Do Egg Spots Influence Levels of Parental Investment in the European Starling *(Sturnus vulgaris)?*

By
Leah Martine Duggan

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Approved: Dr. Colleen Barber

                 _____
                 (supervisor)

Approved: Dr. Ron Russell

                 _____
                 (reviewer)

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Abstract

Parasites can impose fitness consequences onto their hosts by reducing their reproductive success or offspring survival rates. *Carnus hemapterus* are external parasites that feed on the blood of European Starling (*Sturnus vulgaris*) nestlings and leave spots on starling eggs from their feces. Adult starlings might alter their offspring provisioning behaviours to compensate for fitness costs caused by *Carnus hemapterus* parasitism. I examined whether adult male European Starlings use egg spots as a cue to increase their offspring provisioning rates to offset the fitness costs of parasitism. To do so, 33 clutches were assigned to one of four groups: Control Spotted (n = 7), Control Unspotted (n = 6), Experimental Spotted (spots were added) (n = 10), and Experimental Unspotted (spots were washed off eggs) (n = 10). I predicted that nestlings hatching from clutches that were originally spotted before treatment would have lower condition. I also predicted that nestlings hatching from naturally and artificially spotted clutches would be paternally provisioned more than those from unspotted clutches. Nestling condition was determined using the residuals from regressing body mass vs. tarsus length on Day 11 of the nestling period. There was no significant difference in either nestling condition or paternal provisioning rates detected across all treatments. *Carnus hemapterus* did not impose any fitness costs in this population of European Starlings, and thus condition remained constant across all groups. Because of this high nestling condition, parents did not have to compensate for any harm caused by *Carnus hemapterus* parasitism, and as a result, fed at a similar rate as those in nests with no *Carnus*.

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Introduction

Ectoparasites are free-living parasites found on the outside of their host's body in contrast to endoparasites, which live inside the host's body (Heeb et al. 2000). Fitness costs can be imposed by parasites at all stages of host life by reducing reproductive success and/or survival (Avilés et al. 2009, Hoi et al. 2010). The energetic costs can vary considerably across natural populations if there are varying extents of parasitism; more intense parasite prevalence usually leads to higher costs (Goater and Holmes 1997). Natural selection may then act upon behavioural mechanisms, such as amount of time spent grooming and physiological mechanisms such as varying intensity of immune responses, to improve host fitness (Avilés et al. 2009, Hamilton and Zuk 1982).

Despite these direct fitness costs, there is not much existing research as to the determinants of ectoparasite infection; however, large colony size, larger brood sizes and younger nestling age appear to play important roles (Hoi et al. 2010). One hypothesis proposes that ectoparasites generally have a minor impact on their hosts because of a long term co-evolutionary arms race occurring between them (Alexander 1981). More recent research suggests, however, that certain hematophagous (blood-sucking) ectoparasites can occasionally have greater impacts when they parasitize the rapidly growing nestlings of some altricial avian species (Boulsama et al. 2002, Lehmann 1993). Altricial offspring are those who hatch in a relatively underdeveloped state and require constant care from parents during their nestling phase (Boulsama et al. 2002). Ectoparasites can have greater effects on altricial nestlings because very young nestlings lack protective feathers (Boulsama et al. 2002, Lehmann 1993).

Carnus hemapterus is a blood-sucking ectoparasitic fly that parasitizes nestlings in over 50 species of birds (Grimaldi 1997, Brake 2011). Adult flies are nest-based and lose their wings

after finding a suitable host's nest (Roulin 1998). During incubation, *Carnus hemapterus* feeds

on the brood patch of the parents (Feare 1984).

When parasitizing the European starling (*Sturnus vulgaris)*, it has been suggested that

Carnus hemapterus leave reddish-brown spots on the bright blue eggshells from their feces

(Avilés et al. 2009, Feare 1984, Kessel 1953, López- Rull et al. 2007). Another study confirmed

that spot density increased with the level of the infestation in the Spotless starling (*Sturnus*

unicolor) (Avilés et al. 2009). Yet another study confirmed that these spots are a direct result of

the presence of *Carnus hemapterus* within the nest (López-Rull et al. 2007).

European Starlings are a cavity-nesting passerine bird that exhibit biparental care (Fauth

et al. 1991, Weitzel 1988). They are found throughout Europe, which is their native range, as

well as North America, where they are a prevalent invasive species (Feare 1984, Kessel 1957).

They are facultatively polygynous, but are socially monogamous (Cabe 1993).

Egg laying occurs between March 15th and July 15th, with two clutches of eggs (early and

late) commonly laid (Kessel 1957). Clutch size is typically 4-6 eggs, with smaller numbers

during the later part of the season (Feare 1984, Kessel 1957). European starlings often

intermingle fresh herbs into their dry nesting material (Gwinner et al. 2000). It has been

hypothesized that this is an adaptation use to reduce parasite levels by their volatile compounds

(Gwinner et al. 2000).

Factors Affecting Carnus hemapterus

Carnus hemapterus is the most prevalent hematophagous ectoparasite of European Starling

nestlings (Liker et al. 2001). They parasitize younger nestlings that have less feather

development, as higher feather density impedes their movement (Liker et al. 2001). Various

biotic and abiotic environmental factors such as host population density or moisture levels appear to influence *Carnus hemapterus* abundance within individual nests in a population (Hornsby et al. 2013). It has been found that higher levels of *Carnus hemapterus* present in the nests of the Spotless Starling can lead to overall lower nestling body mass (Avilés et al. 2009). Lower body mass in nestlings often leads to poorer condition, which can then lead to lower rates of survival (Feare 1984).

Changes in parental investment are likely to occur in response to the negative effects of parasitism (Christe et al. 1996). Parents may either provision their offspring more, or at a higher rate to compensate for the negative effects, or feed less to save energy to invest in future, healthier clutches (Christe et al. 1996). A change in parental provisioning rates in response to changes in egg spot concentration would occur if egg spots were used by the parents as a signal of future offspring fitness (López-Rull et al. 2007).

It has been proposed by the parental food compensation hypothesis that parents of parasitized broods will attempt to compensate for the negative effects of the parasitism by increasing their provisioning with high quality food to increase offspring survival (Tripet and Richner 1997). It has also been suggested that parasitized nestlings might beg more, thus stimulating the parents to provision them more frequently (Christe et al. 1996). In some species, a reduction of parental effort might occur due to negative effects of ectoparasitism on the parents (Avilés et al. 2009). An example of these parental costs could be due to more time spent grooming to remove parasites, leaving less time for provisioning offspring (Brown et al. 1995).

Previous studies have found mixed results related to parental provisioning rates of nestlings that were infected with *Carnus hemapterus*. One study has found that high levels of *Carnus hemapterus* in the nest can increase the rate of provisioning by adult males, but not by

females in Spotless Starlings, who did not alter their rate (Avilés et al. 2009). This might occur because males are often the father of offspring at more than one nest, and adjust their rates of feeding depending on their perception of the extent of paternity between these nests (Alivés et al. 2009). Moreno and Osorno (2003) surveyed many studies and found that even levels of blue pigmentation in eggs can affect levels of male parental investment. Hornsby et al. (2013) found no correlation between *Carnus hemapterus* levels and parental provisioning rates. The present study differs from that of Hornsby et al. (2013) in that it experimentally manipulate the levels of egg spots across different clutches.

In this study, I hypothesized that adult European Starlings use egg spots as a cue to increase their provisioning rates to offset the nestling fitness costs of parasitism by *Carnus hemapterus*. I sampled nests with varying levels of natural egg spots and experimentally applied or cleaned egg spots from unspotted and spotted clutches respectively. I then determined the condition of nestlings over two stages of the nestling period and examined paternal provisioning rates. I predicted that nestlings hatching from clutches that were originally spotted before treatment (Control Spotted and Experimental Unspotted) would have significantly lower condition due to being more infested with *Carnus hemapterus*. I also predicted that paternal provisioning rates would be significantly higher in naturally and artificially spotted clutches (Control Spotted and Experimental Spotted) than in clutches without spots.

Methods

Field Experiment

This study was conducted on the campus of Saint Mary's University in Halifax, Nova Scotia, Canada (44° 39' N, 63° 34' W) in the spring and summer of 2018. Nests were checked every two to three days after 11am from April until the end of July 2018 to determine egg-laying, as

well as clutch size, hatching success, and fledging success. Four to six days after egg-laying was complete, each nest box was assigned to one of four treatment groups: 1) Control with naturally occurring spots, indicating *Carnus* infestation 2) Control with no spots, indicating no infestation 3) Experimental treatment with spots removed from spotted eggs (cleaned of spots treatment), and 4) Experimental treatment in which spots were added to the eggs (added-spot treatment), to mimic the effect of *Carnus* infestation.

Nests were randomly assigned on Day 2 of the incubation period into their corresponding treatment by blindly selecting nest box numbers based on eggshell appearance. A total of thirty-three nest boxes, placed approximately 2m or more above the ground housed the clutches that were studied. After each brood had fledged, old nest material was removed from the boxes. Nest boxes from both early and late clutches were sampled together due to a lack of occupied nests during the late brood (early brood n=25 vs. late brood n=8). Clutches containing eggs with no spots were assigned to either the control-no spots group (n=6) or to the experimental added-spots treatment (n=10). Nest boxes containing eggs with natural spots were assigned to either the control-with-spots group (n=7) or the experimental cleaned-egg treatment (n=10). Due to the absence of naturally unspotted eggs in late clutches, I could not assign any nests to the control-no spots group or to the experimental added spots group.

On Day 4 of the incubation period (Day 0 is hatch day), the treatments were applied. All eggs were carefully handled to check for spotting on the day that the treatment was to be applied. For the cleaned-spot treatment, cotton balls were moistened with tap water and rubbed over the eggs to gently remove any spots. For the add-spots treatment, medium-tipped, non-toxic red Faber-Castell ® markers were used to add 60 spots to each egg, approximating the natural spot pattern. These clutches were checked every 1-3 days for the duration of the incubation period.

For the control-without-spots group, if spots developed during incubation, they were moved to the control-spotted group. For the experimental cleaned-egg treatment, clutches were checked every second day for spots over the incubation period. If new spots developed, they were cleaned off using a cotton ball and water.

Nests were checked every one to two days to ensure the eggs retained their respective treatment conditions. If the add-spots treatment eggs developed natural spots, they were cleaned off using the method of the clean spots treatment, leaving only the permanent artificial spots. These treatment checks were performed until one day before the estimated date of hatching.

On days five and eleven after hatching (day zero is hatch day), nestlings were weighed using a Pesola spring scale to the nearest 0.50 grams. Tarsus length was measured using Fowler Sylvac digital calipers to the nearest 0.01mm. Nestling condition was estimated by running a regression of mass against tarsus length and using the residuals to estimate the nestling condition index.

Nestlings were banded on days five and eleven. On day five, nestlings were given one temporary coloured band to distinguish their identity from that of their siblings. On day eleven, the temporary colour band was removed, and a permanent metal Canadian Wildlife Service (CWS) band was placed on the right tarsus. A black plastic band was placed on the nestlings' left tarsus to indicate that they fledged in 2018.

Adult starlings were also banded and measured upon capture in the nest box using a mo-trap when their nestlings were 5-13 days old (Stutchbury and Robertson 1986). Adults were sexed based on beak colouration; males have blue on their lower mandible while females have pink on their lower mandibles (Kessel 1951, Feare 1984). Adults were also sexed using the eye-

ring method; males have no eye-rings while females have light brown eye-rings (Kessel 1951, Feare 1984).

Adults were banded with a Canadian Wildlife Service band on their right tarsus, along with a pink or yellow band on top to distinguish females from males respectively. They were banded with a unique combination of two coloured bands on their left tarsus to distinguish them from other adults. Measurements for weight and tarsus length were also taken in the same manner as with nestlings.

On days seven or eight as well as thirteen or fourteen of the nestling period, parental provisioning watches were conducted on each nest box for one hour starting before 11am, beginning when a parent arrived with food. Using 10 x 42 Celestron ® binoculars, parents were observed to determine provisioning frequency; the observer sat at least 20m away from the nest box. The number of visits as well as the time and duration of occurrence were recorded, as well as the sex of the visiting parent.

Data Analysis

All statistical analysis was performed using GraphPad Prism Software 6.0 (GraphPad Software Inc., La Jolla, CA, USA). Parametric statistics were used to analyze Normally distributed data, and non-parametric statistics were used to analyze non-Normally distributed data. Variables were tested for normality using the D'Agostino & Pearson omnibus Normality test. A nestling condition index was created by running a linear regression of body mass against tarsus length on Day 11 of the nestling period. Results were considered significant when $P \leq 0.05$.

Results

There was no significant difference in brood condition across all treatments on Day 5 of the nestling period (H = 3.194, df = 3, P = 0.36, Fig. 1). Similarly, no significant difference was detected in brood condition across all treatments on Day 11 of the nestling period (H = 4.849, df = 3, P = 0.18, Fig. 2). No significant difference was detected in the proportion of male provisioning visits to offspring across all treatments on Day 7 after hatching (H = 1.652, df = 3, P = 0.65, Fig. 3.) or on Day 13 after hatching (H = 1.065, df = 3, P = 0.79, Fig. 4.).
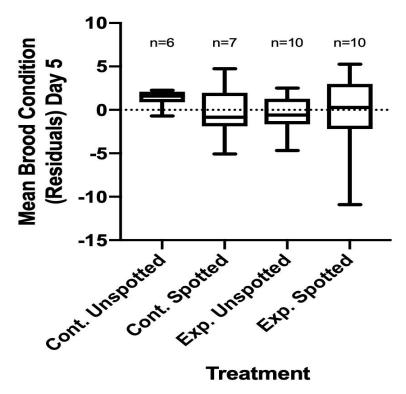

Figure 1. Condition of broods from each of four treatments determined from residuals of nestling body mass vs. tarsus length on Day 5 of the nestling period.
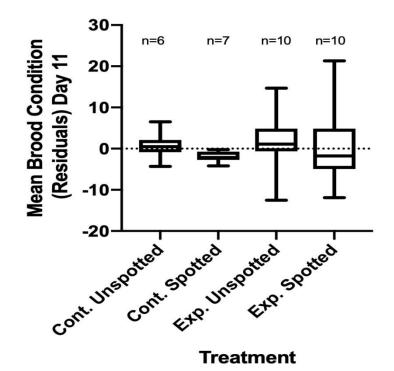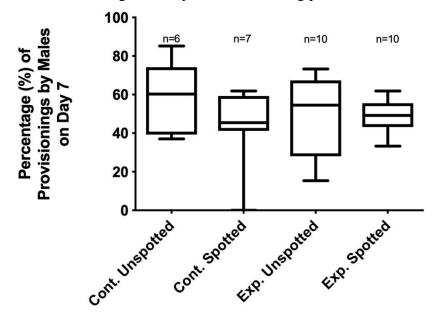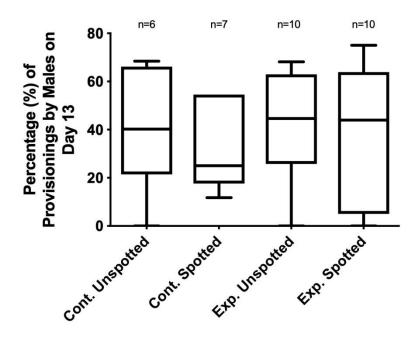
Figure 2. Condition of broods from each of four treatments determined from residuals of nestling body mass vs. tarsus length on Day 11 of the nestling period.

Figure 3. Percentage of male provisioning visits to offspring for each of the four treatments on Day 7 of the nestling period.

Figure 4. Percentage of male provisioning visits to offspring for each of the four treatments on Day 13 of the nestling period.

Discussion

Condition did not vary significantly in response to the presence of egg spots on either Day 5 or

Day 11 of the nestling period and so my prediction of originally spotted clutches being in worse

condition was not supported. The lack of significant difference in condition appears to indicate

that there was little difference in fitness costs imposed by *Carnus hemapterus* in this population

of European Starlings, and therefore parents would not need to alter their provisioning rates.

Parental provisioning rates in this study also did not appear to be related to the presence

of spots on European Starling eggs at either stage of the nestling period (Day 7/8 or 13/14)

(Figures 3 and 4). These findings do not support my prediction that male starlings would

provision spotted clutches more often and are different from findings by Avilés et al. (2009),

who found that male starlings provisioned nestlings from unspotted-egg clutches more

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frequently. Because there was no difference in condition caused by *Carnus* infestation, male European Starlings did not need to increase their provisioning rates to compensate.

Responses to ectoparasitism can be influenced by life history trade-offs and may vary among species and populations (Avilés et al. 2009). Avilés et al. (2009) found both a lowered condition and lower provisioning rates by males in response to increased *Carnus hemapterus* parasitism in the Spotless Starling. Christe et al. (1996) found that Great Tit (*Parus Major*) nestlings infected with the ectoparasitic Hen flea (*Ceratophyllus gallinae*) were in poorer condition and begged more than those who were not. Tripet and Richner (1997) observed that Blue Tit (*Parus Caeruleaus*) nestlings infested with the hen flea did not have significantly lower body condition, but were provisioned more by adults.

Environmental conditions were also likely a factor in *Carnus hemapterus* prevalence within nests. Great Tits (*Parus major*) breeding in areas with high levels of humidity had significantly higher ectoparasite loads (Heeb et al. 2000). The European Pied Flycatcher (*Ficedula hypoleuca*) experienced higher levels of ectoparasite infestation during cold and wet weather (Merino and Potti 1996).

Merino and Potti (1996) also found that reduction in nestling growth and survival caused by ectoparasites varied over the years in their study population. This finding mirrors the phenomenon where Hornsby et al's (2013) study found a difference in condition, with unspotted clutches being in worse condition than spotted clutches, whereas mine did not, even though we used the same study site, but in different years. It is likely that parasite success varies with host reproductive success due to changing environmental conditions. If a host experiences fitness costs due to adverse environmental conditions, then the parasites likely do so as well.

Some studies that report a difference in parental provisioning levels focused on the Great Tit and other members of the family Paridae (Christe et al. 1996, Tripet and Richner 1997). These birds tend to have relatively short lifespans (generally 1-4 years) (Perrins 1980), which limits future chances at reproduction. In comparison to the European Starling, which has a relatively longer lifespan (up to 15 years), it would be beneficial to members of family Paridae to increase their provisioning rates to counteract any possible parasitism. Because of life history trade-offs, members of the family Paridae would need to invest more in their current brood due to limited potential for future reproduction, as opposed to the longer-lived European Starling, that would have more future reproductive potential.

My study's findings were somewhat limited by low sample size. There were far fewer late brood nests than in previous years, which decreased the sample size considerably. Similarly, there was a general lack of naturally unspotted nests which affected the sample size for the control unspotted group.

Future research is needed to determine the environmental effects on the fitness of *Carnus hamapterus*. Such studies could track humidity and temperature over several years in different study sites to determine if any patterns emerge. These studies could reveal insights into how environmental perturbations associated with climate change affect host-parasite relationships.

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