Marina Ayala
EBIO 4410
Independent Project

# The effect of a brood-size manipulation and GPS tag on *Hirundo rustica* blood glucose levels

**Background:**

Life history of an organism gives insights to its quality of life. A way to examine an organism's health is by looking at its stress levels. The best way to quantify stress is through measuring blood glucose, which can be an indicator of stress. In avian species, stress causes an elevation in corticosterone as well as blood glucose (Blas, 2015; Braun & Sweazea, 2008).

One of the most costly endeavors an avian will pursue in its life is reproduction. Reproduction is a personal cost, since it requires so much energy and investment. A way to quantify the cost of reproduction, can be examined through blood glucose. Avians in the genus *Hirundo*, exhibit an exceedingly high blood glucose concentration relative to their body mass, and yet they do not exhibit oxidative stress damage (Braun & Sweazea, 2008; Sweazea et al., 2008). Blood glucose regulation in avian populations is regulated by endocrine hormones that uphold homeostatic control (Dawson, 2000; Vágási et al., 2020). Small avian species, like *Hirundo rustica*, have increased metabolic demand, which in turn leads them to exhibit an increased glucose concentration in blood plasma which is likely due to diet composition and avian physiology (Basile et al., 2018; Braun & Sweazea, 2008) The aim of this study is to look at possible elevation of blood glucose due to the stress of reproduction.

This study examines *Hirundo rustica* blood glucose by adding experimental measures. It is hypothesized that both a brood size-manipulation and addition of a GPS tag will have an effect on the blood glucose levels in Barn Swallows due to possible added stress.

**Methods:**

*Study System*

*Hirundo rustica,* commonly known as Barn Swallows, are small colorful passerines that migrate each year from S. America to N. America in order to nest and brood young (Brown & Brown, 2020). Nest-building occurs from May to mid-June, and adults breed throughout the summer into the early fall months. Barn Swallows create a distinctive mud-cup nest, commonly seen in agricultural barns and across cityscapes. Their preferred habitat is near agricultural fields, which provide a food source for these aerial insectivores (Scordato & Safran, 2014).

*Field methods*

In this study, we collected data from adult barn swallows during the breeding season in Boulder County, CO between the months of May-September (*2019-2020)*. We collected physiological samples from a population of n=37 adult female barn swallows. The sites selected are local agricultural barns, horse ranches, and underpasses. The different study sites reflect both rural and urban habitats with 16 sites total. 6 sites were underpasses in urbanized areas, and the other 10 were barns in agricultural areas mostly near livestock.

This study is a part of an ongoing research study in which nesting individuals are studied and catalogued each year in Boulder County by *Dr. Rebecca Safran's* lab at the *University of Colorado Boulder*. The design of the long-term study is that study sites among Boulder County are long-term nesting sites of Barn Swallows in which data from this specific population is collected annually. The population examined migrates annually to these long-tem sites in which they are monitored and tagged between the months of May-September.

In the study, individual birds were identified at a nest at each site through distinctive color bands, as well as USGS tag number. Brooding females were of key interest, so each capture physiological measures were assessed as well as adding a color and USGS metal band (if not already present). The same was done for male birds, but since brooding females were the main study for this experiment, throughout the whole population females had physiological measurements taken throughout the study. This included measures such as wing length, tail streamer length, mass, USGS tag number, color bands, and feathers (which were collected from both the body and tail). Brooding females that successfully incubated eggs into nestlings were able to be candidates for the experiment.

All of the brooding females were randomly placed into one of four groups: experimental and control; experimental females were subjected to both a brood size manipulation and the placement of a GPS tag on their back using a Rappole leg-loop harness. We tagged females when chicks were 4-6 days old, and we manipulated brood size when chicks were 8 days old. Nests were either enlarged (E) or reduced (R) through randomized selection. Each female served as her own control, since measurements were taken before and after tag addition and brood size manipulation. We collected blood from the brachial vein using a capillary tube. After blood was extracted, a cotton ball was applied with pressure to the prick site to prevent further bleeding. Blood glucose was measured in the field using a Freestyle Lite ® Glucose Monitor and strips. The Blood glucose reading was immediately recorded in μg/ dL and sampled once the bird was out of the cloth bag. Blood glucose was recorded before the tag was placed on and after the tag was removed. The bird's physiological data was also collected when blood glucose was measured, which included: mass, feather samples, measurements, and wing faults.

Females that did not receive a GPS tag were captured and handled to be subjected under the same stress conditions administered to experimental females with a GPS tag. Females that had a GPS tag were also captured and handled in a similar manner, except the GPS tag was placed on. In order to measure the blood glucose level of a bird, birds were placed in a cloth bag for 15 minutes (Montoya et al., 2020). Other covariates measured included mass (g) that was collected using a scale, time the bird was captured was recorded, type brood treatment (enlarged or reduced), year , and site were recorded. These variables are used to isolate the effect of treatment on blood glucose, since a bird's mass, the time of day the measurement was taken, year, and brood treatment may all have an effect on the blood glucose measure.

*Statistical methods*

The data was first cleaned in Excel. The filtering of the data was done in RStudio and birds that had two paired glucose measurements before and after treatment were only used. The data was analyzed by first assessing the univariate distributions, bivariate descriptive statistic stats and associations, and finally the full generalized mixed model. The mixed model included change in blood glucose (treatment 1- treatment 2), tag, brood treatment, mass (before treatment), year, and site. The independent variables are tag and brood treatment. One of the dependent variables is blood glucose, specifically the change in blood glucose. A residual model was created for change in blood glucose to account for time of day. Time of day was recorded in 24 hour format, which allowed for transformation of this data. The sin of the time data was taken, which is a method used to transform circular data using the R package 'circular' (Cremers and Klugkist, 2018). The other dependent variable is mass. The random effects are site and year. The software used was RStudio (version 1.2.1335) and the packages used were broom, dotwhisker, dplyr, emmeans, car, circular, ggplot2, lme4, lmerTest, lubridate, and tidyverse.

**Results:**

In this study, we found that there is no effect of brood size manipulation or the addition of GPS tag on blood glucose levels in Barn Swallows. The brood size manipulation showed no significant effect ($p = 0.54$) on within-individual change in blood glucose. The addition of a GPS tag showed no significant effect ($p = 0.58$) on within-individual change in blood glucose. Therefore, the null hypothesis cannot be rejected. The implications of these findings is that this research provides valuable insights for future studies in which stressed blood glucose is measured in a way to control for external factors such as foraging schedule, time of day, and activity levels. For future research, creating a standardized measure for blood glucose (such as measuring fasted blood glucose in the birds) may help control for variation.

**Tables and Figures:**
**(on next page)**

Link to GitHub page with code: https://github.com/maay21/EBIO_4410

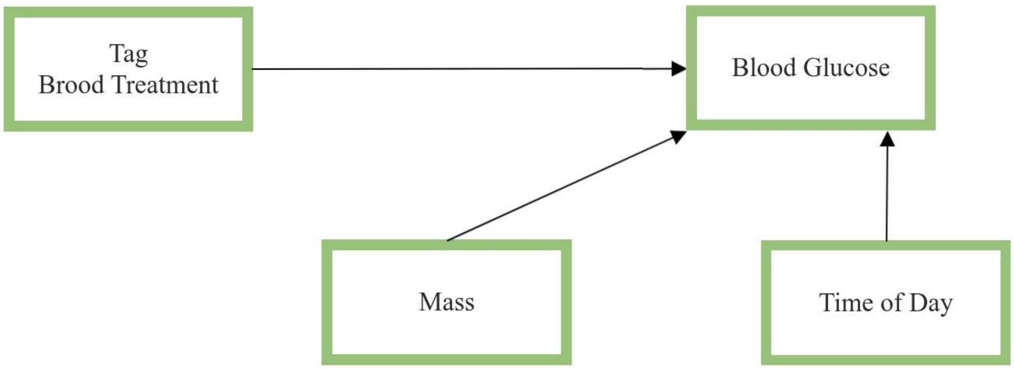


**Figure 1.** A DAG Directed-acyclic graph (DAG) showing the relationship between variables and blood glucose (Laubach et al. 2021). Blood glucose (specifically the change) is the dependent variable. Mass and time of day are precision covariates. The independent variables are tag and brood treatment.

$$\Delta\textbf{Blood Glucose} \sim \textbf{tag} + \textbf{broodSize} + \textbf{tag*broodSize} + \text{mass1} + \text{year} + \text{time} + (1|\text{site})$$

**Figure 2.** Equation used to model the change in blood glucose.

| Table 1. Mean and Standard Deviation for Blood Glucose and Mass | Mean | Standard Deviation |
|---|---|---|
| | | |
| Change in Blood Glucose | 19.10811 | 42.90868 |
| Initial Blood Glucose (Before Treatment) | 217.4595 | 31.91969 |
| Final Blood Glucose (After Treatment) | 236.5676 | 39.28566 |
| Change in Mass | -0.7683784 | 0.8600146 |
| Initial Mass (Before Treatment) | 17.96108 | 0.9339694 |
| Final Mass (After Treatment) | 17.1927 | 1.07599 |
| Time of Day (Radian Hours, Before Treatment) | 0.255361 | 0.7089235 |
| Time of Day (Radian Hours, After Treatment) | 0.00649219 | 0.7089235 |

**Table 1.** Mean and standard deviation for blood glucose, mass, and time of day as radian hours.

| Table 2. Brood Treatment and Tag | Sample Size | % of Individuals |
|---|---|---|
| | | |
| **Brood Treatment (Enlarged)** | 20 | 54% |
| **Brood Treatment (Reduced)** | 17 | 46% |
| **Tag** | 20 | 54% |
| **No Tag** | 17 | 46% |

**Table 2.** Brood treatment vs. tag. The sample size is indicated for each variable, as well as the percentage of individuals in the sample population that received each treatment.

| Table 3. Estimate and Confidence Intervals Categorical | Estimate | Standard Error | Degrees of Freedom | P-value | Lower CI | Upper CI |
|---|---|---|---|---|---|---|
| | | | | | | |
| **Change in Blood Glucose and Tag** | 12.61 | 14.2 | 35 | 0.3806 | -16.214881 | 41.42665 |
| **Change Blood Glucose and Brood Treatment** | 10.9 | 14.24 | 35 | 0.449 | -18.00256 | 39.80256 |

**Table 3.** Estimate and confidence intervals for the categorical variables: brood treatment and tag. Both p-values >0.05, so the relation to blood glucose is not significant. 95% confidence intervals used.

| Table 4. Change in Blood Glucose and Tag | SE | df | lower CI | upper CI |
|---|---|---|---|---|
| | | | | |
| **No Tag** | 10.44 | 35 | -8.9 | 33.5 |
| **Tag** | 9.62 | 35 | 5.36 | 44.4 |

**Table 4**. Tag vs. no tag. 95% confidence intervals used.

| Table 5. Change in Blood Glucose and Brood Treatment | SE | df | lower CI | upper CI |
|---|---|---|---|---|
| | | | | |
| **Enlarged** | 9.65 | 35 | -5.49 | 33.7 |
| **Reduced** | 10.47 | 35 | 3.75 | 46.2 |

**Table 5.** Change in blood glucose vs. brood treatment. 95% confidence intervals used.

**Time of Day That Data Samples Were Collected Before Treatment**

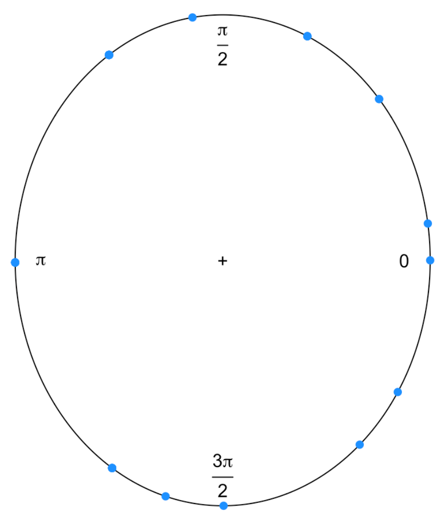


**Figure 3.** Time of day as radian hours before treatment (initial measure of blood glucose). Time was converted to radian hours by first converting time to 24-hour time, then taking the sin of each hour. $\frac{\pi}{2}$ represents noon/midnight (Cremers and Klugkist, 2018). Each point represents one measure of blood glucose.

**Time of Day That Data Samples Were Collected After Treatment**

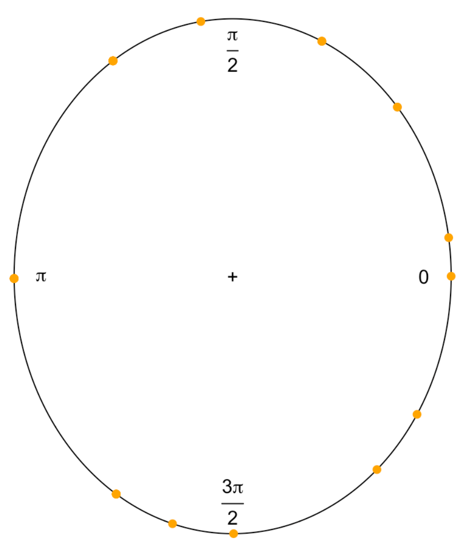


**Figure 4.** Time of day as radian hours after treatment (final measure of blood glucose). Time was converted to radian hours by first converting time to 24-hour time, then taking the sin of each hour. $\frac{\pi}{2}$

represents noon/midnight (Cremers and Klugkist, 2018). Each point represents one measure of blood glucose.

**Tag vs. Change in Blood Glucose**

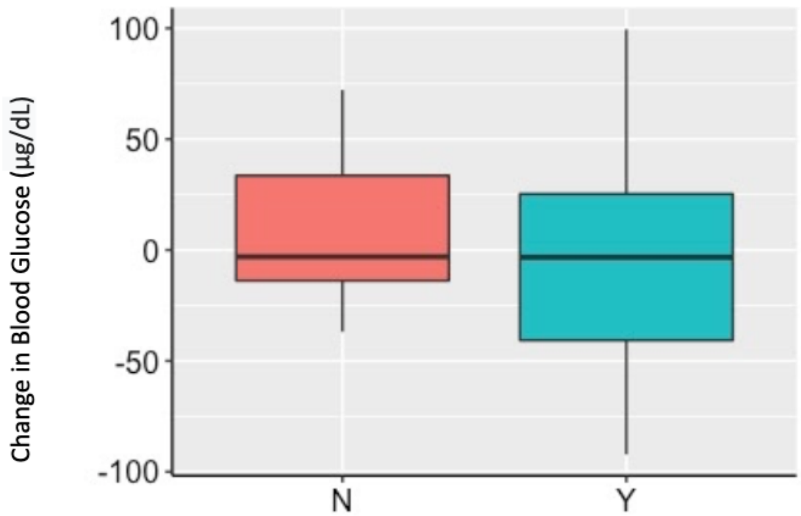


**Figure 5.** Tag (N- no tag, Y- tag) vs. change in blood glucose (measured in µg/ dL). Middle line indicates median value, top and bottom represent 75th and 25th percentile, respectively.

**Brood Treatment vs. Change in Blood Glucose**

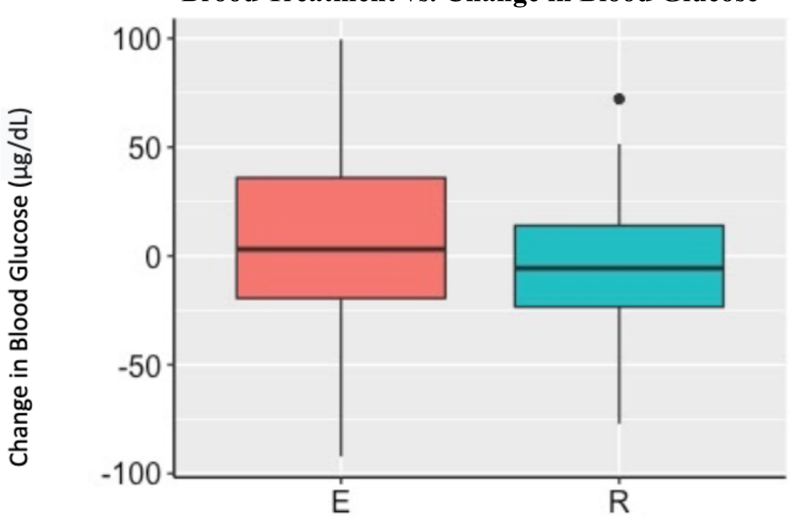


**Figure 6.** Brood treatment (E- enlarged, R- reduced) vs. change in blood glucose (measured in µg/ dL). Middle line indicates median value, top and bottom represent 75th and 25th percentile, respectively.

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