

Effect of blood parasites on incubation efficiency of *Z. l. oriantha* in the Colorado Rocky Mountains

Cassandra McCuen

Mentor: Ross Conover

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Abstract

Haemoparasites are common in passerines and have known mild to detrimental effects on populations. It is important to understand how these effects influence nest incubation in females, as it could reflect future reproductive success of that species as vectors and transmission increase with climate change. We investigated how incubation temperatures varied in female mountain white-crowned sparrows in the presence and absence of haemoparasites to determine if these parasites are influencing efficiency. There was a trend showing that parasitized females showed more variance in nest temperature, but it was not significant. A larger sample is necessary to draw conclusions from this study.

Introduction

Haemoparasites are common in passerines (Merino et al. 2000), and can cause detrimental effects on populations. Most notably, many Hawaiian Honeycreeper species have been lead to extinction or extirpated from many parts of the Hawaiian Islands as a result of an introduced mosquito vector of malaria (Warner 1968). In geographical areas where passerines coevolved with these parasites, there are lesser, but potentially ecologically relevant effects that are poorly understood (LaPointe 2012). Haemoparasites can pose a variety of fitness challenges, such as lower reproductive success, lower overall body condition (Parus major; Merino et al 2000), or may influence sexual selection by altering male ornaments (Zuk 1992). These effects likely arise from the known increased metabolic demands for immunocompetence against infection (Sheldon et. al 1996), but as no effects on body mass have yet been found (Bennett 1988), it is likely these birds are increasing foraging efforts to offset this effect (Marzal 2004).

Haematzoa is a subclass of avian blood parasites that include four main genera, Leucocytozoon, Haemoproteus, Plasmodium, and Trypanosoma. This study focuses on six common species within these genera, *L. fringillinarum*, *L. majoris*, *H. coatneyi*, *P. relictum*, *P. vaughni*, and *T. avium*. These parasitic protozoa are vectored by dipteran such as mosquitos (Culicidae), black flies (Simuliidae) and biting midges (Ceratopogonidae; Murdock 2009). As climate change continues and temperatures begin to warm, these vectors are expanding their range, and parasite development within these vectors is increasing (Loiseau et al 2013, Caminade et. al 2018). This causes concern for many bird populations, as infections are also likely to increase as a result (Loiseau et al 2013).

The mountain white-crowned sparrow (*Zonotrichia leucophrys oriantha*, MWCS) is a passerine subspecies that breeds in montane ecosystems in the Western United States (Morton 2002). These birds breed in subalpine meadows ranging from 2,500 to 3,500 meters, and build cup shaped nests in dense willow stands (*Salix* spp.) (Morton 2002). After the last of the clutch is laid, females will spend all night and 75% of the day incubating nests (Morton 2002). Incubation typically lasts 11-15 days, maintaining a thermal environment ideal for embryonic development (Morton 2002). This subspecies has been known to be infected by *Leucocytozoon*, *Plasmodium*, and *Haemoproteus*, with *Leucocytozoon* and *Plasmodium* effecting song structure and consistency the most in males (Gilman et al 2006). As bird song in males is a testament to reproductive fitness, we wanted to explore how reproductive fitness in females are affected by these parasites by looking at nest temperature variance. This may cause longer incubation periods and decreased embryonic development, leading to changes in reproductive success in affected birds (Morton 2002).

We hypothesize that infected MWCS females will be more energetically stressed, causing increased need to forage, and therefore more variant nest temperatures.

Methods

Study Area

These data were collected in the Elk Mountain Range of the Colorado Rocky Mountains, near the Rocky Mountain Biological Laboratory (RMBL) from 2017 to 2019, late May through mid-July. Study sites include alpine and subalpine meadows, ranging from lower elevation to higher elevation sites in the East River Valley (2924m), and Schofield Park (3167m) (*Figure 4*). Temperatures at these sites ranged from 29°C during the day and 19°C at night throughout the summer months (NOAA), and snow cover can persist through late June. Plant communities in these areas predominantly include willow (*Salix* spp.), cinquefoil (*Potenilla* spp.) larkspur (*Delphinium* spp.), corn lily (*Veratrum* spp.), and mountain bluebell (*Mertensia* spp.).

Nests are well-hidden and primarily built on somewhat damp ground, in dense willow thickets (Morton 2002). However, nests can sometimes be found in elevated sites such as isolated pines (*Pinus* spp.), spruces (*Picea* spp.) and the crowns of willows (Morton 2002).

Nest Searching and Monitoring

Mountain White-crowned Sparrow nests were found using the rope-drag technique, and by behavioral observation (Martin et al 1993). When a nest was found, the location was immediately marked with a GPS unit, aged, and flagged three meters from the north and to the south of the nest. Nests were checked every three to four days to reduce disturbance of the parents and avoid abandonment (Martin et al 1993). Each time the nest was visited, a different path was taken to reduce disturbance to the surrounding vegetation and to prevent predators from locating the nest due to human influence (Martin et al 1993).

Using the Koford technique, voucher images of egg and nestling development were referred to when eggs were candled and nestlings were monitored (Koford 1999). Thermochron button model DS1921G and DS1922L data loggers were painted brown for camouflage and used to monitor nest temperature every two minutes during incubation. One data logger was placed beneath the nest lining and another 15 cm away from the nest in a similar microclimate as a control. This allowed us to measure off-bouts during incubation, determined by a change in temperature of the nest when the female is not incubating.

Bird Capture and Bleeding

Birds were captured using mist nets. Mist nets were set up near nests, and incubating females were flushed off the nest by walking toward it, into the mist nets and safely extracted.

All captured birds will be uniquely banded with color bands and a US Geological Survey band. If already banded, color combinations will be recorded. Blood will be drawn from each bird's brachial vein using a lancet and capillary tube, and a thin smear will be created using glass slides in the field (Oppliger et. al 1996).

Parasite Load Analysis

Blood slides were fixed in 91% isopropyl alcohol and stained using Wright-Giemsa stain, then rinsed with water (Oppliger et. al 1996). They were then examined using a compound microscope 100x oil immersion lens. To determine blood parasite presence, slides are scanned in a zigzag pattern for a total of ten minutes to confirm presence of any of the six parasite study species (Oppliger et. al 1996). All parasites identified were noted and a photo was taken of the specimen for confirmation.

Nest Temperature Analysis

Nest temperature data were paired with ambient temperature data according to time stamps, and a one-way ANOVA was performed to get both mean and root mean square error for nest and ambient temperature, or variance from the mean, for each nest.

Statistical Analysis/Graphing

Nest temperature variance and mean were compared against nest and infection status to determine the significance of this relationship. Graphs were created comparing parasite status, and mean variance for both nest and ambient temperatures. Additionally, graphs were created comparing variances of each individual nests and their parasite status scored from none, one, to multiple.

Results

For nests we obtained temperature data and parasite data for from 2017-2019, our sample size was 15. In Figure 1 we see that nest temperatures were warmer than ambient temperatures. In Figure 2 we see that ambient temperatures are more variant than nest temperatures for both infected and uninfected birds. Additionally, we see that infected birds are slightly more variant than uninfected birds, but not significantly. In Figure 3 we break down Figure 2 to explore the variance among nests with and without parasites. We see again that variance in nests where one or multiple parasites were present, the variance is slightly more but not significantly.

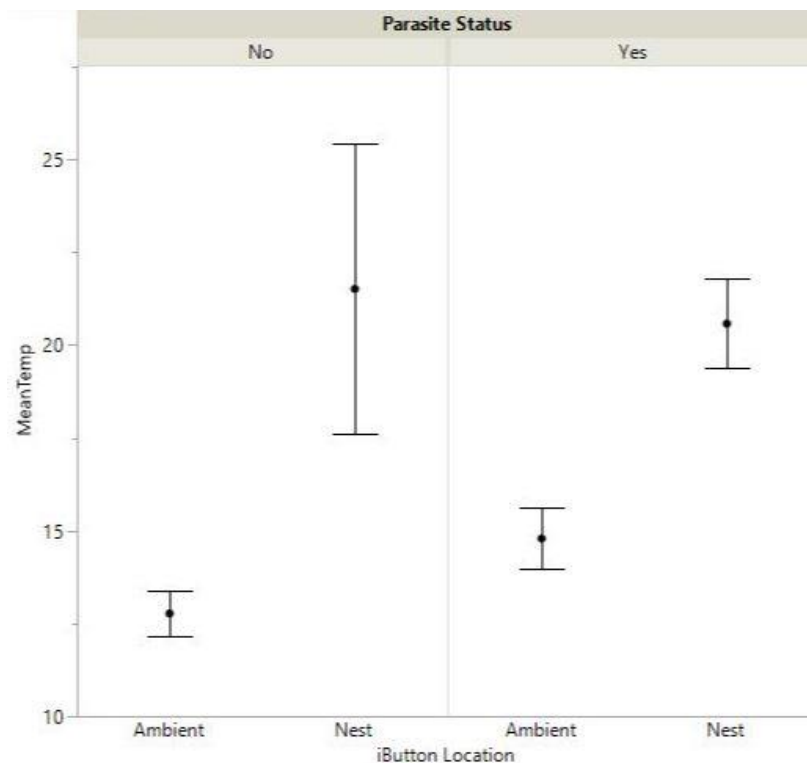


Figure 1. Nest and ambient temperatures from each nest collected were grouped by parasite status and averaged out to create mean temperatures, standard error bars were applied. For uninfected birds $n=4$, and infected birds $n=11$. The x-axis represents ambient or nest location grouped by parasite status (yes, no), and the y-axis is the mean temperature.

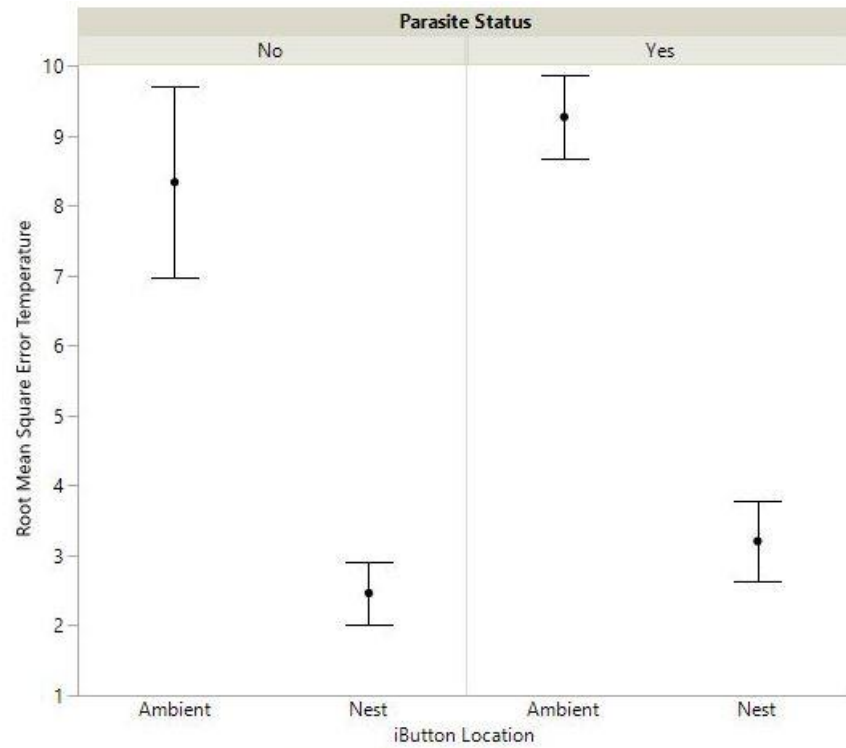


Figure 2. Variances for nest and ambient temperatures for all nests were grouped by infected and uninfected, and averaged out. Standard error bars were applied. For uninfected $n= 4$, and infected $n= 11$. X-axis represents nest or ambient temperature grouped by parasite status, and y- axis represents variance from the mean.

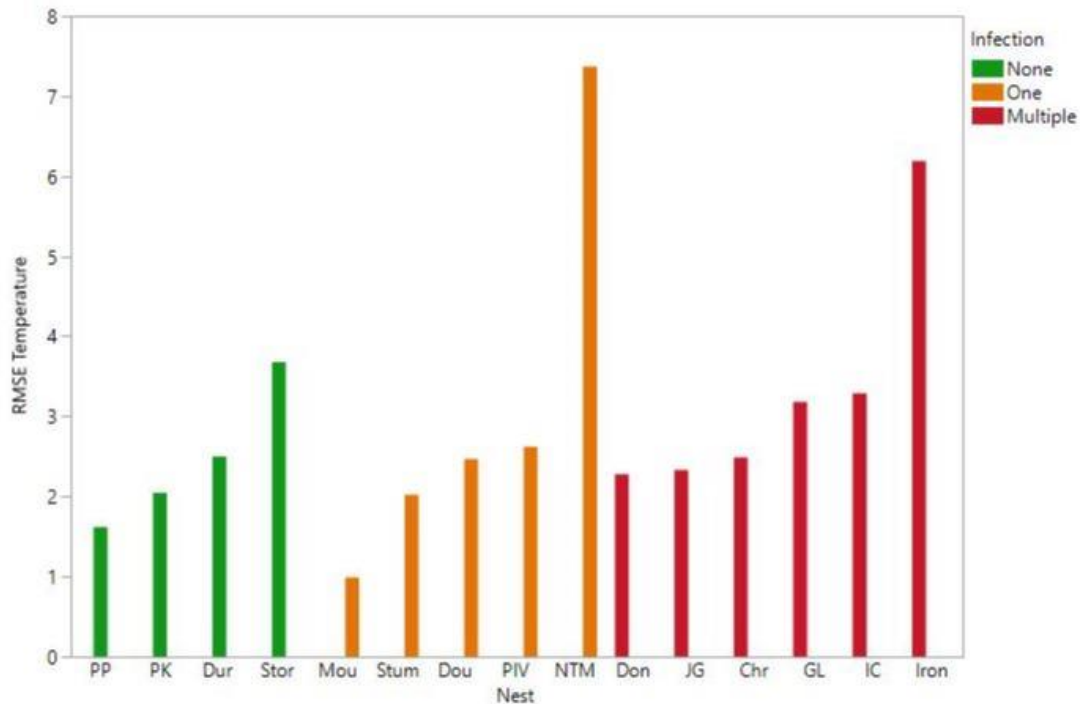


Figure 3. Figure 2 was broken down to compare variances of each individual nest and include infection status. The x-axis represents nests, the y-axis represents variance from the mean, and all were scored for parasites. Green= none, orange= one, red= multiple parasites present.

Discussion

Results

Overall, our results were not significant. Mean nest temperatures were warmer than ambient temperatures, which is to be expected if the female is putting effort into incubation. Additionally, larger variance between ambient temperatures versus nest temperatures were expected because during incubation the female needs to keep the nest at optimal temperature for embryonic development (Morton 2002). A slight trend showed that variance in nest temperatures were greater for parasitized versus non-parasitized birds, but it was not a significant result. A larger sample size is needed to draw any conclusions from this study.

Future Considerations/Questions

The original intent of this study was to use frequency of off-bouts during incubation to determine incubation efficiency. However, the methods used for incubation data collection made this difficult to analyze. The nest iButtons were placed under the nest lining, avoiding direct contact with the female brood patch and providing the iButton with some insulation. Therefore, completely accurate nest temperature data was unable to be obtained, and the data quality was too low to run through incR (Lasheras 2017). Additionally, we had poor means of confirming

our off-bout analysis with the methods used. Ideally, iButtons in conjunction with nest cameras would be a good option to compare and confirm when off-bouts are occurring. iButtons should also not be placed under the nest lining, but instead attached to a button and weaved into the lining of the nest (Smith et al 2016). For this study species, we were unable to put the iButtons in alongside the eggs, as we had many iButtons removed from the nest and data lost. Other possible methods to determine off-bouts include using pressure sensors that will sense when the female is on the nest (Smith et. al 2016) These methods, however, need to be investigated for their practicality with the study species to ensure there will be no negative effects such as nest abandonment.

When incubation off-bouts can be confirmed and measured, and sample size increases, it will be interesting to explore how each individual parasite and combinations of two or more will affect off-bout frequency. This, in conjunction with a less variable method of determining nest stage, would help us make the connection of parasite presence and incubation duration to explore if there is any effect.

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Figure 4. Map of study sites 2017-2019, including lower East River Valley and Schofield Park.
[map deleted for size. J.reithel]