The Effect of Ectoparasite Load on the Condition of Nestling European Starlings (*Sturnus vulgaris*)

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Abstract

Nest-dwelling ectoparasites are commonplace among-cavity nesting birds such as the European starling (*Sturnus vulgaris*), and can be detrimental to the health of nestlings. To determine if this was also the case for *Sturnus vulgaris*, ectoparasite load and evidence of ectoparasites such as tiny blood spots found on eggs were compared to brood condition in 12 nests. Nests were divided into early and late broods to determine if time of breeding played a role in ectoparasite load. Egg spot and nestling data were collected between April and July of 2021 from nest boxes found throughout the Saint Mary's University campus. Nest material from six early brood nests and six late brood nests were taken for analysis once nestlings had fledged and frozen for a week before dissection. Nest material was filtered through a 250 micrometer sieve with water and ectoparasites were identified, documented and removed under a dissecting microscope. There were significantly more ectoparasites found in the later broods than in early broods, possibly due to the colder temperatures in April and early May. The number of egg spots was positively correlated with *Carnus* spp. load, and positively correlated to other ectoparasites as well but this could be due to all ectoparasites increasing in the later broods. While there was a trend towards lower condition with increased ectoparasite load, no significant effect of ectoparasite load on nestling condition was found.

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Introduction

Ectoparasites and Nestling Condition

Hematophagous ectoparasites describe a wide variety of animals, and hematophagy has convergently evolved in many different taxa (Krasnov et al. 2008). Ectoparasites can either be host-specific, feeding on one species or genus of animal, or host-opportunistic, feeding on many different species from the same taxa (Krasnov et al. 2008). Hematophagy has been found to lower the hematocrit levels of the host, which can lead to a lower overall immune response and condition of the host (Benton and Shatrau, 1965; Hoi et al., 2017; Mason, 1944; Saino et al., 1998; Fairn et al., 2014).

Many bird species across the world deal with a multitude of ectoparasites, especially within their nests (Gwinner and Berger, 2005; Hoi et al., 2017; Mason 1944; Merino and Potti, 1996; Pirrello et al., 2015). The number of these ectoparasites (ectoparasite load) can vary within the nest, and can have negative effects on the health of offspring (Gwinner and Berger, 2005; Hoi et al., 2017; Mason 1944; Merino and Potti, 1996; Pirrello et al., 2015).

The number of ectoparasites in a nest plays a role in the condition, development, and mortality of nestlings (Mason, 1944). This has been particularly well documented for cavity-nesting birds, whose altricial nature and long development time, along with ease of study, make for great study species (Martin and Li, 1992). The most common effect of ectoparasites on nestlings is on their hematocrit levels, which decrease with an increase in nest ectoparasite load. This is most likely due to the fact that most nest ectoparasites are hematophagous (Benton and Shatrau, 1965; Hoi et al., 2017; Mason, 1944; Saino et al., 1998; Fairn et al., 2014). One study even found that nestlings of Barn Swallows (*Hirundo rustica*) may have developed a counteradaptation to the lowered hematocrit levels and nestling condition by fledging early, whereby nestlings who experienced higher ectoparasite numbers developed feathers and left the nest sooner than those who had lower ectoparasite loads (Saino et al., 1998).

By contrast, several studies have found that an increase in ectoparasite load did not have an effect on nestling condition. Mazgajski (2007a) performed an experiment to see if the addition of old nest material (in which many nest ectoparasites overwinter and/or lay their eggs) had any effect on ectoparasite abundance and if so, whether ectoparasite abundance in turn had any impact on nestling condition in European Starlings (*Sturnus vulgaris*). While a positive relationship was found between the addition of old nest material and ectoparasite abundance, an increase in ectoparasite abundance had no effect on nestling condition. Similarly, Stephenson et al. (2009) examined if feathers in the nest had an effect on nestling condition and ectoparasite abundance by acting as a barrier to ectoparasites, they tested this by comparing control (untouched) nests to nests that they artificially removed feathers from and nests that they had sprayed insecticide into to lower ectoparasites. They found a positive correlation between feather abundance and nestling health, however, they also found that feather abundance had no impact on ectoparasite abundance and all nests had the same number of ectoparasites, suggesting that their insecticides did not work and their results may be equivocal. Roby et al., (1992) found that in both Eastern Bluebirds (*Sialia sialis*) and Tree Swallows (*Tachycineta bicolor*), blowfly prevalence within nests had no detectable effect on nestling condition or mortality rate. When examining the trophic systems of mites inside European Starling nests, Wolf et al. (2012) found no effect of parasitic mite density on the condition of European Starling nestlings. These studies' cast doubt on the once universal belief that increased ectoparasite loads have negative fitness consequences for nestling birds.

Instead, variation of the effect of ectoparasite load on host condition could be explained by external/abiotic factors (Mazgajski, 2007a; Roby et al., 1992; Stephenson et al., 2009; Wolfs et al., 2012). For example, Veiga and Valera (2020) found that nest location played a significant role in which ectoparasites were present in the nests of the European Roller (*Coracias garrulus*). Carnid flies (*Carnus hemapterus*) and ticks (Fam. *Argasidae*) preferred to occupy nests on cliffs and in nest boxes, whereas mites (*Dermanyssus* spp.), and blackflies (Fam. *Simuliidae*) preferred nests in tree cavities (Veiga and Valera, 2020). In addition to types of ectoparasites present, ectoparasite load itself can be affected by external factors (e.g., the weather). Merino and Potti (1996) found that climate at the nesting site of the European Pied Flycatcher (*Ficedula hypoleuca*) played a role in the abundance of nest ectoparasites. More specifically, ectoparasites such as fleas (*Ceratophyllus* spp.) increased in cold, rainy years and bird blowflies (*Protocalliphora* spp.) increased in the hotter years (Merino and Potti, 1996).

Common Cavity-Nesting Ectoparasites

Cavity nesters such as European Starlings are exposed to a wide range of ectoparasites, even in the northern reaches of Canada (Benton and Shatrau, 1965; Fairn et al., 2014). The most common ectoparasites appear to be the hematophagous bird fleas (*Ceratophyllus* spp.), bird mites (*Dermanyssus* spp.), and the Carnid fly (*Carnus hemapterus*) (Fairn et al., 2014).

Bird Fleas

Bird fleas (*Ceratophyllus* spp.) have been known to be a common ectoparasite of the European Starling, along with other passerine birds, for some time (Benton and Shatrau, 1965; Fairn et al., 2014; Merino and Potti, 1996; Roby et al., 1992). They are hematophagous, breed and lay their eggs in old nest material (Heeb et al., 2000; Mazgajski, 2007a), and prefer more rainy and colder climates than other ectoparasites (Merino and Potti, 1996). A study done by Heeb et al. (2000)

found that bird fleas in the nests of the Great tit (*Parus major*) preferred less humid nests for initial infection. These authors also found that the presence of bird fleas increased humidity levels within the nest box compared to those that had fleas removed; they believe this finding was caused by increased evapotranspiration in either the nestling (due to a higher metabolic rate caused by parasitism), or in fleas, as blood diets are higher in water content (Heeb et al., 2000). The former of the two proposals is supported by their results, which showed a positive correlation between nest humidity and number of nestlings (Heeb et al., 2000). In this same study, bird fleas were also found to have a negative effect on nestling condition in Great Tits (Heeb et al., 2000).

Bird Mites

Bird mites (*Dermanyssus* spp.) are found all over the world and are a frequent inhabitant of the nests of birds (Fairn et al., 2014; Murillo and Mullens, 2017; Rendell and Verbeek, 1996). The mites get there by being directly transported by the parent birds, and most will spend their entire lives on the body of the adult or nestlings (Rendell and Verbeek, 1996). Mites lay their eggs, and occasionally overwinter in the nest material, which the parent bird will remove from the nest in preparation for the next breeding season (Rendell and Verbeek, 1996). Overwintering and newly hatching mites emerge in the spring, and latch onto another bird to begin their cycle (Rendell and Verbeek, 1996). They are also a common pest to the poultry industry (Murillo and Mullens, 2017).

Carnus hemapterus

The final and most common ectoparasite found in nest boxes are the Carnid fly (*Carnus hemapterus*) (Fairn et al., 2014). *Carnus hemapterus* are hematophagous Dipteran flies that feed on both nestlings and parent birds (Amat-Valero et al., 2012; Fairn et al., 2014; Valera et al., 2006). Upon reaching adulthood, they fly into the nests of medium to large birds, drop their wings completely and spend the rest of their life inside the nest feeding on the blood of both the nestlings and parents (Valera et al., 2006). Adult Carnid flies will also mate and lay their eggs in the nest, with the larvae hatching 5 days later (Valera et al., 2006). The larvae feed on organic matter within the nest material for 21 days then pupate, which causes a state of diapause that can vary in length, but usually lasts for a couple months to allow them to emerge the following spring to search for new hosts (Valera et al., 2006). *Carnus hemapterus* undergo long diapauses and bivoltinism (two generations per year) within their life cycles (Amat-Valero et al., 2012). While this may be interpreted as a specific adaptation to multi-brooded bird species, these insects also parasitized single brood species, indicating some form of polymorphism in their diapauses (Amat-Valero et al., 2012).

Vega and Valera (2020) found that *Carnus hemapterus* preferred nests in nest boxes or on cliffs compared to tree cavities, but this mostly due to the close proximity of these nests to one another making it easier for *Carnus* dispersal. *Carnus hemapterus* have been shown to have a negative effect on nestlings (Hoi et al., 2017; Pirello et al., 2015). Hoi et al. (2017) found that increased *Carnus hemapterus* levels were associated with decreased hematocrit levels, decreased immune levels, and decreased nestling condition in European Bee-eaters (*Merops apiaster*). Similarly, Pirrello et al. (2015) found that *Carnus* abundance had a direct negative effect on the timing and duration of the first moult of nestling birds and was also associated with decreased nestling condition.

European Starlings

European Starlings are double-brooded, medium-sized passerine birds found throughout multiple continents. They lay both an early and a later clutch and both parents incubate the eggs and provision the offspring. Given their wide distribution and tendency to live and nest in urban areas, several studies have been conducted on them and their ectoparasites (Avilés et al., 2009; Fairn et al., 2014; Gwinner and Berger, 2005; Pirrello et al., 2015). However, given the conflicting results regarding the fitness consequences associated with ectoparasite loads in other species, it is not clear what the effect, if any, ectoparasites may have on European Starling nestlings in Canada.

Starlings have been found to prefer clean nesting sites and will invest more in broods with less ectoparasites (Gwinner and Berger 2005; Mazgajski, 2007b; Mazgajski, 2003; Mazgajski, 2013). In fact, males of the Spotless Starling (*Sturnus unicolor*) adjust their feeding effort to nestlings that hatched in nests whose eggs had a high number of *Carnus* spots (*Carnus* egg spots are believed to be the feces of Carnid flies parasitizing the parents who are incubating the eggs) (Avilés et al., 2009). European Starlings themselves have shown preference for nest cavities that lack older nest material (Mazgajski, 2007b; Mazgajski, 2003). Male Starlings have also been found to put fresh herbs into their nests (Gwinner and Berger, 2005). While this did not lower ectoparasite load, these herbs had anti-bacterial properties and decreased the negative effects that the ectoparasites had on nestling condition (Gwinner and Berger, 2005).

Egg Blood Spots

The eggs of European Starlings are laid as a solid pale blue colour, but can become laden with blood spots as incubation progresses (Avilés et al., 2009). These spots have been suggested by recent studies to be the feces of Carnid flies which were parasitizing the brood pouch of the parents (Avilés et al., 2009). With egg spots being a sign of *Carnus*, it would seem intuitive that an increase in egg spot numbers would signify an increase in ectoparasite load, specifically that of *Carnus*, and subsequently, a decrease in brood condition. Past studies have found a positive correlation between egg spots and ectoparasite load (Avilés et al., 2009). In contrast, a previous study found that egg spots not only did not correlate with *Carnus* load but also that clutches of spotted eggs had nestlings in better condition than unspotted clutches of eggs in European Starlings (Hornsby et al., 2013), and a more recent thesis which also took place at this same study site found no such correlation (Duggan, 2019).

Study Objective and Predictions

For this study I wanted to use nestling condition data collected from the field and ectoparasite count data to determine if ectoparasite signs such as egg spots correlated with ectoparasite load (*Carnus hemapterus* and then with other ectoparasites). I tested to see if there were differences between early and late broods in: 1) *Carnus* numbers 2) ectoparasite load, and 3) number of egg spots. I also wanted to assess the effect of ectoparasite load on nestling condition in European Starlings. I predicted that an increase in egg spots would indicate an increase in ectoparasite load, as increased *Carnus* numbers should lead to the creation of more blood spots from *Carnus* on the egg shells. I also predicted that ectoparasite load would be greater in the late broods than the early broods, as more parasites should emerge from diapause and breed as the Starling breeding season progresses. Finally, I predicted that a greater ectoparasite load would have a negative effect on nestling condition, which is supported by many studies (Gwinner and Berger, 2005; Hoi et al., 2017; Mason 1944; Merino and Potti, 1996; Pirrello et al., 2015).

Methods

Field Work

Nestling and egg data were collected during the 2021 breeding season, between April and July. Early broods occurred from late April through late May whereas the late broods occurred from June through July. I studied six early broods and six late broods; of these three broods had the same parents for both early and late broods. Nest boxes were located on tree trunks, two to three metres off the ground around the Saint Mary's University campus (Halifax, Nova Scotia). Egg spots were counted on each egg on the 11th day after all eggs were laid, as Starling eggs typically hatch 12 days after the final egg is laid.

Nestlings were temporarily removed from their nest to assess their condition. Parents may abandon the nest if they return to find their nest empty, so we removed only half the nestlings at a time. Nestling mass was measured with a 100g Pesola spring scale to the nearest 0.50 gram and tarsus length was measured with a Fowler 54-115-330 electronic caliper to the nearest hundredth of a millimeter. Nestlings were measured on their fifth and eleventh day after hatching (day 0 is day of first hatch). Coloured leg bands were put on the legs of the nestlings on the fifth day so that they could be individually identified on day eleven. When the nestlings reached 11 days of age, they were given a Canadian Wildlife Service band with a unique number code for future identification. Nest material was collected within 24 hours of all nestlings fledging the nest and were frozen.

Lab Work

The method for counting ectoparasite load of the nests was adapted from Stephenson et al. (2009) and Fairn et al. (2014). Briefly, nest material was placed into large Ziploc bags and placed in a freezer kept at -20° C. Material was kept in the freezer for at least a week to kill all the ectoparasites within the sample. The nest material was weighed for total weight and a quarter of the material was subsampled for ease of analysis. Nest material was filtered through a 250 µm sieve, and the material was kept hydrated throughout the examination process to prevent loss of specimens. Specimens collected were identified, documented and stored in glass vials containing 95% ethanol.

Statistical Analyses

Nestling condition was determined by doing a linear regression of mass against tarsus length for all 11-day old nestlings on the study site, and the residuals were used as an index of condition. Brood condition per nest was calculated by taking the average brood condition of all nestlings in that nest. The average number of egg spots within a nest was correlated with 1) the number of *Carnus hemapterus* found in the nest and 2) nestling condition to determine if any relationship existed. All data were tested for normality using a D'Agostino & Pearson omnibus normality test and all tests were two-tailed. Results were considered significant if $P \leq 0.05$.

Results

Brood and Ectoparasite Load Correlation

There was a significant difference in the number of *Carnus* between early and late brood nests with late nests having more *Carnus* in them (Mann-Whitney U = 1.0, $n_1 = 6$, $n_2 = 6$, P = 0.004, Fig. 1). Similarly, total ectoparasite load, including *Carnus*, was found to be significantly higher in the late than the early brood nests (Mann-Whitney U = 0.0, P = 0.002, Fig. 2). Total ectoparasite counts from the early and late brood nests are documented in Table 1 and percentages of each of the three ectoparasites found were documented in Figure 6.

Table 1. Total number of *Carnus*, fleas, and mites found in early and late brood nest material.

Ectoparasites	Early Broods	Late Broods
Carnus spp.	38	1042
Fleas	23	91
Mites	50	213
Total	111	1346

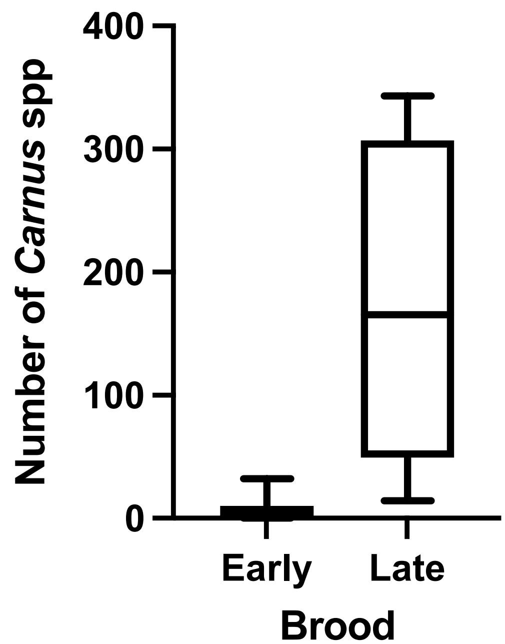

Figure 1. Number of *Carnus spp.* found in early (n=6) and late brood nests (n=6).

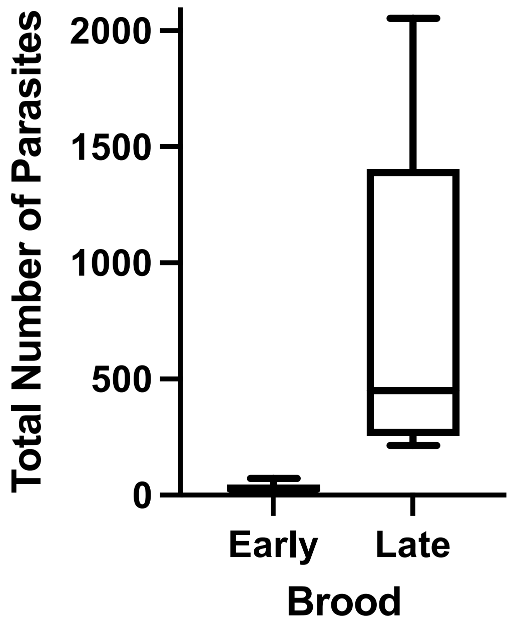

Figure 2. Total number of ectoparasites found in early (n=6) and late brood nests (n=6).

Egg Spots and Ectoparasite Load

The number of egg spots was significantly higher in late clutches than in early ones (Mann U = 0, n_1 = 6, n_2 = 6, P = 0.002, Fig. 3). Number of egg spots was significantly and positively correlated with the number of *Carnus hemapterus* in the nest (r = 0.6814, n = 12, P = 0.01, Fig. 4). The number of egg spots was also significantly correlated with the number of fleas and mites in the nest (r_s = 0.7070, n = 12, P = 0.01, Fig. 5). *Carnus hemapterus* had the highest percent occurrence of all ectoparasites in the nests (Fig. 6).

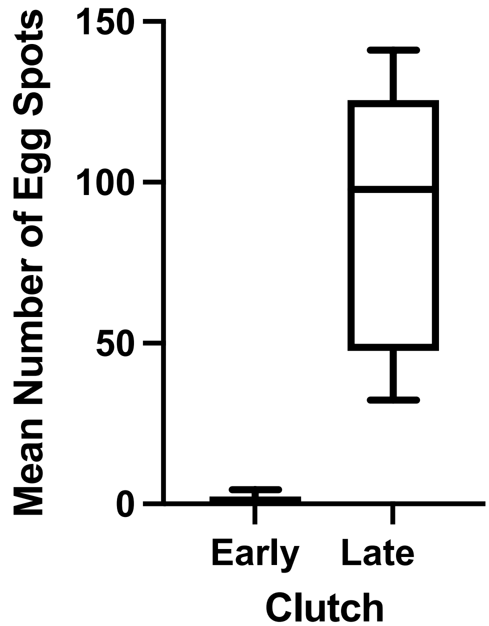

Figure 3. Mean number of egg spots per nest in early brood (n=6) and late brood (n=6) nests.

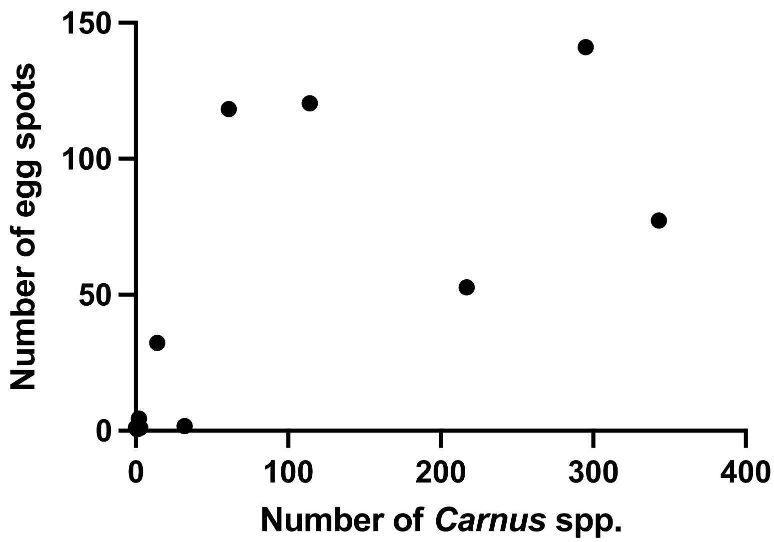

Figure 4. Mean number of egg spots in nest vs. number of *Carnus* spp. found in each nest.

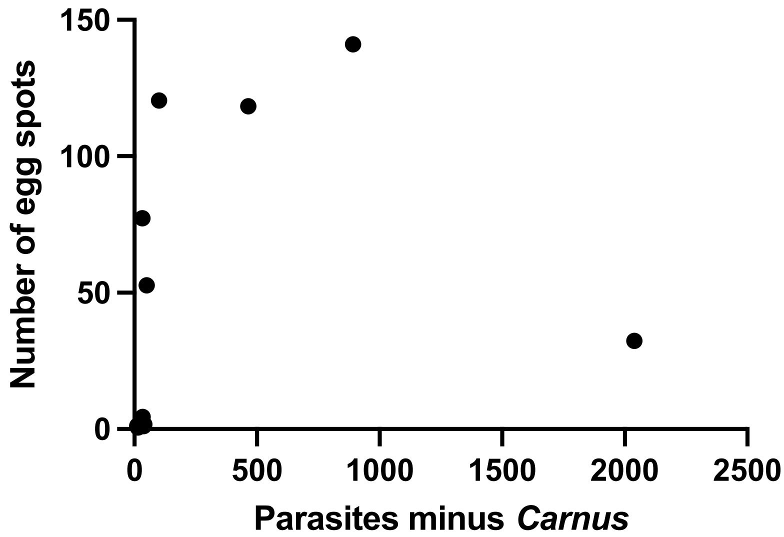

Figure 5. Mean number of egg spots in nest vs. total number of ectoparasites per nest aside from *Carnus* spp.

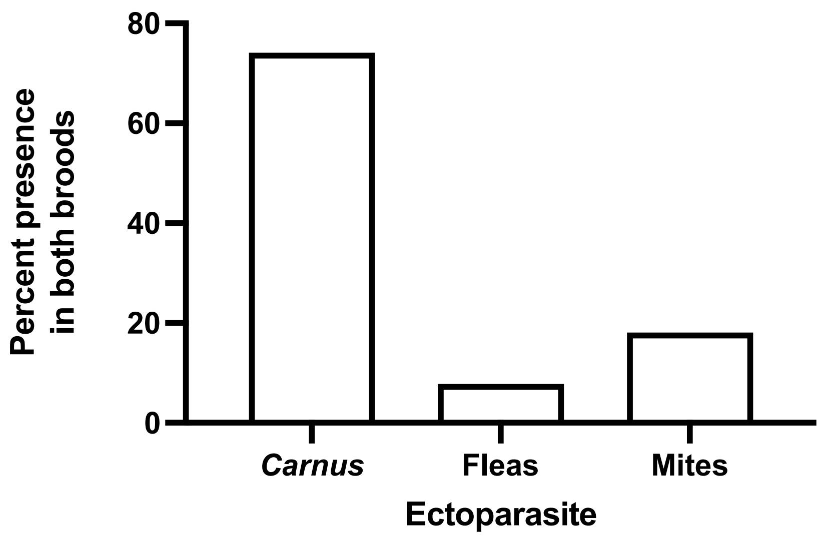

Figure 6. Overall percentage of *Carnus*, fleas, and mites found in all nest material.

Ectoparasite Load and Brood Condition

No significant correlation between ectoparasite load and brood condition was found (r_s = -0.1888, n = 12, P = 0.56, Fig. 7).

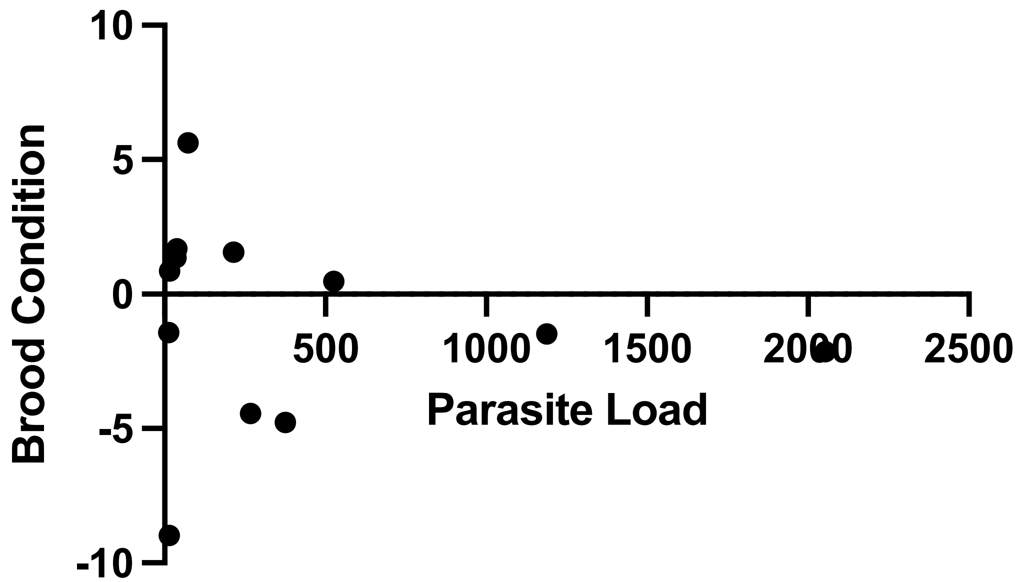

Figure 7. Brood condition of each nest vs. total number of ectoparasites in each nest (n=12).

Discussion

As predicted, the ectoparasite load was significantly higher in late brood nests than the early brood nests. This increase in ectoparasite load in the later broods may be caused by changes of the external environment, e.g., humidity, as the summer progressed. The environment in which the nests are located has been shown to have a direct effect on ectoparasite load in nests (Heeb et al., 2000; Merino and Potti, 1996). Ectoparasites such as the Hen Flea (*Ceratophyllus gallinae*) use the increased temperature of spring as a trigger to emerge from their dormant stages (Oppliger et al., 1994). Oppliger et al. (1994) even found that Great tits (*Parus major*), when faced with flea-infested nesting sites, would delay their breeding time by eleven days to wait for the fleas in the nest to emerge and leave the nest in search of food (Oppliger et al., 1994). Heeb et al. (2000) found that low humidity conditions led to increased number of Hen Fleas, suggesting that fleas require a low specific nest humidity to successfully reproduce. However, Merino and Potti (1996) determined that cold and wet ambient conditions resulted in higher overall ectoparasite counts (mites, blowfly larvae and fleas) within Pied Flycatcher nests. I did not examine the effects of humidity on ectoparasite loads in this study, but it would be a good question to examine due to potentially conflicting results amongst studies.

As predicted, the average number of eggs spots per clutch was also significantly higher in late clutches as compared to early clutches. The number of egg spots was also positively correlated with the number of *Carnus hemapterus* found in the nests, supporting the findings of Avilés et al. (2009) who found that egg spots were an indicator of *Carnus* load. Lopez-Rull et al. (2007) also found egg spots as a reliable indicator of *Carnus* load in Spotless Starlings (*Sturnus unicolor*). However, I also found a positive correlation between egg spot number and ectoparasite load that did not include *Carnus* (bird fleas and bird mites). Surprisingly, neither

Avilés et al. (2009) or Lopez-Rull et al. (2007) examined the abundance of other ectoparasites in the nest. Studies in the past have assigned the creation of egg spots to solely *Carnus*, probably due to it being the most common ectoparasite of the European Starling (Liker et al., 2001). However, if it is caused by blood excrement or blood splatter then there is an opportunity of it being created by other hematophagous parasites, which should have been taken into account. Hornsby et al. (2013) also suggested in their study that the number of egg spots could be attributed to the presence of all these ectoparasites, after they found no correlation between egg spot abundance and *Carnus hemapterus* abundance.

Counter to my prediction, and the findings of various studies (Benton and Shatrau, 1965; Hoi et al., 2017; Mason, 1944; Saino et al., 1998; Fairn et al., 2014), there was no significant relationship between ectoparasite load and brood condition. Small sample sizes and large variation in brood condition at lower ectoparasite loads seem to account for the lack of statistical significance, as suggested in a previous study (Duggan, 2019). There was also a considerable amount of variation of ectoparasite numbers within the later broods that were examined, which partnered with a low sample size, could cause results to appear non-significant. However, these results do support those of other studies that also found no effect of ectoparasite load on nestling condition (Duggan, 2019; Mazgajski, 2007a; Roby et al., 1992; Stephenson et al., 2009; Wolfs et al., 2012), and they all provide different theories on why that is. Mazgajski (2007a) concluded that his results showed that the cost of ectoparasite load was not caused by feeding but instead the energy expenditure in removing old nest material and site preparation. Roby et al. (1992) believed that density dependant constraints were the major factor in the ectoparasites negligible effect on nestling condition. Stephenson et al. (2009) found that the insecticide they used to experimentally manipulate ectoparasite numbers was ineffective, which caused them to question the validity of their own results. Wolfs et al. (2012) looked at the interactions of the ectoparasite *Dermanyssus gallinae* and a predatory mite which hunts it *Androlaelaps casalis*, and their effect on European Starlings nestling development. They provided multiple reasons why they found no correlation between *Dermanyssus* numbers and decreased nestling development, which include not taking parental quality into account, the disparity between when they measured nestling development and when they measured mite density, or the lack of a standard growth curve in Starling development (Wolfs et al., 2012).

Future research could examine humidity and ambient temperature during the incubation and nestling stages in conjunction with ectoparasite loads. Ambient and nest humidity were significant variables in ectoparasite load in two other studies (Heeb et al., 2000; Merino and Potti, 1996), but was not examined in this study. Another possible topic for future study could be to build upon what Hornsby et al. (2013) found and see if ectoparasites other than *Carnus hemapterus* are playing a role in the creation of egg spots.

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Appendix

Supplementary Table 1: Number of *Carnus*, bird fleas, and bird mites within each of the nest boxes (NB) in both the early and late broods.

	Early Brood Count						**Late Brood Count**					
Nestbox	NB 4	NB 14	NB 22	NB 34	NB 36	NB 49	NB 22	NB 26	NB 29	NB 33	NB 36	NB 49
Carnus	3	2	32	0	1	0	217	14	295	114	343	59
Fleas	5	13	0	0	1	4	17	0	24	12	23	15
Mites	3	0	7	0	7	33	7	0	4	4	0	198
Total	11	15	39	0	9	37	241	14	323	130	366	272