to determine the population of Blowflies previously within a nest (Cantarero *et al.*, 2013a; 1.3.7.2).

To make sure nests were precisely weighed, nests were weighed in the “zip-lock” bag and then the individual bags weighed without any material (López-Arrabé *et al.*, 2014), with this being deducted to give the nest weight. To remove feather dust, nest material was sieved through a 2mm and 1mm sieve into a clean white tray. Goodenough, (2007a) method used soft-tipped paint brushes to go through sieved material and remove any individuals found. Seeker and entomological forceps have been used in this study for similar effect. To find any remaining arthropods or ectoparasites, including Blowfly Pupae, the rest of the material was pulled apart using tweezers (Reynolds *et al.*, 2016). Identifying individuals, the naked eye was unable to, required the use of a Nikon SMZ800 dissection microscope at 10x magnification. Individuals of each ectoparasite species are were counted throughout nest dissection as well as any arthropods, which grouped according to taxa. Counted individuals were then transferred to clear tubes and labelled with box number, species or taxa, number of individuals. Individuals were preserved using 95% ethanol solution (Hebda and Wesolowski, 2012) and stored in the same location and conditions as the nests. When unhatched eggs were discovered in a nest while in the laboratory, the whole nest was autoclaved, as collection of bird eggs was, and still is, illegal under the Wildlife and Countryside Act 1981.

Some studies do take the nest straight after the nestlings have fledged. Cantarero *et al.*, (2013a) removed nests soon after fledging in sealed plastic bags. Some methods in this study were similar to what was used by Cantarero *et al.*, (2013a) such as removing Blowfly pupae removed directly from the nest material. However, for removal of Hen Fleas and Mites, Berleses funnels were used for 48 hours with the contents in jars identified using an Olympus SZX7 stereoscopic microscope. Berleses funnels were not used in this study as this method relies on nest being removed soon after fledging and the ectoparasites still being alive to move into the jars below.

### 3.3.2 Nest composition

Nest material was identified to allow for analysis on ectoparasite load and nest composition. Material was initially divided into organic or inorganic and was recorded along with a ranking using the DAFOR scale (D=dominant, A=abundant, F=frequent, O=occasional and R=rare) (Fowler and Cohen, 1990). Moss species identification was conducted using Atherton *et al.*, (2010). To allow for comparison with other mosses in different nests, a sample of each species found in each individual nest was placed into a container (Reynolds *et al.*, 2016). Hair and/or wool was identified to species of Badger (*Meles meles*), Sheep (*Ovis aries*), Deer (Cervidae) and Wild Boar (*Sus scrofa*), all of which were present at Nagshead. Tree material, including leaves and bark, was also recorded. Inorganic material, such as the outer fabric from tennis balls, which had previously been noted to be in nest from RSPB Nestbox species surveyor, was also recorded.

### 3.3.3 Data analysis

Using the nest material data, the different nest materials were analysed. When entering the nest material data into SPSS the different abundance levels were given a number 1-5, with 1 being rare and 5 being dominant. This allows for the data to analysis along with nest ectoparasite data. A mean average abundance of each nest material for the nests of the avian study species were calculated and entered into a table. This then showed which materials had the highest mean abundance within the nest composition of the different species.

Nest weight was an important factor to consider when analysing ectoparasites as more nest material could lead move available habitat for ectoparasites. The mean nest weight was calculated for each species made into a column graph. To test if differences in nest weight occurred between the study species: a Kruskal-Wallis test was conducted. This determined if there was a statistically significant difference in the nest weight between all study species types. Mann-Whitney U tests were then carried out to find which between which species specifically the difference was occurring in.

Analysis of the abundance of adult Hen Fleas and Blowfly pupae initially involved calculating descriptive statistics to compare mean number of these ectoparasites for different species and in different box types. This allowed for a comparison of to see if either ectoparasite was more prevalent, while also allowing comparison of the ectoparasites between the avian study species and nestbox types. This gave an indication of which avian species and box type had a higher ectoparasite load. However, to discover if there were significant differences in the ectoparasite load within the nests of the different box types, Generalized Linear Models (GLM) were conducted on the adult Hen Flea and Blowfly pupae data.

With multiple factors potentially influencing the ectoparasite load, a multivariate test was needed to analyse the data. Separate Generalized Linear Models (GLM) were conducted firstly on the abundance of Hen Fleas as the dependent variable and secondly on Blowfly pupae as the dependent variable. A Poisson distribution loglinear model was used as the dependent variables were count data, making it non-parametric data (Burnham and Anderson, 2004). Bird species and box type were added as factors (i.e. discrete category variables) and nest parameters (e.g. nest weight) were added as covariates (i.e. continuous variables). An interaction between bird species and box type was also included in the model. This showed whether box type is having an impact on bird species interaction with the two ectoparasite species. The models also allowed for a comparison within and between factors using estimated marginal means (EMM). The EMMs showed a mean abundance of ectoparasites for the different bird species and box type with covariates factored in. This ensured that any differences that were occurring between species and nestbox type were not due to these covariates. P values were used to identify any significant differences against a critical significance of 0.05 for factors and covariates and 0.10 for interactions (Burnham *et al.*, 2011).

To avoid overparameterization of the model by entering too many factors and covariates the Akaike's Information Criterion (AIC) score was used to compare different competing models for the same dependent variable



(Burnham and Anderson, 2004). The lower the AIC score the better the model. AIC value as weighs up two different aspects, the model fit and offsetting the model against the number of variables (Burnham and Anderson, 2004).

Overall, this method allowed for the relative abundance of ectoparasite and nest-dwelling arthropods to be collected and analysed, for the different types of nestboxes at Nagshead. It allowed for effective storage and analysis of nest material, while keeping in the laws set out by the Wildlife and Countryside act 1998. Although there was the potential for the loss of ectoparasites with the delay of nest collection, the reasons previously outlined for Hen Fleas and Blowfly Pupae would mitigate this.

### 3.4 Results

#### 3.4.1 Baseline findings: Nest composition

A number of different materials were found when examining the nest composition of the different species. Nine different moss species were found. *Brachythecium rutabulum*, *Hypnum andoi*, *Isothecium myosuroides*, *Kindbergia praelonga*, *Polytrichastrum formosum*, *Mnium hornum*, *Pseudoscleropodium purum*, *Rhytidiadelphus squarrosus* and *Thuidium tamariscinum*. Animal material was also found in the form of Deer hair, Badger hair, Boar hair, Sheep wool and Bird feathers. Plant material was also found as leaves, roots, wood/sticks and bark. Other material included compact soil, stones and synthetic material categorized as non-natural. Lichen was found but only in the nest of Pied Flycatchers. A list of these nest materials can be seen in table 5 with a mean DAFOR value for each of the different materials within the nests of the different species.

**Table 5.** Mean DAFOR value for different nest material found in the nests of secondary-cavity nest birds at Nagshead.

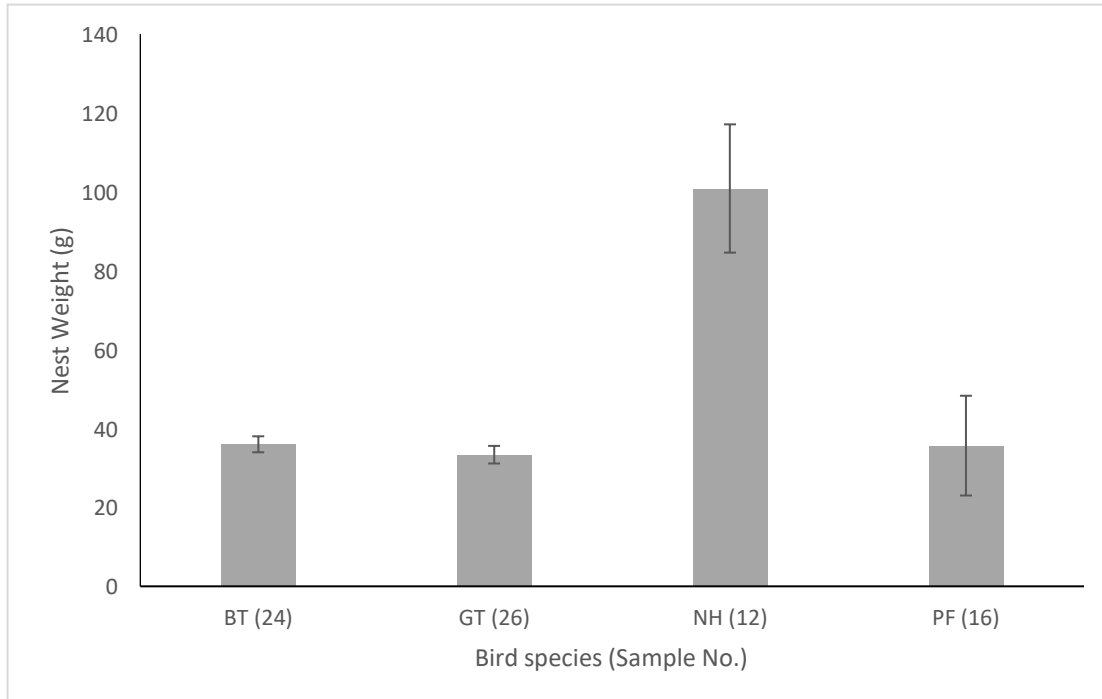
Nest Material	Bird Species (Mean DAFOR value)			
	Blue Tit	Great Tit	Nuthatch	Pied Flycatcher
<i>Brachythecium rutabulum</i>	1.33	0.88	0.15	0
<i>Hypnum andoi</i>	2.42	0.58	0.08	0

<i>Isothecium myosuroides</i>	0.58	0.19	0	0
<i>Kindbergia praelonga</i>	3.29	2.85	0.15	1.88
<i>Polytrichastrum formosum</i>	0	0.74	0	0
<i>Mnium hornum</i>	0	0.15	0	0
<i>Pseudoscleropodium purum</i>	0.25	0.62	0	0
<i>Rhytidiadelphus squarrosus</i>	0.83	0.12	0	0
<i>Thuidium tamariscinum</i>	0	2	0	0
Deer hair	0.38	1.27	0	0.25
Badger hair	0.66	1.23	0	0.25
Boar hair	1.33	2.08	0.08	0.31
Sheep wool	0.21	2.96	0	0
Unidentified white hair	2.21	1.62	0	0
Feathers	1.62	0.46	0	0.06
Leaves	0	0.19	1.62	1.63
Roots	0	1	0	1.06
Wood/Sticks	0.04	0.65	2.23	0
Bark	2.13	0.08	4.77	3.19
Compact Soil	0	0	0.85	0
Stones	0	0.04	0.31	0
Non-Natural	0.04	0.27	0	0.13
Lichen	0	0	0	0.13

*Kindbergia praelonga* was found to have the highest mean occurrence for the nest material found in Blue Tits nests. Sheep wool was found to have the highest mean occurrence for nest material found in Great Tits nests. Bark was the material with the highest mean occurrence for the nest material in Nuthatch and Pied Flycatcher nests.

#### 3.4.2 Baseline findings: Nest weight

Figure 11 shows the mean nest weight for the different study species. Great Tit was found to have the lowest mean nest weight, with Blue Tit and Pied Flycatcher being around the same value. Nuthatch had the highest nest weight; this could be due to the material used in Nuthatch nests as it was the only species to use compact soil.



**Figure 11. Bar graph of mean nest weight from Blue Tit, Great Tit, Nuthatch and Pied Flycatcher, for nests take from Nagshead Nature reserve. With standard error bars.**

To find if the differences in nest weight between species seen in figure 11 are significant a Kruskal-Wallis test was conducted. Nest weight differed significantly between the four species (Kruskal-Wallis test:  $\chi^2 = 22.891$ ,  $df = 3$ ,  $P = 0.000043$ ).

To find where the significant differences were occurring Mann-Whitney U tests were carried out between the species as a form of non-parametric post-hoc test. There was no statistically significant difference between Blue Tit and Great Tit (Mann-Whitney U test:  $U = 235.5$ ,  $n_1 = 24$ ,  $n_2 = 26$ ,  $P = 0.137$ ).

There was a statistically significant difference between Blue Tit and Nuthatch (Mann-Whitney U test:  $U = 27$ ,  $n_1 = 24$ ,  $n_2 = 12$ ,  $P < 0.001$ ).

There was no statistically significant difference between Blue Tit and Pied Flycatcher (Mann-Whitney U test:  $U = 185$ ,  $n_1 = 24$ ,  $n_2 = 16$ ,  $P = 0.847$ ).

There was a statistically significant difference between Great Tit and Nuthatch (Mann-Whitney U test:  $U = 24$ ,  $n_1 = 26$ ,  $n_2 = 12$ ,  $P < 0.001$ ).

There was no statistically significant difference between Great Tit and Pied Flycatcher (Mann-Whitney U test:  $U = 156$ ,  $n_1 = 26$ ,  $n_2 = 16$ ,  $P = 0.178$ ).

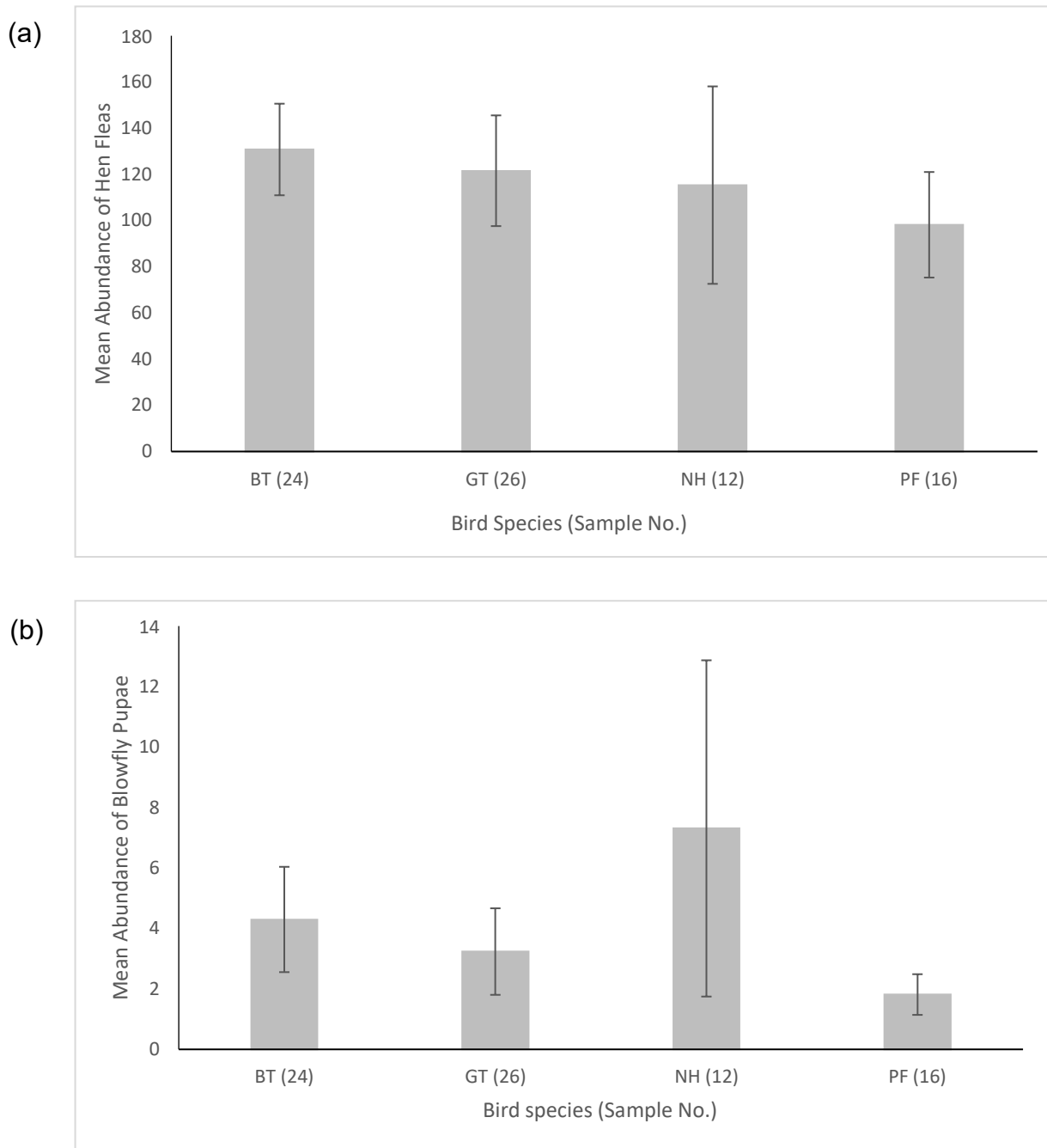
There was no statistically significant difference between Nuthatch and Pied Flycatcher (Mann-Whitney U test:  $U = 16$ ,  $n_1 = 12$ ,  $n_2 = 16$ ,  $P < 0.001$ ).

From the Mann-Whitney U tests it can be seen that Nuthatch nest weight differs significantly compared to the other species.

#### 3.4.3 Baseline findings: Parasites

Multiple different macroinvertebrates were found in the nests collected from Nagshead. These included species of Beetle (Coleoptera) (larvae and adults), Moths (Lepidoptera), Arachnids, Woodlice (Isopoda). Histeridae were also found in some of the nests. Bumblebee (*Bombus*) nests were also found in two of the bird nests surveyed. The ectoparasites that were found in the nests were Hen Fleas (adult stage), Blowflies (larval and pupae stages) and Ticks. Blowfly larvae and Ticks were only found in a few of the nests and when found consisted of only one or two individuals. This led to the conclusion that analysis would focus upon adult Hen Fleas and Blowfly pupae.

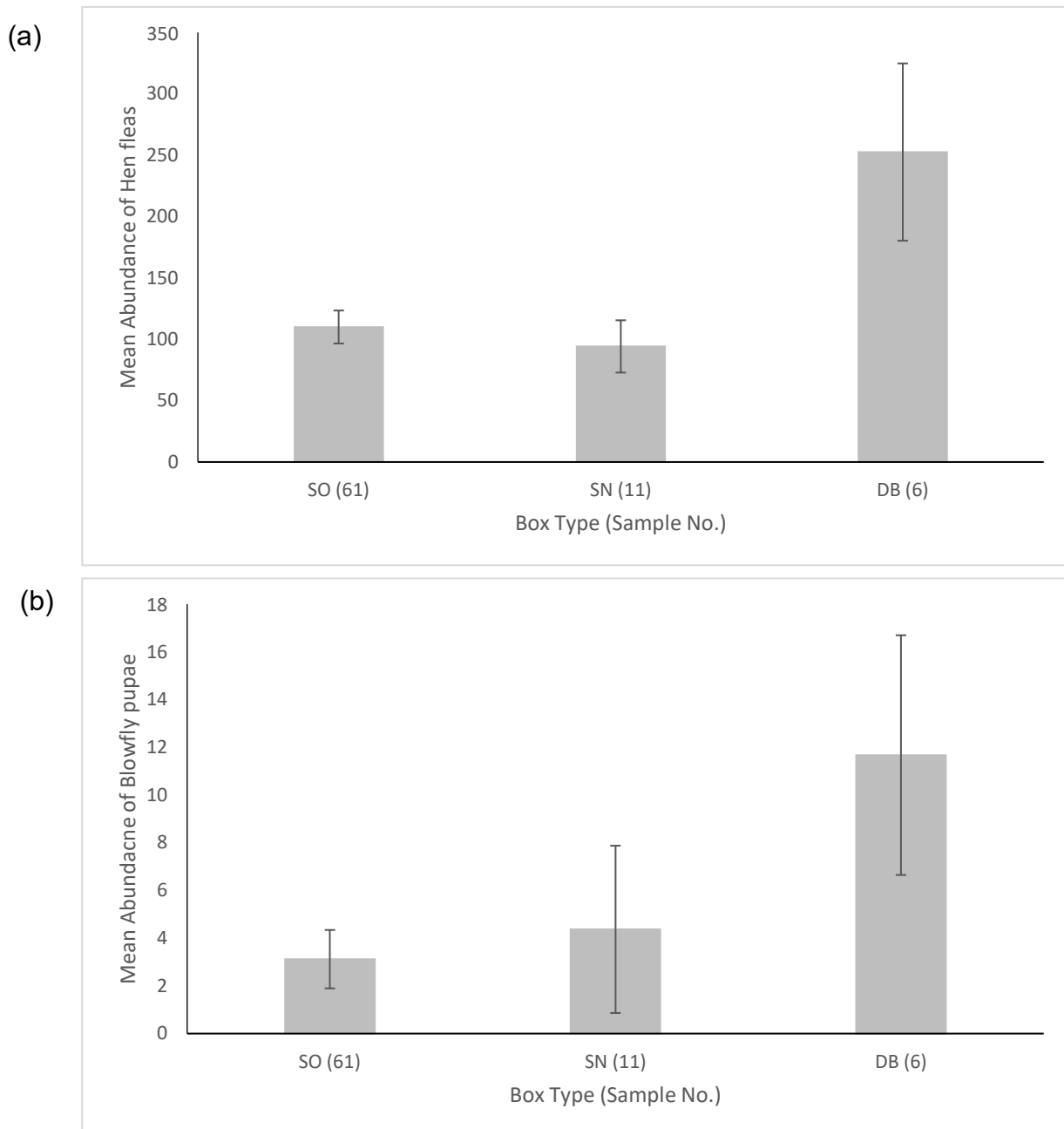
There were differences occurring between the abundance of the two-parasite species outlined between the bird species. Figure 12(a) shows the difference in mean abundance of adult Hen Fleas found in the nest of the bird species. Blue Tits were found to have the highest mean abundance, while Pied Flycatchers were the lowest. Figure 12(b) shows the difference in mean abundance of Blowfly pupae found in nests of the bird species. Nuthatches were found to have the highest mean abundance, while Pied Flycatchers had the lowest Blowfly mean abundance. When comparing figure 12(a) and figure 12(b) it was seen that there is a higher abundance of adult Hen Fleas compared to Blowfly pupae in the nests of the bird species.



**Figure 12.** (a) Mean abundance of adult Hen Fleas found in the nests of secondary cavity nesting birds at Nagshead. (b) Mean abundance of Blowfly pupae found in the nests of secondary cavity nesting birds at Nagshead. Standard error bars on both graphs.

Differences also occurred in the abundance of the two-parasite species between the different nestbox types that had been erected at Nagshead, Forest of Dean. Figure 13(a) shows the difference in mean abundance of adult Hen Fleas found in the nest of the different box types. Deep boxes had

the highest mean abundance, while standard new boxes had the lowest mean abundance. Figure 13(b) shows the difference in the mean abundance of Blowfly pupae found in the nests of the different box types. Deep nestboxes had the highest mean abundance, while standard old nestboxes had the lowest mean abundance. Figure 13(a-b) also shows that there is a higher abundance of adult Hen Flea present compared to Blowfly pupae in the nests found at Nagshead.



**Figure 13.** (a) Mean abundance of adult Hen Fleas found in the nests of the different box types at Nagshead. (b) Mean abundance of Blowfly pupae found in the nests of the different box types at Nagshead. Standard error bars on both graphs.

### 3.4.4 Generalized Linear Models (GLM)

A list of the independent variables (factors and covariates) which were the candidate factors in competing GLMs can be seen in table 6. Some nest materials were not considered as factors in the GLM as frequency of occurring and abundance was low.

**Table 6.** Factors, covariates and interaction considered for the Generalized Linear Models on adult Hen Flea and Blowfly pupae data

Factors	Covariates	Interactions
Bird species	<i>Kindbergia praelonga</i>	Bird Species*Box Type
Box type	<i>Rhytidiadelphus squarrosus</i>	
	<i>Hypnum andoi</i>	
	<i>Brachythecium rutabulum</i>	
	<i>Pseudoscleropodium purum</i>	
	<i>Thuidium tamariscinum</i>	
	<i>Polytrichastrum formosum</i>	
	<i>Mnium hornum</i>	
	Nest weight	
	Deer hair	
	Badger hair	
	Boar hair	
	Sheep wool	
	Leaves	
	Compact soil	
	Roots	
	Wood/sticks	
	Bark	
	Feathers	

Multiple models for both the adult Hen Flea and the Blowfly pupae abundance data were created using these factors, to identify the optimum model. As previously mentioned, AIC was used to determine the optimum model and prevent selection bias (3.3.3).

## 3.4.4.1 Adult Hen Flea

A generalized linear model was carried out on the adult Hen Flea data with bird species and box type as the factors. This showed if the differences previously mentioned from Figure 12(a) and 13(a), occurred within a complex model and if the differences were significant. A generalized linear model on the abundance of adult Hen Fleas found a significant difference occurred (AIC=5164.385, df=17, n=78, P=<0.01). The optimum model (with the lowest AIC of 5164.385) contained nest weight, deer hair, badger hair, unidentified animal hair, leaves, *Kindbergia praelonga*, *Rhytidiadelphus squarrosus*, compact soil and roots as covariates. An interaction between bird species and nestbox type was also included in the model. Table 7 shows the factors, covariates and interactions included in the model and if they were significant.

**Table 7.** Factors, covariates and interactions included in the optimum Generalized Linear Model for Adult Hen Flea abundance.

	P	Correlation (+/-)
<b>Factors:</b>		
Bird Species	<0.001	N/A
Box Type	<0.001	N/A
<b>Interactions</b>		
Bird Species*Box Type	<0.001	N/A
<b>Covariates:</b>		
Nest weight	<0.001	+
Deer Hair	<0.001	+
Badger hair	<0.001	+
Unidentified animal hair	<0.001	+
Leaves	<0.001	-
<i>Kindbergia praelonga</i>	<0.001	+
<i>Rhytidiadelphus squarrosus</i>	<0.001	+
Compact Soil	<0.001	-
Roots	<0.001	+

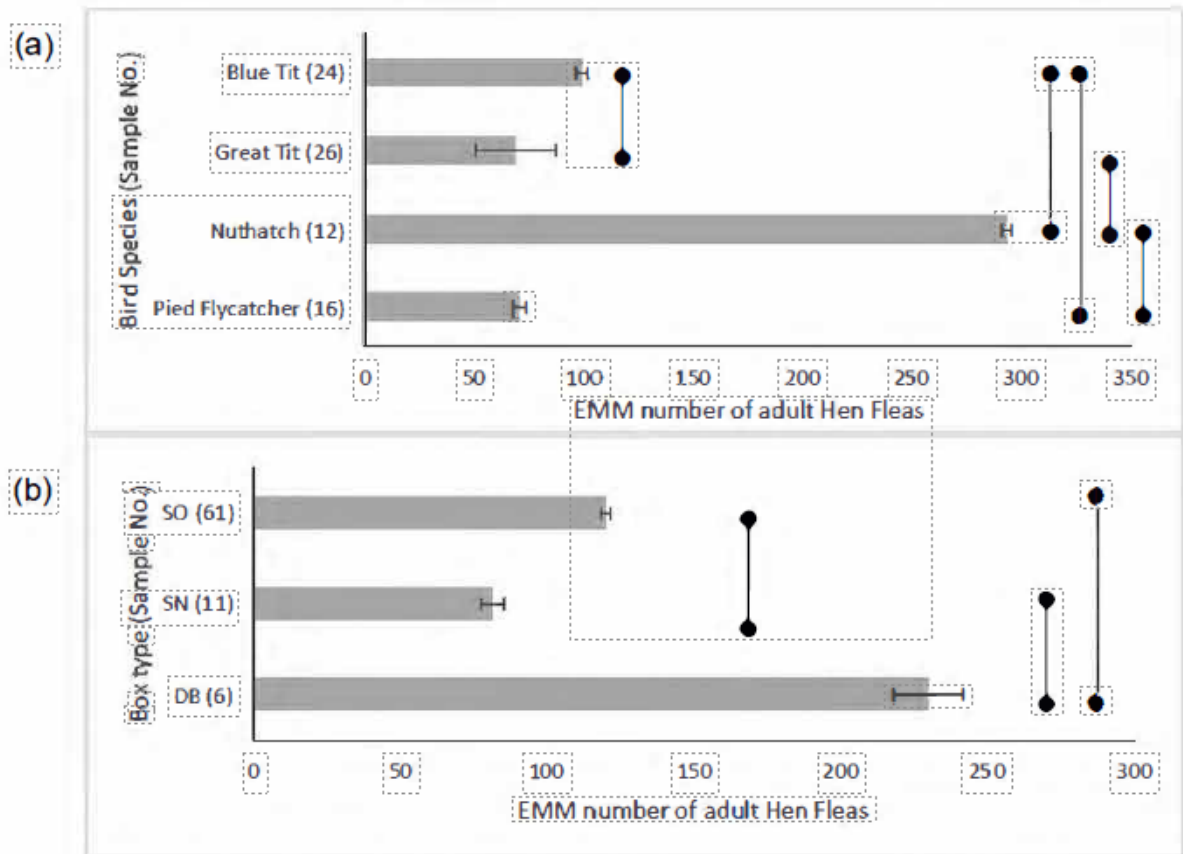
All covariates were found to be having a significant influence on the abundance of Hen Fleas.



Nuthatch had the highest abundance, while Great Tit had the lowest. This differs to what was seen in figure 12(a) where Blue Tit was found to be highest and Pied Flycatcher lowest. Statistically significant differences in the abundance of adult Hen Fleas were found between different bird species; Blue Tit and Great Tit ( $P < 0.001$ ), Blue Tit and Nuthatch ( $P < 0.001$ ), Blue Tit and Pied Flycatcher ( $P < 0.001$ ), Great Tit and Nuthatch ( $P < 0.001$ ) and Pied Flycatcher and Nuthatch ( $P < 0.001$ ). There was no statistically significant difference between Great Tit and Pied Flycatcher ( $P = 0.548$ ).

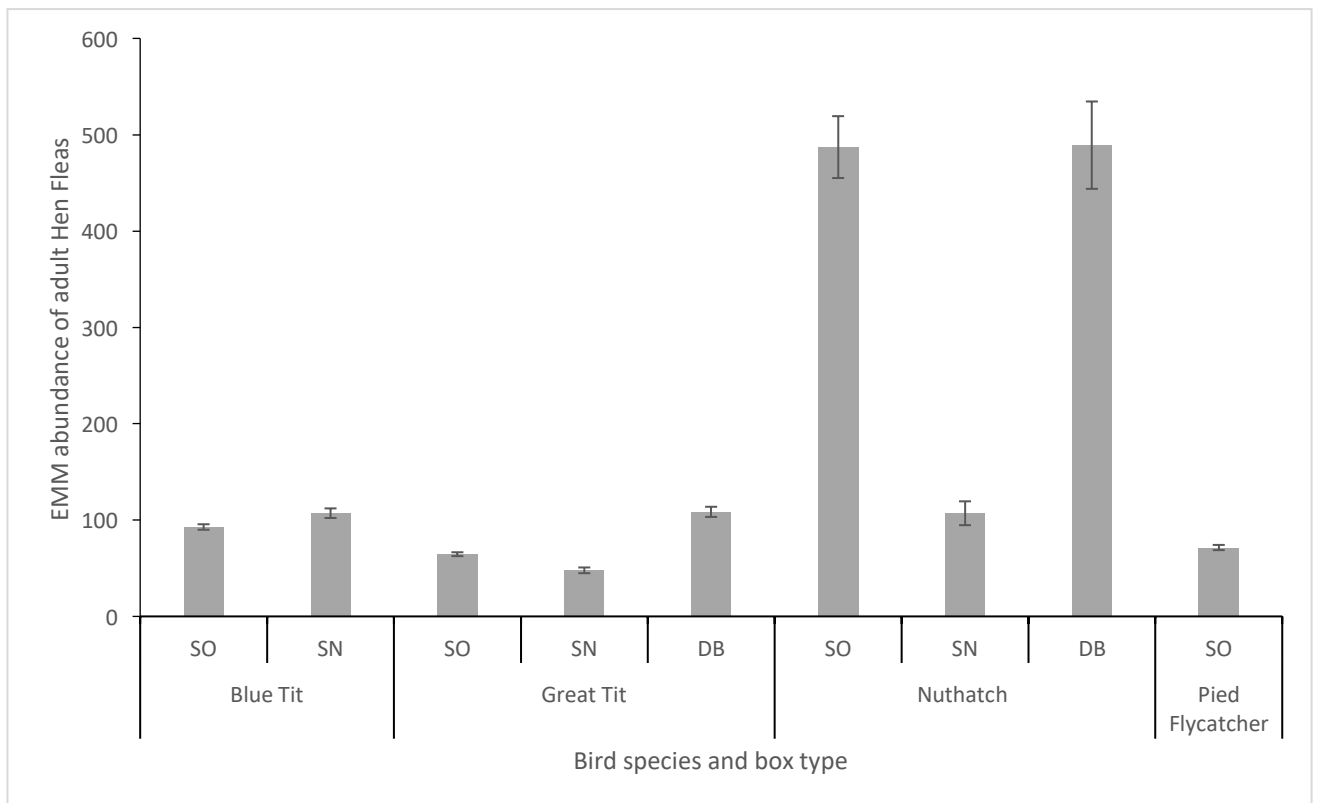
For nestbox type deep boxes had the highest abundance, while lowest was standard new boxes. This was the same as previously seen in figure 13(a). Adult Hen Flea abundance was found to be significantly different between all of the box types. Standard old and standard new ( $P < 0.001$ ), standard old and deep ( $P < 0.001$ ), standard new and deep ( $P < 0.001$ ).

Figure 14(a-b) shows the EMM of adult Hen Flea abundance for the study species and box type. Statistically significant differences are represented by solid black lines, representing if  $P < 0.001$ , and dotted black lines representing if  $P < 0.05$ . No line between the bars meaning no statistically significant difference occurred.



**Figure 14.** (a) EMM abundance of adult Hen Fleas found in the nests of secondary cavity nesting birds at Nagshead. (b) EMM abundance of adult Hen Fleas found in the nests the different nestbox types placed at Nagshead. Standard error bars for the different species and nestbox types were added.

Figure 15 shows the EMMs for each of the study species when nesting the different nestbox types. The interaction between box type and bird species was found to be significant as previously seen in table 6. Nuthatch had a higher abundance of adult Hen Fleas in standard old and deep nestboxes compared to the standard new. It was also higher than all the other species in the different nestbox type. This could be what was driving the significant interaction between nestbox type and bird species. Deep nestboxes for Great Tit and Nuthatches had the highest abundance of Hen Fleas for their species. Blue Tit was the only species to have a higher abundance of Hen Fleas in standard new boxes than in standard old. However Pied Flycatchers were only found in standard old nestboxes, not allowing for comparison with this species when in standard new nestboxes.



**Figure 15.** EMM for adult Hen Fleas, for each of the study species when nesting in the different nestbox types found at Nagshead. Standard error bars for the different species in the different nestbox types were added.

#### 3.4.4.2 Blowfly Pupae

A GLM was carried out on the Blowfly pupaedata with bird species and box type as the factors. This showed if the differences previously mentioned from figure 12(b) and 13(b) occur within a complex model and if the differences are significant. The generalized linear model was carried out on the abundance of Blowfly Pupae and found a significant difference was occurred (AIC=408, df=18, n=78,  $P < 0.01$ ). The optimum model (with the lowest AIC score of 408) contained nest weight, badger hair, leaves, *Kindbergia praelonga*, *Hypnum andoi*, *Brachythecium rutabulum*, compact soil, roots, bark and feathers as covariates. An interaction between bird species and nestbox type was also added into the model. Table 8 shows the factors, covariates and interactions included in the model, if they were significant, and whether they have a positive or negative correlation with Blowfly pupae.

**Table 8.** Factors, covariates and interactions included in the optimum Generalized linear model for Blowfly pupae abundance.

	P	Correlation (+/-)
<b>Factors:</b>		
Bird Species	<0.001	N/A
Box Type	<0.001	N/A
<b>Interactions</b>		
Bird Species*Box Type	0.001	N/A
<b>Covariates:</b>		
Nest weight	<0.001	+
Badger hair	<0.001	+
Leaves	0.002	-
<i>Kindbergia praelonga</i>	<0.001	+
<i>Hypnum andoi</i>	<0.001	+
<i>Brachythecium rutabulum</i>	<0.001	+
Compact Soil	0.002	+
Roots	<0.001	-
Bark	0.003	-
Feathers	<0.001	+

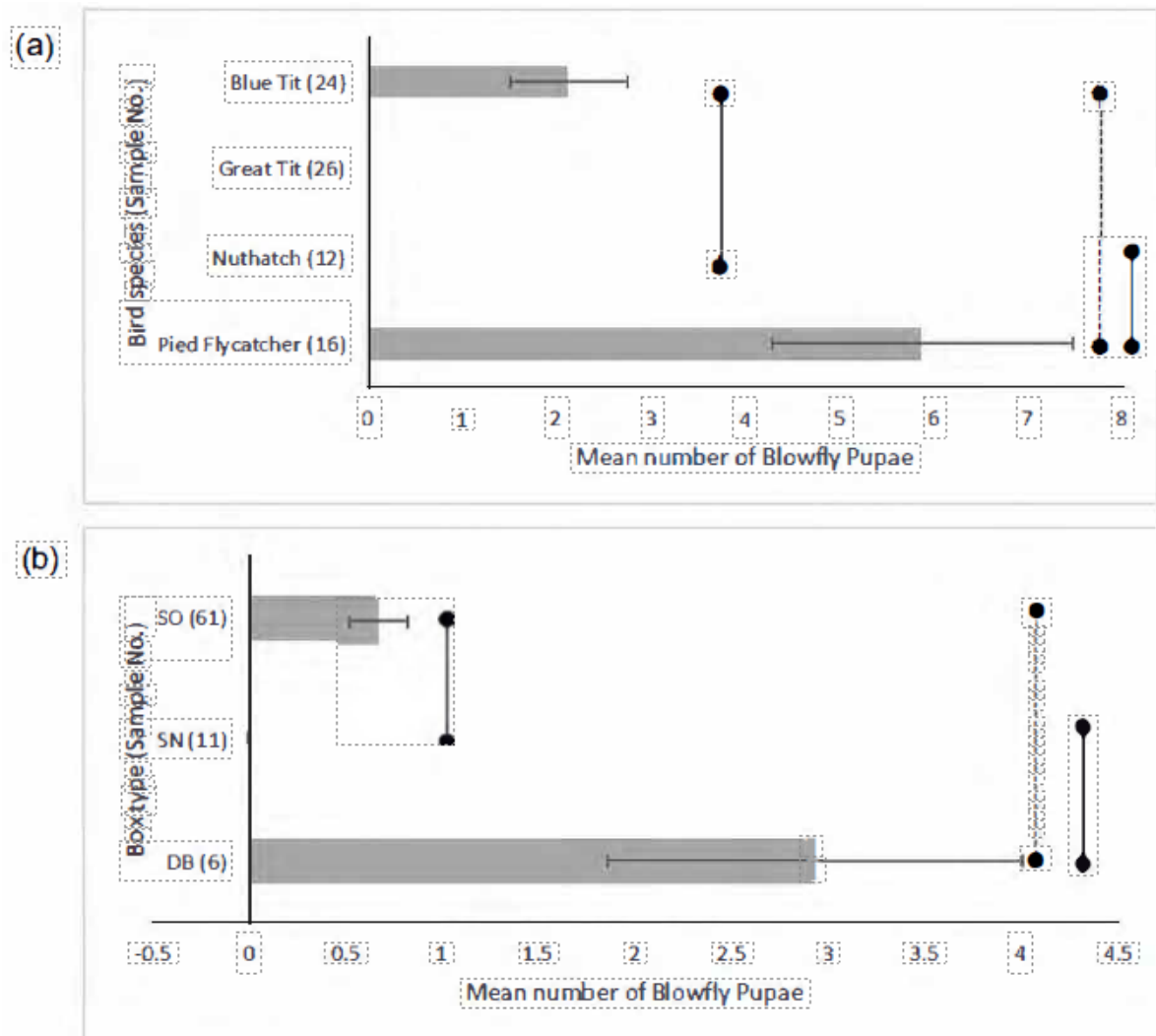
All covariates were found to be having a significant influence on the abundance of Blowfly pupae.

Pied Flycatcher was found to have the highest abundance of Blowfly pupae with an while lowest was Nuthatch. This differs from what was previously found in figure 12(b). Statistically significant differences were found between Blue Tit and Nuthatch ( $P=0.001$ ), Blue Tit and Pied Flycatcher ( $P=0.042$ ), and Nuthatch and Pied Flycatcher ( $P<0.001$ ). No statistically significant differences were found between Blue Tit and Great Tit ( $P=0.954$ ), Great Tit and Nuthatch ( $P=1.000$ ) and Great Tit and Pied Flycatcher ( $P=0.874$ ). Figure 11(a) shows the EMMs of the different study species. The Standard Error bar for Great Tit in figure 11(a) was removed for due to it skewing the graph. This was likely due to the majority of Great Tit nests having no blowfly pupae along with three nests having 11, 16 and

33 Blowfly pupae, and the EMM abundance for Blowfly pupae in Great Tit nests was 0.0004.

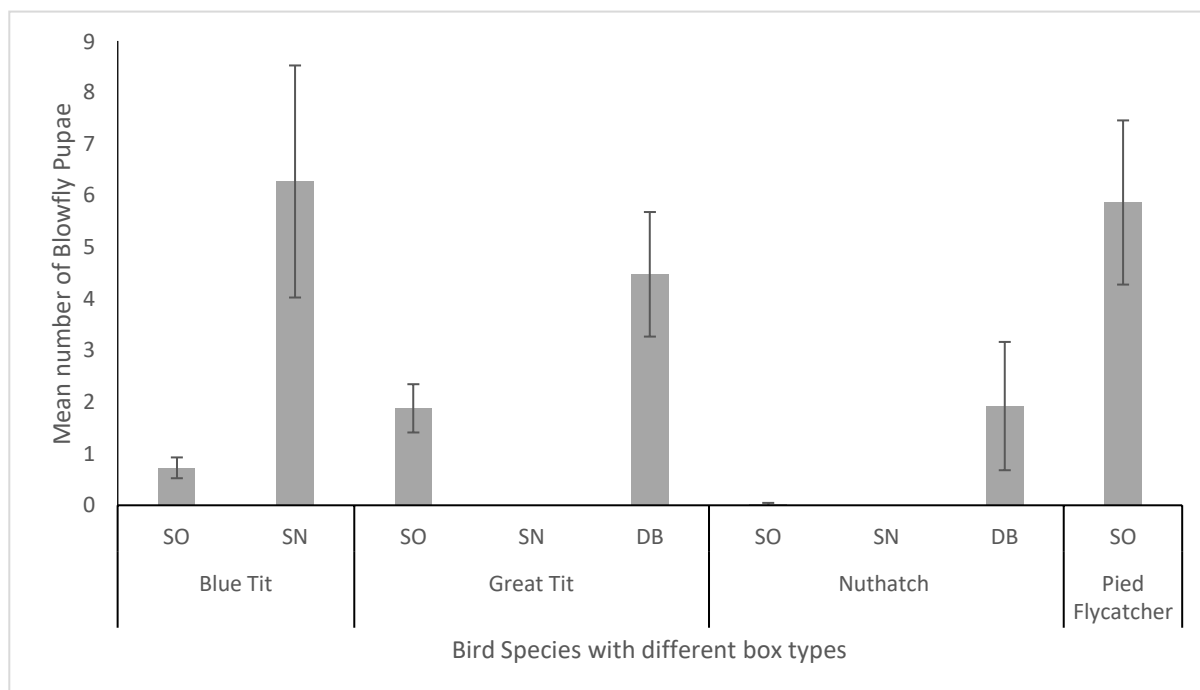
For nestbox type deep nestboxes had the highest abundance, while standard new nestboxes had lowest. Deep nestboxes are shown to have the highest abundance of Blowfly pupae in figure 13(b). Standard old has the lowest abundance in figure 13(b) which differs from these findings. A significant difference was found between all box types; standard old and standard new ( $P < 0.001$ ), standard old and deep ( $P = 0.032$ ), standard new and deep ( $P = 0.006$ ). It should also be noted that the EMM for standard new nestboxes was 0.

Figure 16(a-b) shows the EMM of Blowfly pupae abundance for the study species and box type. Statistically significant differences are represented by solid black lines, representing if  $P < 0.01$ , and dotted black lines representing if  $P < 0.05$ . No line between the bars meaning no statistically significant difference occurred.



**Figure 16.** (a) EMM of Blowfly pupae abundance found in the nests of secondary cavity nesting birds at Nagshead. (b) EMM of Blowfly pupae abundance found in the nests the different nestbox types placed at Nagshead. Standard error bars for the different species and nestbox type were added.

EMMs of Blowfly pupae abundance for different species within different box type were shown in figure 17. The interaction between the two factors was found to be significant which can be seen in table 8. Standard new boxes for Blue Tit had a higher abundance of Blowfly pupae than the standard old. This was the same as what was found for adult Hen Fleas. Blue Tit in a standard new box had the highest abundance of Blowfly pupae. Great Tit and Nuthatch deep nestboxes had the highest abundance of Blowfly pupae. When just looking at species in standard old boxes, Pied Flycatchers had the highest abundance of Blowfly pupae.



**Figure 17.** EMM for Blowfly pupae abundance, for each of the study species when nesting in the different nestbox types found at Nagshead. Standard error bars for the different species in the different nestbox types were added.

### 3.5 Discussion

From the GLMs with adult Hen Fleas and Blowfly pupae as the dependent variable it can be seen that there are significant differences between some species and between all nestbox types. Nuthatch and Pied Flycatchers had a significantly higher abundance of the adult Hen Fleas and Blowfly pupae respectively, than the other study species. This is with the exception of Pied Flycatcher and Great Tit for Blowfly pupae. Nuthatch was also found to have high a significantly higher nest weight than the other species. This could be due to the type of nest material or Nuthatch having a small sample size but with nests from deep nestboxes, increasing the mean nest weight. Nest weight was included as a covariate with the GLM for Hen Fleas so the increase in this ectoparasite in Nuthatch nests was not due nest weight. Deep nestboxes were found to have the highest abundance of both ectoparasite species. This increase in ectoparasite load could cause a reduction in the fledgeling success rate.

When comparing the results found in the single variate and multivariate tests some differences occurred. The results from the two GLMs found different study species

had the highest abundance of ectoparasites compared to figure 12(a-b), which showed the results in the single variate tests. When looking at the abundance of ectoparasites in nestbox types, GLM with Hen Flea as the dependent variable was found to have the results found in figure 13 (a). There were differences found when comparing results in figure 13 (b) to the results for nestbox type in the GLM with Blowfly pupae as the dependent variable. Using single and multivariate testing is useful for comparison but using multivariate tests is key to analysing data from nests, as they are biological complex with multiple variables influencing the ectoparasite load.

All the nestbox types were found to be statistically significant different, with deep nestboxes having the highest abundance of both adult Hen Fleas and Blowfly Pupae. Species which used the deep nestboxes, were found to have the highest EMM for abundance of adult Hen Fleas and Blowfly pupae compared to when in standard old or new nestboxes. Deep nestboxes having a significantly higher EMM for adult Hen Fleas and Blowfly pupae indicates that the increase nest material in deep nestboxes increases ectoparasite load. This links with what was previously mentioned that birds which have an increased weight of nest material, could be providing more habitat for ectoparasites (1.3.9.2). Rendell and Verbeek, (1996) previously found that there was a significantly positive correlation between the number of fleas per nest and the total volume of nest material. Other studies have suggested that the increased ectoparasite load could decrease host fitness and reproductive success (Tomás *et al.*, 2007). If breeding success is significantly lower in deep nestboxes, compared to the other nestbox types, then it could be linked to the significantly increased ectoparasite load. It would also mean deep nestboxes would be a less viable option for creating the optimum nesting site.

The difference between standard old and standard new nestboxes boxes showed that standard old boxes have significantly higher number of both ectoparasites. This is with the exception of when a Blue Tit is in the nestbox and it is the reverse. This could potentially mean that the ectoparasite load within nestboxes increases with the age of the nestbox. Vilka (2003) conducted research to test the importance of nestbox age by analysing nestbox occupancy against nestbox age. Vilka (2003) found a significant correlation between declining occupancy rates and nestbox age. When old nestboxes were replaced with new ones Pied Flycatcher broods increased



the following year. However, Pied Flycatcher clutch size increased in older nestboxes. All avian study species were seen to have a preference to standard old nestboxes in this study. If this preference continued the antipredator proof nestboxes would be used less compared to the older nestboxes. Old nest material has been previously mentioned to have a negative effect on Blue Tits (3.1). Other studies have looked into the impact nest re-use can have including, one by López-Arrabé *et al.*, (2012) looking at if re-use of nest material had a negative effect on Pied Flycatcher. Blowfly and Hen Flea abundances were found to be significantly higher in old nests than in new nests.

Leaves as a covariate in both GLMs were found to have a significant influence on the ectoparasites and were found to have a negative correlation with both ectoparasite (table. 7 and 8). Leaves could be being used by the study species to reduce the ectoparasite load in nests. Roots and bark were also found to have a negative correlation with Blowfly pupae (table. 8). Factors effecting choice of nest material and how nest materials have different properties has previously been mentioned (1.3.6). It was also previously mentioned that fresh plant matter has been suggested reduce the ectoparasite load in nest (Mennerat *et al.*, 2009). This is similar to the nest protection hypothesis. This posits that birds place in the nest green material, rich in volatile compounds to reduce the abundance of parasites and pathogens (Dubiec *et al.*, 2013). This works on the principle that the metabolites from plants that are known for their antimicrobial properties and effects on herbivorous insects, may also have in effect on avian parasites. Tomas *et al.*, (2012) tested this hypothesis by introducing French Lavender (*Lavandula stoechas*) and cotton lavender (*Santolina chamaecyparissus*) into Blue Tit nests in Valsaín, Spain. The nest of yearling females with the two plants added were found to have a significant decrease the abundance of ectoparasites in their nests. However, it was noted that older females could be using other methods of parasite removal which are more effective for nests with higher ectoparasite loads (Tomas *et al.*, 2012). A study on the nest protection hypothesis by Scott-Baumann and Morgan (2015), which looked to review the hypothesis by looking at multiple studies on the hypothesis, including Tomas *et al.*, (2012) study. Scott-Baumann and Morgan (2015) concluded that bird behaviour of seeking out and incorporating fresh aromatic herbs for a

beneficial value such as reducing parasite load, could show a self-medication strategy carried out by birds if more evidence could be produced.

### 3.6 Conclusion and next steps

Multiple findings can be taken from the information gathered in chapter 3. When testing nest data multivariate test are needed as they have several factors influencing results. Nuthatches and Pied Flycatchers have the highest Hen Flea and Blowfly pupae abundance, respectively, when compared to the other study species. Deep nestboxes have the highest abundance of both ectoparasites, compared to the other nestbox types. Standard old nestboxes have a higher abundance of both the ectoparasite species, used in this study, compare to standard new nestboxes. Nestbox age could play a factor in ectoparasite load. Leaves when in the nests are found to be a significant covariate and are negatively correlating with the two ectoparasite species.

The next steps which will be carried out in chapter 4 will be to analyse the breeding success rate is influenced by ectoparasite load and nest parameters. Comparison with the findings of ectoparasite load will then show if any of the findings in chapter 3 cause a difference in the breeding success of the study species, while in the different nestbox types.

## Chapter 4

# Do ectoparasites and nest parameters influence the breeding success of secondary nesting passerines at Nagshead?

### *Summary*

*In this chapter the influence ectoparasite load and nest parameters influence breeding success in the avian study species is explored. Nesting data collect by the Nestbox Species Surveyors is used to calculate the breeding success rate of each nests. GLM is used to analyse if there is any difference in the breeding success rate between the different nestbox types and avian study species. Any significant differences in breeding success between nestbox type and avian study species are identified. Any nest material influencing breeding success is identified and discussed. Comparison of results with those found in chapter 3 determine if there is any link between ectoparasite abundance and breeding success rate.*

### 4.1 Introduction

Chapter 3 found significant differences in the ectoparasite load between some of the avian study species and all of the nestbox types. This chapter will focus on finding if the breeding success differs between the avian study species and nestbox types. Comparisons will then be made to see if any differences in ectoparasite load links to differences in breeding success rate. An increased breeding success rate is the optimum outcome for birds when reproducing as it increases the chance of the parental genes continuing in a population.

Previous studies have looked into how ectoparasite load alters the fledgling success. One study by Richner *et al.*, (1993), in Switzerland, looked at the effect that Hen Fleas have on reproduction in Great Tits. The fledgling success in infected nests was found to be 30% lower than that in parasite-free nests. The body mass of the chicks was also measured, 14 and 17 days after hatching, and was found into be significantly lower than in infected nests. Richner *et al.*, (1993) study supports that an

increase in ectoparasite load reduces the fledgling success and body mass of hosts for one of the avian study species involved in this study. Deep nestboxes have previously found to have an increased ectoparasite load compared to the other types, which indicates a potential for a reduced breeding success.

Some studies have gone into detail on how ectoparasites could reduce fitness in cavity nesting species. López-Arrabé *et al.*, (1996) theorised that ectoparasites expose cavity-nesting birds to an oxidative challenge which could long term compromise individual fitness. Using heating treatment, they reduced the ectoparasitic load of a sample of Pied Flycatcher (*Ficedula hypoleuca*) nests. Markers in plasma for total antioxidant capacity (TAS), red blood cells for total levels of glutathione (tGSH) and plasma lipids for oxidative damage were used to assess if there was a difference. TAS and tGSH were higher in the heat-treated nests, while a negative correlation was found between TAS and MDA in nestlings, supporting López-Arrabé *et al.*, (1996) theory.

Research in how the certain nestboxes cause different microclimates which could be more or less favourable to nestlings has also been conducted. García-Navas *et al.*, (2008) conducted a study on whether Tree Sparrows (*Passer montanus*) are affected by the presence of old nests when using a nestbox and their reproductive parameters. The study occurred between September 2006 and August 2007 in Toledo, central Spain. Fifty pairs of nestboxes found in gardens, suburbs zones and undisturbed parts of forest next to the river Tagus were used. Tree sparrows showed a preference to woodcrete nestboxes compared to wooden nestboxes during breeding season. It was stated that woodcrete boxes provide more insulation to nesting birds and created a higher internal temperature therefore a more favourable microclimate. However, Bueno-Enciso *et al.*, (2016) study on the effect of nestbox type on the breeding performance of Blue and Great Tits did not support woodcrete boxes cause a more favourable microclimate. This study was conducted in three forests near San Pablo de los Montes, central Spain over three years (2011-2013), using 180 wooden nestboxes and 60 woodcrete nestboxes. It was found in woodcrete nestboxes maximum and minimum temperature were significantly higher and lower than in standard wooden boxes. This could cause a more variable microclimate with rapid fluctuation in temperature within woodcrete nestboxes. Areas with a more stable and favourable microclimate is preferable in secondary cavity-

nesting passerines as it allows for temperature of eggs or nestlings to be maintained (Maziarz and Wesółowski, 2013).

Grooming nestlings and nest maintenance are behaviours carried out by adult birds to remove nest material and ectoparasites. Tripet, F *et al.*, (2002), in Switzerland, looked at Blue Tit behavioural responses to Hen Flea load. An increase in grooming and a significant increase in sanitation bouts by females was found in nests with an increased density of Hen Fleas. High Hen Flea density also reduced the nestling weight in early nestling periods. However, this was compensated by females increasing feeding efforts. Similar research has also been conducted on the behavioural responses by Pied Flycatchers to ectoparasite load (Cantarero, A *et al.*, 2013b). It was found adults in nests which had received heat treatment reduced the frequency and intensity of grooming and nest sanitation behaviours. Nestlings in nests with reduced parasites were significantly larger, with wing length and tarsus length increased, as well as an increased fledgling success. The treatment did not influence incubation or provisioning rates (Cantarero, A *et al.*, 2013b). These studies provide evidence behaviours carried out by Great Tit and Pied Flycatchers are influenced by the ectoparasite abundance and nests sanitation and grooming behaviours will increase as ectoparasite load increases.

Another method which adult birds carry out to potential reduce ectoparasite load is the use of fresh plant matter in nests (Mennerat *et al.*, 2009). This was further expanded on by Dubiec *et al.*, (2013), Tomas *et al.*, (2012) and Scott-Baumann and Morgan (2015) (3.5). More recent studies have looked into whether fresh, aromatic plants in nests influences the physiological conditions of nestlings. Gładalski *et al.*, (2020) experimentally added fresh, aromatic plant fragments into a random sample of Blue Tit nests in Poland. It was found that in the supplemented nests, nestlings had increased levels of haemoglobin when compared to control nests. This may provide evidence that fresh aromatic plants added by adults improve nestling body conditions and their overall fitness. Gładalski *et al.*, (2020) concluded that more research on properly sensitive physiological indicators is needed to confirm if this is the case. Leaves, when entered as covariates were, found to be significantly influencing and negatively correlating with abundance of both ectoparasites. This could indicate that leaves are being used as fresh plant material to reduce

ectoparasite load by the avian study species. Analysing if leaves link to an increased breeding success rate will add more evidence to this.

Behaviours such as grooming, nest maintenance, and adding fresh plant material show that adults are actively trying to improve conditions for the nestlings. These behaviours bare an energy cost which could be used for other behaviours such as provisioning which would increase survival rate of nestling. By finding and eliminating any nestbox types that link to a reduced breeding success rate, the optimal artificial nesting site can be created. If the breeding success rate is significantly reduced in the deep nestboxes, there could be a link with significantly increased ectoparasite load found in chapter 3.

This study will compare the results from chapter 3 on ectoparasite load with the breeding success rate calculated using the data collected by the nestbox checkers in 2019, in order to find if a difference in breeding success rate occurs between the nestbox types and/or species.

#### 4.2 Study site

All nests were retrieved from Nagshead Nature Reserve (Gloucestershire, UK), near Parkend in the Forest of Dean. Management at Nagshead as well as the overall fauna and flora present had been discussed in the previously (2.1:2.3.1-2). A total of 78 nests was used in this study. This included 24 Blue Tit nests, 26 Great Tit nest, 12 Nuthatch nest and 16 Pied flycatcher nests. There were 61 nests form standard old boxes, 11 from standard new boxes and 6 from deep boxes. When selecting nests for use all nests from deep and standard new were selected regardless of species and all Pied Flycatcher and Nuthatch nests were selected due to low sample sizes. Table 4 (3.2) shows the number of nestboxes used in this study from each of the study species and the different nestbox types.

All nests were taken from nestboxes after the breeding season of 2019 by Nestbox Species Surveyors throughout August 2019. Nests were checked for eggs and dead chicks before removing and put into a ziplock bag with minimal air left in. They were then taken to the University of Gloucestershire where they were frozen at -18°C as soon as possible.

#### 4.3 Methods

##### 4.3.1 Breeding success rate.

To calculate the breeding success rate of the secondary cavity nesting birds nesting in 2019, data collected by Nestbox Species Surveyors (2.3.2) was analysed. Organised by the RSPB nestbox checking was carried out from the end of April to the end of August depending on the number of broods. Nestboxes were checked once a week to initially determine if a nestbox was being used. Once nesting was established monitoring continued until fledging or failure of nestlings. Two volunteers were responsible for one route and survey the nestboxes once a week. One person used a ladder, ensuring it is stable first, to access the nestbox keeping disturbance to a minimum. The second person then recorded species, number of eggs/nestlings, nestling feather development, incubation by adults and success of the nest were recorded during the bird breeding season. Once the breeding season was over the nests were removed to prevent an increase of ectoparasites in future nests. To make sure that all nests could be compared fairly, as different nests have different numbers of nestlings, the percentage breeding success rate was calculated. This involved dividing the number of successful fledged nestlings by the total number of eggs laid and multiplying by 100.

##### 4.3.2 Data analysis

Analysis of the breeding success rate within the nests of the study species (2.2) and the different box types (1.3.9.1-3:2.3), consisted initially of descriptive stats comparing mean breeding success. This allowed for a basic comparison of the breeding success rate, giving an indication of which species and box type had a high success rate of eggs to fledglings. However, to discover if a significant difference was occurring in the breeding success, within the nests of the different box types, Generalized Linear Models were conducted on the data.

With multiple factors potentially influencing the breeding success, a multivariate test was needed to analyse the data. A GLM was conducted on the breeding success. A Poisson distribution loglinear model was used as the dependent variables were count data, making it non-parametric data

(Burnham and Anderson, 2004). Bird species and box type were added as factors (i.e. discrete category variables) and nest parameters (e.g. nest weight) were added as covariates (i.e. continuous variables). An interaction between bird species and box type was also included in the model. This showed if box type was having an impact on bird species interaction with breeding success.

EMMs were not used in this chapter as they were not appropriate to use with percentage data. The EMM when calculated went above 100% which was not possible as you cannot have over 100% of the eggs fledging. For this chapter the difference between species and box type was included but the EMMs were not.

To avoid overparameterization of the model by entering too many factors and covariates, the Akaike's Information Criterion (AIC) score was used to compare models with the same dependent variable (Burnham and Anderson, 2004). The lower the AIC score the better the model. AIC value weighs up two different aspects, the model fit and offsetting the model against the number of variables (Burnham and Anderson, 2004). This study was not overparameterized as the variables were not entered to find if a significant difference but instead to get the lowest AIC score.

## 4.4 Results

### 4.4.1 Baseline findings: Parasites

The differences in the ectoparasite load between the different species and box types have previously been found (3.4.3) and the influence of these factors on breeding success analysed. The influence of the abundance of adult Hen Fleas and Blowfly pupae on breeding success has been considered to understand if there is a simple, direct effect of the ectoparasites regardless of bird species, box type, nest weight, or nest materials. Spearman's correlation coefficient tests were conducted to determine this.

There was no significant correlation between abundance of adult Hen Fleas and breeding success (Spearman's rank-order correlation:  $r_s = 0.136$ ,  $N = 78$ ,  $P = 0.236$ ).

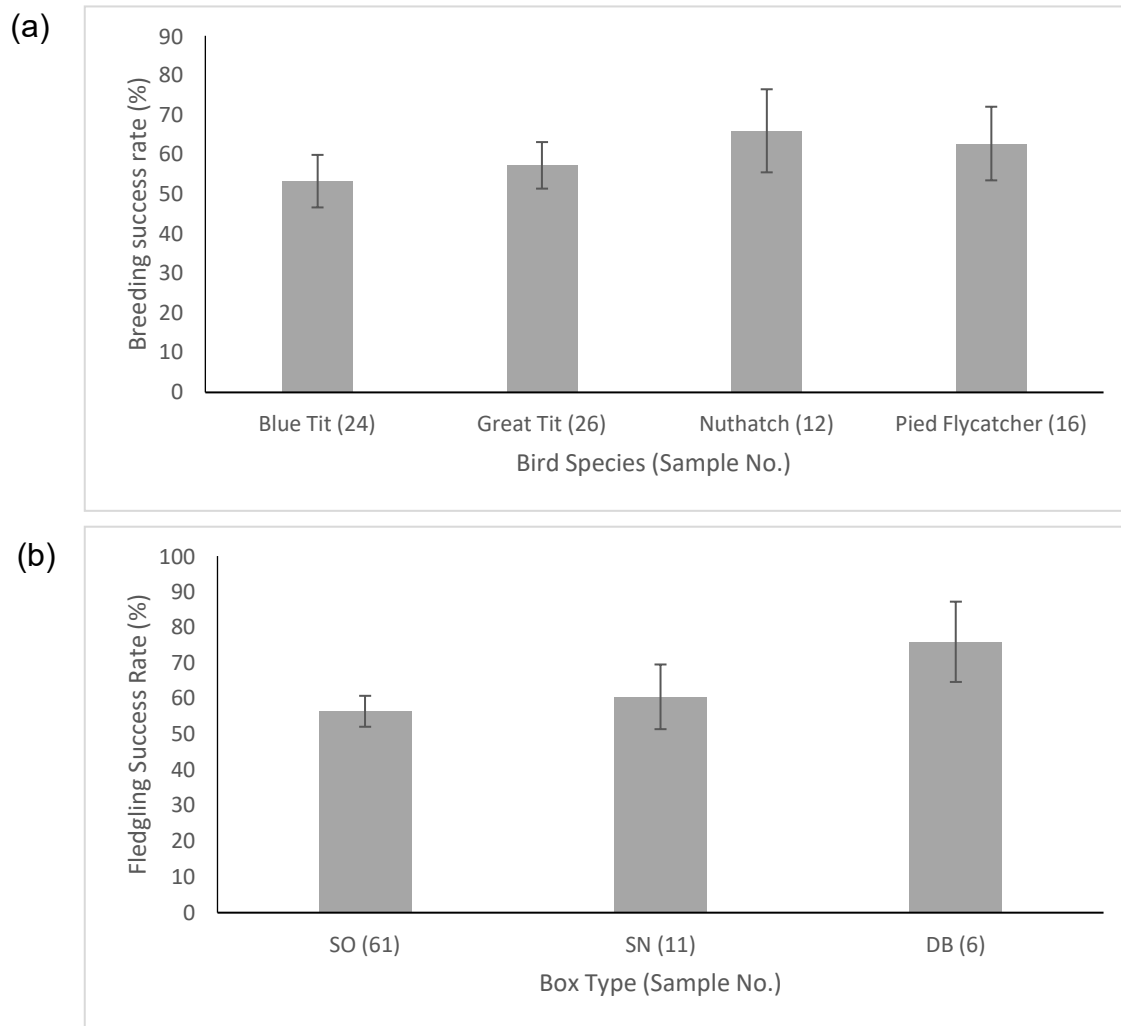


There was no significant correlation between abundance of Blowfly pupae and breeding success (Spearman's rank-order correlation:  $r_s = 0.129$ ,  $N = 78$ ,  $P = 0.261$ ).

#### 4.4.2 Baseline findings: Breeding success

Visualising the data in more detail, Figure 18 (a-b) shows the mean breeding success rate within the nests of the study species and box types. Nuthatch was found to have the highest breeding success, while Blue Tit was the lowest. Deep nestboxes were found to have the highest mean breeding success rate, while standard old boxes had the lowest.

Nuthatch and deep nestboxes were both found to have a significantly higher abundance of one or both of the ectoparasites species. A multivariate test was needed to see if these differences still occur and were significant.



**Figure 18.** (a) Mean breeding success rate within the nests of the study species and box types. (b) Mean breeding success rate within the nests of the nestbox types placed at Nagshead. Standard error bars on both graphs.

#### 4.4.3 Generalized Linear Model (GLM)

A list of the independent factors which were considered for the GLMs can be seen in table 6 (3.4.4). Some nest materials were not added as candidate variables as frequency of presence, and abundance when present, were low. Multiple models for breeding success data were created using these factors, to create the optimum model. Previously mentioned AIC was used to determine the optimum model and prevent selection bias (4.3.2).

##### 4.4.3.1 Breeding success

A generalized linear model was conducted on the breeding success rate with bird species and box type as factors. This showed if the differences

found in figure 15 (a-b) occur if the differences were significant, in a multivariate model. A significant difference was found to be occurring within the model (AIC=1002.788, df=15, n=78,  $P < 0.001$ ). Nest weight, *Brachythecium rutabulum*, *Pseudoscleropodium purum*, *Rhytidiadelphus squarrosus*, compact soil, feather and leaves were entered as covariates in the model and were found to be significant. Table 9 shows the factors, covariates and interactions included in the GLM, if they were significant and whether they have a positive or negative correlation with breeding success.

**Table 9.** Factors, covariates and interactions included in the optimum Generalized Linear Model with breeding success rate .

	P	Correlation (+/-)
<b>Factors:</b>		
Bird Species	<0.001	N/A
Box Type	<0.001	N/A
<b>Interactions</b>		
Bird Species*Box Type	<0.001	N/A
<b>Covariates:</b>		
Nest weight	<0.001	+
<i>Brachythecium rutabulum</i>	<0.001	-
<i>Pseudoscleropodium purum</i>	<0.001	-
<i>Rhytidiadelphus squarrosus</i>	<0.001	-
Compact Soil	<0.001	-
Feathers	<0.001	+
Leaves	<0.001	+

Great Tits had the highest breeding success in the model while Nuthatch have the lowest. This is different to what was seen in Figure 18 (a) which saw Nuthatch have the highest mean breeding success rate. Statistically significant differences in the breeding success rate were found between the study species. Great Tit had a significantly higher breeding success rate than all the other species ( $P < 0.001$  in all cases); Blue Tit breeding success was significantly higher compared to Nuthatch ( $P < 0.001$ );

Nuthatch breeding success was significantly lower than Pied Flycatcher ( $P < 0.001$ ) and there was no statistically significant difference between Blue Tit and Pied Flycatcher ( $P = 0.975$ ).

When comparing the breeding success rates within the different nestbox types it was found that standard old had the highest, and standard new had the lowest. This is different to what was found in figure 18 (b).

Breeding success in standard new nestboxes was found to be significantly lower than standard old and deep nestboxes ( $P < 0.001$  and  $0.047$  respectively). There was no significant difference in the breeding success rate between standard old and deep nestboxes ( $P = 0.877$ ).

#### 4.5 Discussion

From the GLM with breeding success as the dependent variable it can be seen that there are significant differences between all but one pairing of avian species, Blue Tit and Pied flycatcher. Standard new nestboxes were also found to have a significantly lower breeding success rate compared to standard old and deep, with no significant difference occurring between the latter two nestbox types. These findings differed from the what was found in figure 18(a-b), providing more evidence that multivariate models should be performed on data regarding nests and nestboxes as there are multiple variable influence breeding success.

Nuthatch breeding success rate was found to be significantly lower than all the other species. Figure 14(a) and 15 showed that Nuthatches have a higher abundance of adult Hen Fleas than the other species. This shows a potential link to a large adult Hen Flea load causing a reduction in the breeding success in Nuthatches relative to other species. Nuthatches are territorial and carry out other behaviours such as using mud in nest building by narrowing the entrance of cavities (Cantarero *et al.*, 2015). Female Nuthatches have also been found to spend more time outside of nestboxes, and have a lower frequency of nest sanitation behaviours compared to female Blue Tits (Cantarero *et al.*, 2013a). Territorial behaviour could be causing a reduction in the amount of time Nuthatches spend on sanitation behaviours. This could cause an increase in the adult Hen Fleas abundance found in this study.

Future studies on the differences in territorial behaviour between avian study species, at Nagshead would be needed to see if these factors link.

Leaves have previously been noted to be having a significant influence on the abundance of adult Hen Fleas and Blowfly pupae (3.4.4.1;3.4.4.2). Table 7 and 8 also show that there is a negative correlation between leaves and the two-parasite species. Table 9 shows that leaves were found to be significant with the GLM for breeding success and a positive correlation was occurring. This adds further evidence that leaves are being used as method of reducing ectoparasite load with fresh plant matter (1.3.6;3.5;4.1).

From the results in the GLM (4.4.3.1) deep nestboxes have no significant difference in breeding success compared to standard old and have a significantly higher breeding success than standard new nestboxes. The increased ectoparasite load in deep nestboxes is not causing a reduction in breeding success rate. Previous research at Nagshead by Goodenough *et al.*, (2007b) looked at the ectoparasite load in Great Tit nests and how this influenced offspring quality. It was found that in 40% of nests, adult *C. gallinae*, larval *C. gallinae*, larval *Protocalliphora* spp., and biting lice of the suborder Ischnocera all occurred. Further analysis found that the occurrence of ectoparasites did not related to offspring quality in the Great Tit nests surveyed. The results from this study and Goodenought *et al.*, (2007b) show that an increased ectoparasite load does not always cause a reduced level of fitness or breeding success rate.

Goodenough *et al.*, (2008) also observed other factors which may be influencing the fledgling success rate of secondary-cavity-nesting passerines that use nestboxes at Nagshead. Research on whether nestbox orientation had an influence upon nestbox selection or breeding success in Great Tit, Blue Tit and Pied Flycatcher. This study used the breeding data, collected by volunteers at Nagshead, from 1990-2004 and found that nestbox orientation did influence the number of Pied Flycatcher young to fledge. North-northeast (0–29°) facing nestboxes were found to have the highest mean number of young to fledge, while southwest (210–239°) facing nestboxes were found to have the lowest. Understanding what factors do influence the fledgling success rate in nestboxes, be it the type of nestbox type or nestbox orientation, is key to ensuring that the optimum artificial nesting site is created.

Breeding success rate in different nestbox types has also been previously mentioned in when discussing the woodcrete nestbox type (2.3.2). This type was not tested in this study due to a low sample size as too few of the study species chose to nest in this box type. Bueno-Enciso *et al.*, (2016) found that breeding success in Blue and Great Tits was significantly worse in woodcrete nestboxes and wing length of fledglings was also found to be shorter in both species. Bueno-Enciso *et al.*, (2016) suggested this was due to the warmer, hermetic and crowded conditions within woodcrete nestboxes. Further study into the woodcrete nestboxes at Nagshead is needed to discover if results replicate what Bueno-Enciso *et al.*, (2016) found.

#### 4.6 Conclusion

The results indicate that if a standard new nestbox is used by avian species there will be a reduction in breeding success compared to the other nestbox types. Deep nestboxes were not found to have a significantly decreased breeding success compared to the other nestboxes, although having a significantly higher abundance of both ectoparasite species. Nuthatch breeding success is significantly lower compared to the other avian study species, which could link to the significantly higher abundance in adult Hen Fleas found in Nuthatch nests. Leaves as a covariate was significantly influencing the dependent factors in the three GLMs, as well as negatively correlating with the both ectoparasites and positively correlating with breeding success. Indicating leaves are used by the avian study species as fresh plant matter to reduce ectoparasite load.

Chapter 5 will conclude on the findings that can be made using the informatin from both the chapters. It will also make recommendations for potential future studies that will add more knowledge on how to create the optimum artificial nesting site for areas needing to increase the amount of nesting sites.

## Chapter 5

### Conclusions and Recommendations

#### *Summary*

*This chapter summaries the findings found throughout the thesis. Key findings from chapter 3 and 4 are outlined and discussed in further detail. Improvements and recommendations for this study are outlined. Potential future studies on this topic, at Nagshead, are discussed.*

#### 5.1 Conclusions

From the result found in chapters 3 and 4 there are a number of conclusions which can be made about how nestbox type and other nest parameters are influencing the ectoparasite load and breeding success of secondary-cavity-nesting passerines at Nagshead, Forest of Dean. Statistically significant differences can be seen to be occurring in the abundance of both ectoparasite species surveyed between bird species and box type. Deep nestboxes were found to be having a significantly higher abundance of both parasites compared to the other nestbox types. This increase in ectoparasite load did not cause a reduction in the breeding success rate of deep nestboxes. Deep nestboxes did not have a significantly lower breeding success compared to standard old nestboxes however had a significantly higher breeding success compared to standard new.

This study found that nest weight was significant in both the GLM with ectoparasite abundance as the dependent variable and had a positive correlation with abundance of both ectoparasite species. Other studies have found positive correlations between the volume of nest material and the number of Hen Fleas and Fowl Mite (Kaliński *et al.*, 2014; Rendell and Verbeek, 1996). An increased volume of nest material in deep nestboxes may not be causing a reduced breeding success, due to ectoparasites, but the antipredator properties may be being negated. Kaliński *et al.*, (2014) study found that in Blue and Great Tits built significantly taller nests in deep nestboxes and when the nests were over 6cm they were more likely to be predated by Pine Martens (1.3.9.2). Kaliński *et al.*, (2014) goes on to state that the female Blue and Great Tits

have to adjust the nest size due to a number of opposing factors. The first factor being the risk predation by Pine Martens or any other predator species such as the Great Spotted Woodpecker (*Dendrocopos major*). The opposing factors were maintaining a proper level of comfort, humidity, sanitary and thermal conditions. This all improved in taller nests and are need to be maintained for a successful brood. It can only be found out if the individuals which use nestboxes at Nagshead will adjust nest size due to Pine Marten predation, once predation by Pine Martens occurs. This is reliant of Pine Martens to move to Nagshead in the future which is uncertain. These finding do show that there is a higher ectoparasite abundance in deep nestboxes and increases knowledge for futures areas creating the optimum nesting site, for secondary-cavity nesting passerines.

Leaves have been previously noted as potentially being used to reduce the ectoparasite load (3.5;4.5). GLMs for adult Hen Fleas and Blowfly pupae (3.4.4.1;3.4.4.2) found that leaves have a significant influence on the abundance of both ectoparasite species, while Spearman's correlation coefficients found negative correlations between leaf abundance and the abundance of both ectoparasites. A positive correlation was found between leaf abundance and breeding success. Leaves were found to be significantly influencing breeding success (4.4.3.1). The use of fresh plant matter, along with the nest protection hypothesis has previously been discussed (1.3.5 ;3.5;4.1). A study by Tomas *et al.*, (2012) was also mentioned which found a significant decrease the abundance of ectoparasites in their nests of yearling females which had two plants added. It was also noted that roots and bark were found to have a negative correlation with Blowfly pupae and were found to be significant covariates in the GLM. However, it has been previously stated that bark is not related to reducing ectoparasite load in Nuthatch nests by Cantarero *et al.*, (2014). An observation was made while going through nests, Blowfly pupae were more secure in nest with large amount of moss, potentially making it harder to remove from the nest by the adult. This suggest that nests with an increased abundance of leaves, bark and roots make removal of Blowfly pupae easier for adult birds than nests with an increased abundance of moss, and leaves are also being used as fresh plant matter to decrease overall ectoparasites load.

Nuthatch were found to have a significantly higher abundance of adult Hen Fleas than the other bird species. They were also then found to have lowest breeding



success rate of study species. It was previously mentioned (2.2.3) that there are less Nuthatch breeding pairs in an area, than the number that the area could support (Bellamy *et al.*, 1998). Nuthatches are territorial in pairs all year round and studies have found that nonterritorial juvenile Nuthatches either disappear from an area or become territorial (Matthysen, 1989). It has also previously been mentioned (4.5) that Nuthatches use mud in nest building to narrow the entrance of cavities (Cantarero *et al.*, 2015) and spend more time outside of nestboxes (Cantarero *et al.*, 2013a). These behaviours along with female Nuthatches having a lower frequency of nest sanitation behaviours compared to Blue Tits (Cantarero *et al.*, 2013a), could be reducing the amount of time and energy spent removing Hen Fleas from within the nest and nestlings.

This study achieves its overall aim to develop knowledge which can then be used to improve breeding conditions for declining species in sites, in the UK and the world. It informs the reader on the different ectoparasite load and breeding success rate, for secondary-cavity nesting species in different nestbox types. This allows informed decisions to be made on what type of nestbox to use when creating artificial nesting sites, for secondary-cavity nesting passerines. This will then help in providing nesting sites and reducing the decline of woodland birds, which has been caused by habitat loss and management practices.

## 5.2 Recommendations

From the result of this study there are a number of recommendations for future studies and management of Nagshead:

- Repeat this study so that nestboxes can become more established and data over a number of years can be collated and compared.
- Carry out a study on the provisioning rates in the different nestbox types.
- Conduct a study looking into the amount of time the different study species carry out territorial, nest maintenance and grooming behaviours. This help understand what is causing the different in ectoparasite load between species.

- Track the movement of Pine martens in the Forest of Dean, and looking into nestbox predation if a population is established at Nagshead.
- Keep all new nestboxes currently at Nagshead, as they provide more nesting sites.

I would first recommend that this study should be repeated over multiple years to see if the results are replicated. With new antipredator nestboxes being within the habitat for a short period of time before the breeding season, it is likely that many individuals of the different study species saw these as foreign objects. They would then be less likely to use them and opt for the standard old nestboxes which would have been used by the same individuals throughout the previous breeding seasons. This meant that this research was restricted by sample size as 61 nests out of the 78 nests sampled were from standard old nestboxes. In the future, the sample size of the antipredator nestboxes would hopefully increase, as the birds which use Nagshead to breed will become used to them and see them as a potential nesting site. Although with age the standard new nestboxes may become more similar to the standard old nestboxes, the erection date is known so studies comparing nestbox age can still be made. Repeating this study over a number of years would also allow for spatial and temporal effects on parasite load to be studied. These could be things like difference in parasite load on the edge of the woodland compared to the centre for the former and is parasite load a significant factor in poor breeding years for the latter. This study set a base which future studies can build on and compared to, as well as providing insight to what is occurring with these complex ecosystems.

The antipredator nestboxes surveyed in this study are shown to be having no statistically significant influence on the breeding success rate. The sample size of woodcrete nestboxes was too small to analyse with the other nestbox types, so it is not known if this box type will also have no influence on the breeding success. Future studies at Nagshead should be conducted as the sample size of woodcrete nestboxes should also increase. Allowing for this nestbox type to be analysed alongside the other nestboxes. This will be important to carry out as studies have contradicting results on woodcrete nestboxes creating a suitable microclimate for nestlings and could influence the ectoparasite load and breeding success (1.3.9.3). Studies similar to this, and one on the microclimate of the different nestboxes types,

should be conducted at Nagshead to fully understand the influence nestbox type has on nesting passerines.

I would also recommend carrying out a study to find out if there is any difference in the provisioning rates between the different nestbox types. Efficient and effective provisioning by adults is an essential behaviour for successful rearing of nestlings. Provisioning is an energy and time-consuming behaviour and any nestbox type which causes a significant increase in the time it takes to enter and feed nestlings could reduce the size of fledglings, as well as reduce survival once fledged (Mariette and Griffith, 2015). This study was originally going to test the provisioning rates within the different nestboxes during the breeding season of 2020. However, due to the COVID-19 pandemic and lockdown occurring during the 2020 breeding season, the study had to change to having a chapter specifically on the fledgling success rate.

Another study of interest would be to compare the amount of time the different study species carry out territorial, nest maintenance and grooming behaviours. This could then help see if the significantly higher abundance of adult Hen Fleas and significantly lower breeding success rate in Nuthatches, links to the amount of time spent carrying out behaviours that reduce ectoparasites (3.5;4.5). Research into if leaves, bark and roots reduce the amount of time needed to remove Blowfly pupae compared to nest made of predominantly moss, as well as if leaves are used a fresh plant material to reduce ectoparasite load, should also be carried out at Nagshead.

With video footage of Pine Marten kits being shared by Forestry England, Pine Martens have now successfully breed in the Forest of Dean as of July 2020. This mean there is a potential in future years of this species moving into Nagshead. If this happens, other methods of protection for secondary-cavity-nesting passerines using nestboxes should be looked into. One method is used by Sorace *et al.*, (2006) is the long-distance relocation of nestboxes. This study involved moving nests which had previously been predated a short (100m) or long (800-300m) away from the point of predation. Nestboxes which were moved a short distance from the original location suffered higher predation pressure than those moved a long distance.

I would recommend that the nestboxes are current kept at Nagshead, as this will allow further study into nestboxes in the area, including the potential future studies

outlined. Having the nestboxes up also allows Nagshead increase its variety of studies on nestboxes and the species that use them. However, I would not recommend erecting standard new nestboxes, except to replace a damaged standard old nestbox, if the significantly reduced breeding success in standard new nestboxes repeats in future research.

It can be seen that there is a large amount of potential for research in this area by the studies carried out on this topic by Goodenough *et al.* This includes the previously mentioned study on whether nestbox orientation influences breed in success. Others studies include the variation offspring quality with cavity orientation in the Great Tit (Goodenough *et al.*, 2008) and the variation in the relationship between lay date and clutch size in three cavity-nesting woodland passerines (Goodenough *et al.*, 2009). With the historic data collected on nestboxes, the variation of nestbox types and multiple secondary-cavity-nesting species at Nagshead, this site has a large amount of potential for increasing the knowledge on how to create the optimum artificial nesting site. This will then help mitigate for the loss of natural nesting sites which have been lost through deforestation and habitat management.

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## Photography credits

All photos in this thesis are my own except for those specifically listed below:

Figure 2a: Broughton, R. (2017) Nest-boxes are no substitute for the crucial role that tree cavities play in maintaining biodiversity, Available from:

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Figure 2b: Stuckey, P. (2012) Nestlings, Available at:

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Figure 2c: Tomkins, S., Moore, F., Holtmann, B., Santos, E.S. and Lara, C.E. (2015) Finestre-use by Dunnocks (*Prunella modularis*) in New Zealand: An uncommon behaviour revealed through a long-term study. *Notornis*, 62, pp.96-98.

Figure 2d: Low, P., Angus, W., Wagner, A., Wilkin, D., Shiels, M., Dockerill, R. and Hochuli, D. (2013) Use of spider silk for nest building by the Regent Honeyeater *Anthochaera phrygia* and the Helmeted Honeyeater *Lichenostomus melanops cassidix*. *Australian Zoologist*, 36(3), pp.349-354.

Figure 2e: Robinson, R.A., Leech, D.I. & Clark, J.A. (2018) The Online Demography Report: Bird ringing and nest recording in Britain & Ireland in 2017, Available from: <http://www.bto.org/ringing-report> (Accessed: 25 September 2020).

Figure 2f: Larson, K.W. and Kundisch, S. (2012) Willow Warbler *Phylloscopus trochilus* nesting in a juniper during a peak lemming year. *Ornis Svecica*, 22(3-4), pp.141-143.

Figure 3: Loaiza-Muñoz, M.A., Valencia-Montoya, W.A. and Londoño, G.A. (2017) Reproductive Biology of the Tawny-Crowned Tunchiornis (*Tunchiornis ochraceiceps*) In Manu National Park, Perú. *The Wilson Journal of Ornithology*, 129(4), pp.813-819.

Figure 5a Nhbs, (2016) Traditional Wooden Bird Nest Box, Available at:

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Figure 5c: Ark Wildlife, (2019) Woodstone Nest Box, Available at: [https://www.arkwildlife.co.uk/Item/Wildlife\\_Habitats~Bird\\_Nest\\_Boxes/NBWS/Woodstone\\_Nest\\_Box.html](https://www.arkwildlife.co.uk/Item/Wildlife_Habitats~Bird_Nest_Boxes/NBWS/Woodstone_Nest_Box.html) (Accessed 15 June 2019).

Figure 5d: GardenBird, (2019) Bird Guardian, Available at: <https://www.gardenbird.co.uk/bird-guardian.html> (Accessed 25 November 2019).

Figure 7: Google Earth (2020) Available at: <http://earth.google.com> (Accessed 21 August 2020).

Figure 8c: Galván, I. (2017) Condition-dependence of pheomelanin-based coloration in nuthatches *Sitta europaea* suggests a detoxifying function: implications for the evolution of juvenile plumage patterns. *Scientific Reports*, 7(1), pp.1-11.

Figure 10: O'Meara, D. (2014) *The DNA Toolbox: Development and optimisation of molecular techniques to identify red squirrels (*Sciurus vulgaris*)* (Doctoral dissertation, Waterford Institute of Technology).