To what extent do co-infections influence body condition and immune markers across different bird species?

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Introduction

Bird populations are declining worldwide, with many species experiencing major losses due to habitat loss, climate change and human disturbance (Wilcove & Wikelski, 2008; Hirst & Halsey, 2023). While these causes are well established, disease remains an underexplored contributor to population declines despite being widespread in birds. Parasites are extremely common in wild bird populations (Ricklefs & Fallon, 2002). They can increase energetic demands and elevate chronic stress, they may intensify the effects of habitat disturbance and nutrition limitations (Cornelius et al., 2014). Many bird species carry infections that may weaken individuals and increase their vulnerability to environmental stressors. Because disease can influence survival, reproduction and physiological resilience, understanding how common parasites affect avian health is important for predicting how birds will respond.

Among these threats, avian blood parasites such as *Plasmodium, Haemoproteus* and *Leucocytozoon* are especially prevalent across migratory and resident birds. Single-genus infections are known to affect physiology: *Plasmodium* often reduces hemoglobin and hematocrit by destroying red blood cells (Schoenle et al., 2017; Knowles et al., 2010), *Leucocytozoon* is associated with reduced hematocrit and body condition (Martín-Maldonado et al., 2023) and *Haemoproteus* can influence immune function and energy use (Alvarado-Piqueras et al., 2025b). However, birds are often infected by more than one parasite genus and co-infections are extremely common (Camargo & de Lope, 2021; Loiseau et al., 2012). Simultaneous parasite infections, especially during migration, may heighten immune activation, increase energetic costs and reduce body condition and oxygen-carrying capacity. Yet, the physiological impacts of co-infection remain unclear. Factors such as migration, behaviour, habitat and host exposure to vectors will differ in species and will influence their likelihood of exposure to different parasite genera (Hellgren et al., 2012). These differences may make some species more susceptible or exposed to parasites. While many studies focus on single-genus infections, the impacts of co-infections on multiple health metrics across bird species are still poorly understood.

To address this, we examined whether co-infection by *Plasmodium, Haemoproteus* and *Leucocytozoon* influences fat score, hemoglobin, hematocrit and blood glucose across five migratory bird species sampled at Hueston Woods State Park (Oxford, Ohio). This area is a forested stopover site for migratory birds where vector abundance and bird movement makes co-infections more likely. The research question asks: To what extent do co-infections influence body condition and immune markers across different bird species? We hypothesized that infection with any blood parasite genus would reduce these health metrics and that co-infection with multiple genera would intensify negative effects. We predicted that co-infected birds would have the lowest values across all health metrics because managing multiple infections increases the demand of energy, damages red blood cells and triggers stronger immune and stress responses.

Methods

Species and Sample Collection:

This study used blood samples collected from birds at the Avian Research and Education Institute (AREI) banding station in Hueston Woods State Park (Oxford, Ohio, USA) during the Fall 2025 migration period. Bird species included Northern Cardinal (Cardinalis cardinalis), Swainson's Thrush (Catharus ustulatus), Wood Thrush (Hylocichla mustelina), American Robin (Turdus migratorius) and White-throated Sparrow (Zonotrichia albicollis). Only birds older than After-Hatch-Year (AHY) were included to limit age-related differences in infection and physiology. Birds older than AHY birds have more consistent blood measurements and a higher chance of previous exposure to blood parasites compared to Hatch-Year individuals.

Field Measurements:

The fat score for each individual was recorded using the AREI standardized scoring system which ranges from 0 to 5. A Homedsun was used to measure hemoglobin as well as hematocrit. Blood glucose was determined using an Accu-chek glucose monitor. Additional information such as species, age class, mass and capture date was recorded for each sample.

Parasite Detection and Identification:

DNA was extracted from blood samples stored in ethanol using QIAGEN DNeasy Blood and Tissue kits. Nested PCR tests targeting the cytochrome b gene were used to detect the three genera: Plasmodium, Haemoproteus and Leucocytozoon. This was done following the protocols of Hellgren and Waldenström. Gel electrophoresis on a 1.5-2% agarose gel was used to visualize PCR products and determine the presence of parasites. PCR-positive samples were cleaned up and sequenced using the Sanger method. Resulting sequences were compared against the GenBank BLAST database to determine parasite lineage and determine whether individuals have single-genus infections or mixed genus co-infections.

Data Analyses:

Data was analyzed separately for each species. Continuous variables (hemoglobin, hematocrit and blood glucose) was compared across infection categories (uninfected, single-genus infected, co-infected) using ANOVA or Kruskal-Wallis tests if data does not follow normal assumptions. Fat score was analyzed using a Kruskal-Wallis test across infection categories. All analyses were conducted in R and results were reported with confidence intervals p-values to increase accuracy.

Results

The distribution of infection categories varied across the birds sampled. Most individuals were infected with a single parasite genus, followed by uninfected birds and the fewest categorized as co-infected (Fig. 1).

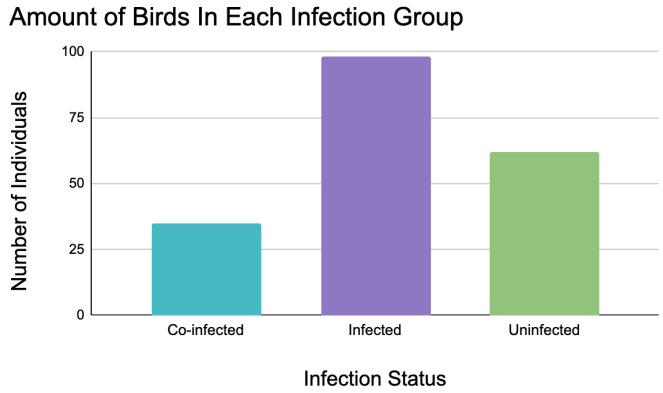

Figure 1: Distribution of birds in each infection category.
Bar chart illustrates the distribution of individuals across infection statuses, with the majority of birds classified as single-genus infected (n = 97), followed by uninfected birds (n = 62) and the fewest individuals categorized as co-infected (n = 34).

Hemoglobin concentration differed across infection categories, showing a decreasing trend from uninfected to co-infected birds. Co-infected individuals showed the lowest hemoglobin values, while uninfected birds had the highest concentrations (Fig. 2).

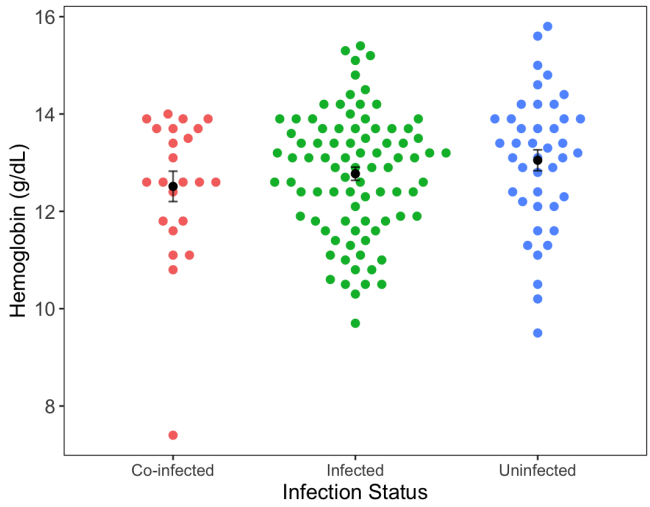

Figure 2: Hemoglobin concentrations (g/dL) across infection categories.
Each point on the boxplot represents an individual bird with mean ∓ standard error shown in black. Co-infected birds exhibit slightly lower hemoglobin values compared to single-genus infected and uninfected individuals, which show higher and more clustered hemoglobin levels.

Hematocrit values varied among the infection categories, with uninfected individuals generally having the highest value (Fig. 3). Single-genus infected birds showed intermediate hematocrit levels that overlapped with both uninfected and co-infected individuals. Co-infected birds had the lowest hematocrit values when compared to the other categories.

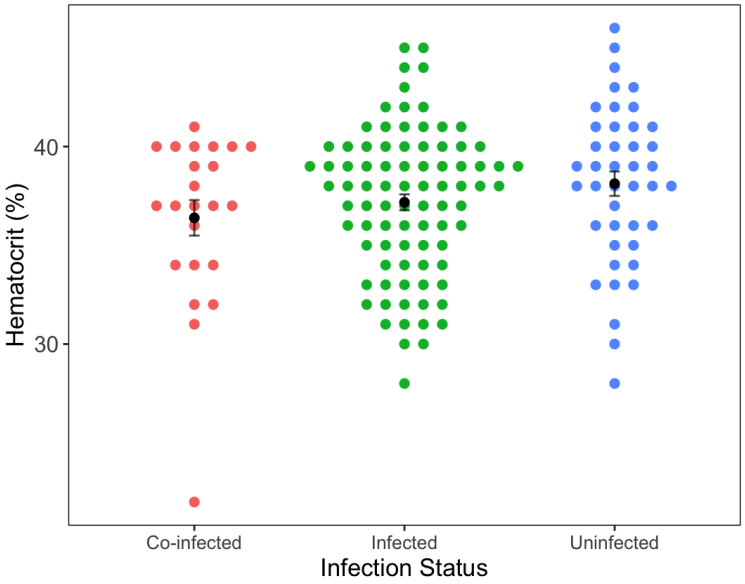

Figure 3: Hematocrit (%) values across infection categories.
Boxplot illustrates a decreasing trend in hematocrit from uninfected to co-infected individuals, with co-infected birds showing the lowest overall values. Mean ∓ standard error are displayed in black, highlighting higher hematocrit in uninfected individuals compared to reduced values observed in co-infected birds.

Blood glucose levels differed slightly across infection categories, with co-infected birds showing similar values to uninfected birds (Fig. 4). Single-genus infected birds clustered at intermediate glucose concentrations. Uninfected birds showed a wide range but overall values remained comparable to those of co-infected individuals.

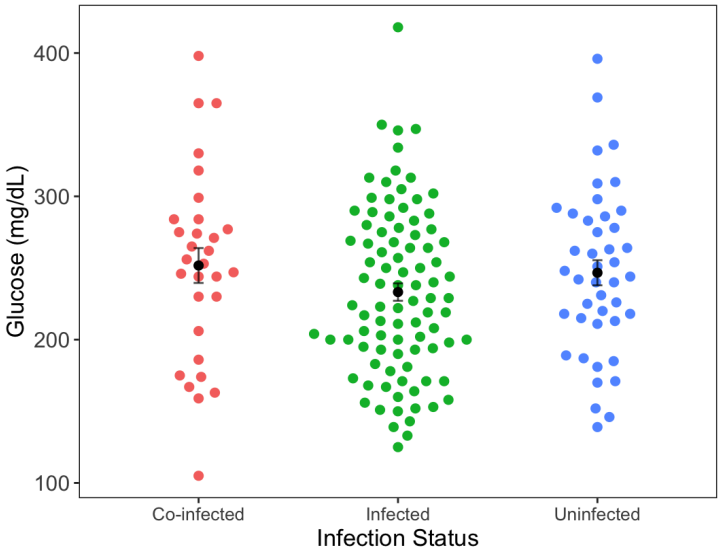

Figure 4: Blood glucose (mg/dL) across infection categories.
Boxplot shows that blood glucose levels were generally similar among the infection categories, with single-genus infected birds displaying slightly higher values. Uninfected and co-infected individuals had comparable glucose levels, though co-infected birds showed the lowest value overall. Mean ∓ standard error are indicated in black.

Fat reserves varied among the infection categories, with each group showing a distinct distribution (Fig. 5). Uninfected birds were generally concentrated at lower fat scores, especially score 0, while singly infected individuals displayed a wider distribution of fat scores. Co-infected birds were mostly concentrated at intermediate fat scores observed in some uninfected and singly infected individuals.

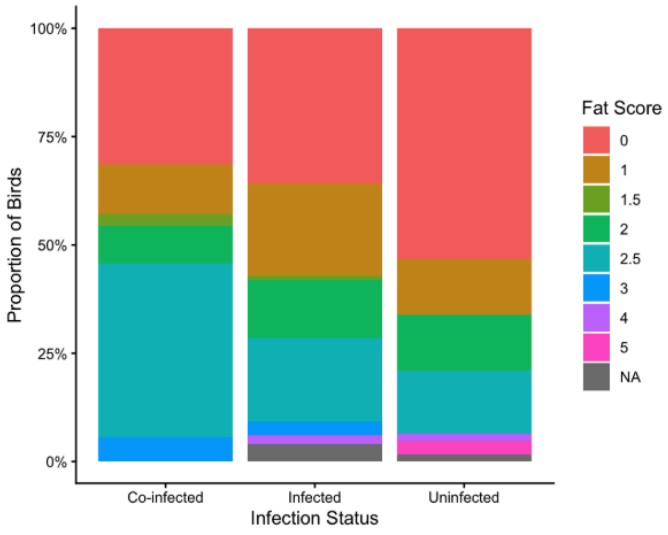

Figure 5: Proportion of individuals in each fat score category across infection statuses. The stacked bar chart shows clear differences in fat reserves among groups: uninfected birds had the highest proportion of individuals with a fat score of 0 and was the only group with scores reaching up to 5. Singly infected birds showed a wider spread of fat scores, including one individual with a score of 4. Co-infected birds exhibited the highest proportions of intermediate fat scores (2.5 - 3), with no individuals exceeding a fat score of 3.

Discussion

This study examined how infection status (uninfected, single-genus infected or co-infected) affected hemoglobin, hematocrit, blood glucose and fat score in migratory birds. Overall, most physiological traits showed no significant differences among infection categories, despite notable differences in fat scores. These findings contribute to our understanding of how blood parasites may affect avian energetic condition during migration.

Hemoglobin and hematocrit did not differ significantly across infection categories, although visual trends showed lower values in co-infected individuals. Kruskal-Wallis tests indicated no statistically significant differences for hemoglobin ($p = 0.378$) or hematocrit ($p = 0.274$). These findings suggest that infection status did not measurably alter oxygen-carrying capacity or packed cell volume in the birds sampled. Previous studies has shown that co-infections can

reduce hemoglobin and hematocrit through inflammation-driven anemia and hemolysis which is the destruction of red blood cells (Ishtiaq et al., 2018; Maldonado 2023). However, other studies report mininal impacts, especially when infections are chronic or at low intensity (Cornelius et al., 2014). The lack of significant differences observed here is more consistent with the latter pattern, suggesting that birds may have been experiencing chronic, low intensity infections which would be too insufficient to cause hematological changes.

Blood glucose levels also did not vary significantly across infection categories, with single-genus infected birds showing slightly higher values but notable overlap across groups. Similar findings have been reported in other studies where chronic infections trigger minimal metabolic responses (Hahn et al., 2018). Although acute infection can elevate glucose through immune activation or stress responses (Ots et al., 1998), the patterns in this study suggest that birds were maintaining metabolic stability despite infection. This aligns with the possibility that sampled birds were not experiencing parasitic stress.

Fat score was the only physiological variable that differed significantly among infection categories ($p = 0.036$). Uninfected birds had the highest proportion of individuals with a fat score of 0 and were the only group with the highest possible score of 5. Single-genus infected individuals showed a broad range of fat scores inlcuding one with a score of 4. Co-infected birds showed the highest concentrations of intermediate fat score (2.5-3) and did not exceed a score of 3. Previous research suggests that avian blood parasites may influence energy balance by reducing fueling efficiency during migration (Hegemann et al., 2022). However, some studies have found that mildly infected birds increase foraging efforts to compensate for energy loss (Emmenegger et al., 2023). The intermediate fat levels observed in co-infected birds may reflect altered energy allocation or limited capacity to store high levels of fat during migration.

Overall, these results suggest that while co-infection may influence fat reserves, hemoglobin, hematocrit and glucose remain relatively stable across infection categories. This could indicate that birds are capable of compensating for many physiological effects of infection or that infection intensities in this population were too low to cause measurable declines. Because migration is energetically demanding, even moderate differences in fat reserves could affect migratory timing or stopover duration.

Future research should incorporate direct measurements of infection intensity rather than presence or absence, as physiological impacts often differ with parasite load. Additional physiological markers such as immune activation indicators and stress hormones would help clarify how co-infections influence metabolism and condition. Sampling across different seasons and age class would also help determine whether infection effects differ between acute and chronic phases or among species with differing migratory strategies. Expanding the dataset to a broader range of species and geographic areas would improve understanding of how co-infection influences avian health across ecological contexts.

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