BAT FLIGHT PROJECT FINAL WRITEUP

BIOLOGY 708: QUANTITATIVE METHODS IN ECOLOGY & EVOLUTION

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INTRODUCTION

Bats are the only mammals capable of powered flight. They achieve flight with their patagia, which are thin, flexible, and weight-bearing flight membranes (Norberg 1972; Anderson & Ruxton 2020). Flight allows for high mobility, which is important for numerous behaviours like roosting, foraging, and migrating (Fenton 1997; Agosta 2002; Fleming 2019). Big brown bats (*Eptesicus fuscus*) are North America's most widespread mammal and rely on flight to forage for insects and roost in both natural and human-made structures (Agosta, 2002).

Big brown bats also survive in captivity (Kurta & Baker, 1990). For almost two decades, McMaster University has housed Canada's only captive big brown bat colony, permitting research on several bat phenomena such as bioacoustics and behaviour. As a member of the McMaster Bat Lab, Renata previously planned a study involving an in-flight foraging task. The project involved bats that had been in captivity for about three months (n = 25). Up until the study's onset, the bats were housed in metal cages (28.0 x 21.0 x 20.5 cm; l x w x h) for quarantine. When the project started, the bats were moved into a larger colony room (2.5 x 1.5 x 2.3 m ; l x w x h) with their food placed in the colony's centre, which was meant to encourage them to fly and exercise. On days eight and nine of the study, Renata attempted to fly the bats and record their flight times. On average, the bats flew 4.09 s on day eight and 3.51 s on day nine. However, these averages are likely somewhat inflated as it was difficult to stop the timer exactly when the bat landed. Regardless, the bats essentially "flopped" to the ground and did not sustain flight. Many of them were also hesitant to take-off from the experimenter's hand and needed multiple downward "shakes" to do so. The study was eventually terminated due to their inability and unwillingness to fly. We suspect the bats could/would not fly because of their decrease in activity levels upon capture (from being housed in the metal cages) and an increase in mass (due to *ad libitum* food access).

The average wild big brown bat mass is 15–25 g (Brigham 1986; Pearce et al. 2008). On the first day after capture, the bats weighed 9.7–16.6 g, so most were below the average wild mass. However, bats tend to overeat when accustomed to captivity (Orr 1958). So, three months later at the study's onset, only six of the 25 bats were in the average big brown bat mass range, while the rest were above—the heaviest of which weighed 43.3 g (***Supplementary Figure 1***).

In light of these observations, Renata has decided to undertake a different experiment regarding captive bat flight. This planned study will examine whether non-flight exercise (i.e. crawling) has an effect on big brown bat flight ability and willingness. The study will use a Promethion Core Metabolic Treadmill to exercise a group of bats, and compare their flight ability and willingness to a control group that is not exercised on the treadmill. Flight time will be a measure of flight ability, whereas the number of "shakes" it takes for a bat to take-off (henceforth termed shakes) will be a measure of flight willingness. We hypothesize that crawling exercise can help captive bats lose mass, and regain their flight ability and willingness. We predict that, compared to control bats, exercised bats will i) decrease in mass, ii) decrease in number of shakes, and iii) increase in flight time. As this is a future study, we do not have any data to analyze yet. Thus, for this project, we have simulated and analyzed data based on expected parameters and observations.

DATA SIMULATION

We simulated data for mass (g), flight time (s), and attempted to simulate shakes. This is a repeated measures study with a sample of 30 captive bats (15 exercised, 15 control), whose flight and weight are recorded every three days over 60 days. Simulating the data involved setting reasonable parameters, checking that the parameters result in simulated data resembling what we expect, then repeating the simulation 2500 times.

Mass

Based on pilot work, we know that bats are not motivated to crawl on the treadmill without a reward. They will, however, crawl if they are fasted and only rewarded with food while crawling to the front of the treadmill. We expect that the bats will all lose mass throughout the study because they will be fasted and only fed during trials, with the exercise group losing slightly more mass because of the extra energy expenditure (***Fig. 1***).

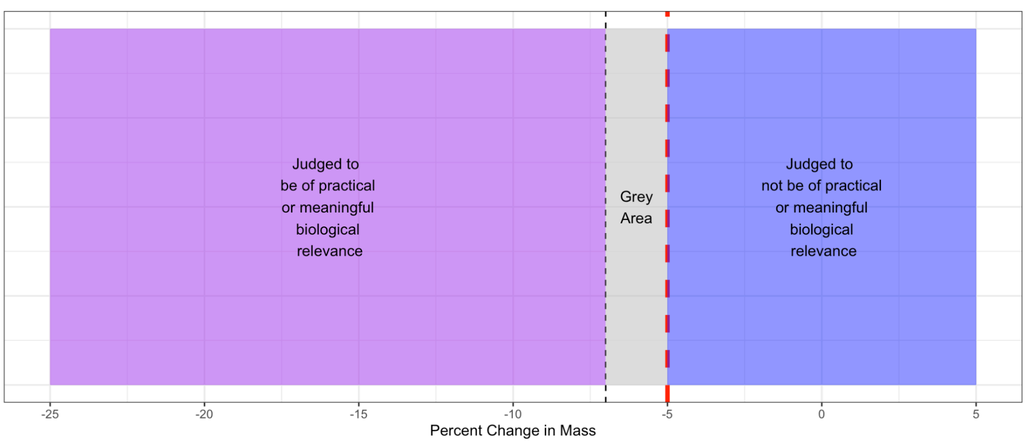

Figure 1. Meaningful percent change in mass for exercise group. *After 60 days, we expect the control group to lose 5% of their original mass on average* (red dashed line). *For the exercise group, a decrease of 5% or any increase in mass would not be of meaningful biological relevance* (blue). *An additional decrease between 5% to 7% would be of some meaningful relevance* (grey area). *An additional decrease >7% would be of meaningful biological relevance* (purple area). *Figure created by the 'BoMm_figure.R' file, in the Batflight repo.*

We simulated bat mass as a continuous response variable to examine how the treatment (exercise vs. control) and the day within the study would affect mass. We used a generalized linear mixed model with a Gaussian distribution with an intercept term, Day, Treatment, and the interaction between Day and Treatment as fixed effects. We included Day nested within Bat ID as a random effect because we expect some correlation across days within the same bat (i.e. individual bat variation in the intercept and slope of the trial day).

For the parameters, we set the initial mass (intercept) to 30 g, average mass loss for the control group to -1.5/60 g, and the average additional mass loss for the exercise group to -3/60 g. For the random effects, we set the standard deviation (SD) of the intercept, slope, and the residual variance to 5, 1/60, and 0.5, respectively. The correlation between the intercepts and slopes was set to -0.75, which was inversely transformed in the simulation. We simulated a data set with these parameters and the plotted data looked reasonable based on our predictions and what we decided were meaningful mass changes (***Fig. 2***).

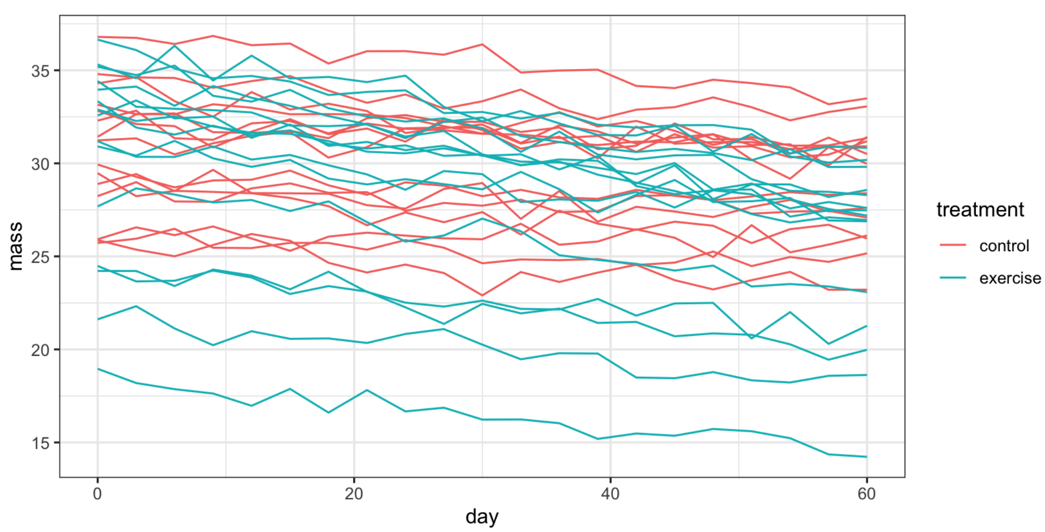

Figure 2. Simulated bat mass (g). *Mass data simulated using set parameters, based on what we expect to see over the 60-day experiment. Both groups lose mass, but the exercised group loses more mass on average than the control bats. Figure created by the 'simulate_mass_data.R' file in the Batflight repo.*

Flight time

The bats will be exercised through crawling, not flight, and so will not necessarily exercise the same muscles. For example, the serratus muscles are stretched and contracted specifically during the upward and downward wingstrokes, respectively (Neuweiller, 2000). Therefore, we do not expect large increases in flight time from crawling exercise. Instead, we expect an increase in the order of seconds. Also, because the control group is exercised somewhat during flight trials, we expect their flight time to increase slightly, but not as much as the exercise group (***Fig. 3***).

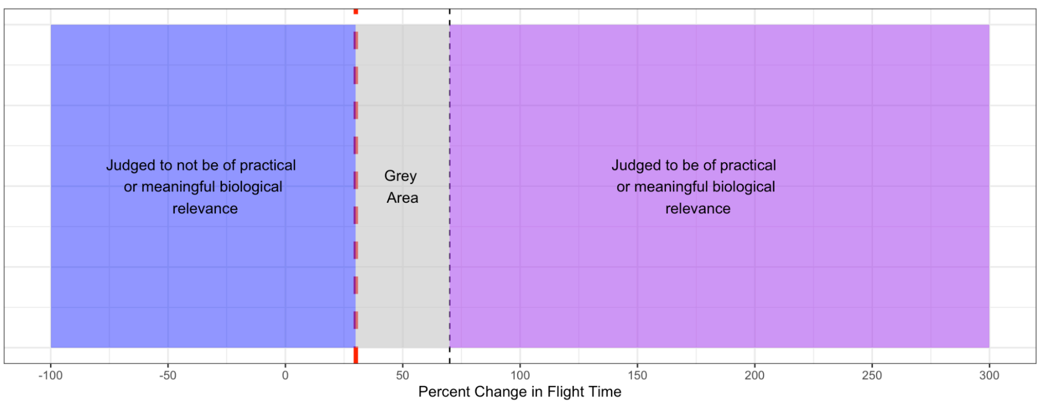

Figure 3. Meaningful percent change in flight time for exercise group. *After 60 days, we expect the control group's flight time to increase by 30% of their original time on average* (red dashed line). *For the exercise group, an increased flight time of 30% or any decrease would not be of meaningful biological relevance* (blue area). *An additional flight time increase between 30% and 70% would be of some meaningful relevance* (grey area). *An additional increase >70% would be of meaningful biological relevance* (purple area). *Figure created from the 'BoMm_figure.R' file in the Batflight repo.*

We simulated flight time as a continuous response variable with treatment and the day of the study as predictor variables. We used the same model format as for the mass data. For the parameters, we set initial flight time (intercept) to 2 s, average flight time increase for the control group to 0.5/60 s, and the average additional flight time gain for the exercise group to 2/60 s. For the random effects, we set the SD of the intercept, slope, and the residual variance to 0.5, 0.5/60, and 0.1, respectively. Finally, we set the correlation between the intercepts and slopes to 0.75, which was inverse transformed in the simulation. Again, we simulated a data set with these parameters and the plotted data looked reasonable based on our predictions and what we decided were meaningful flight time changes (***Fig. 4***).

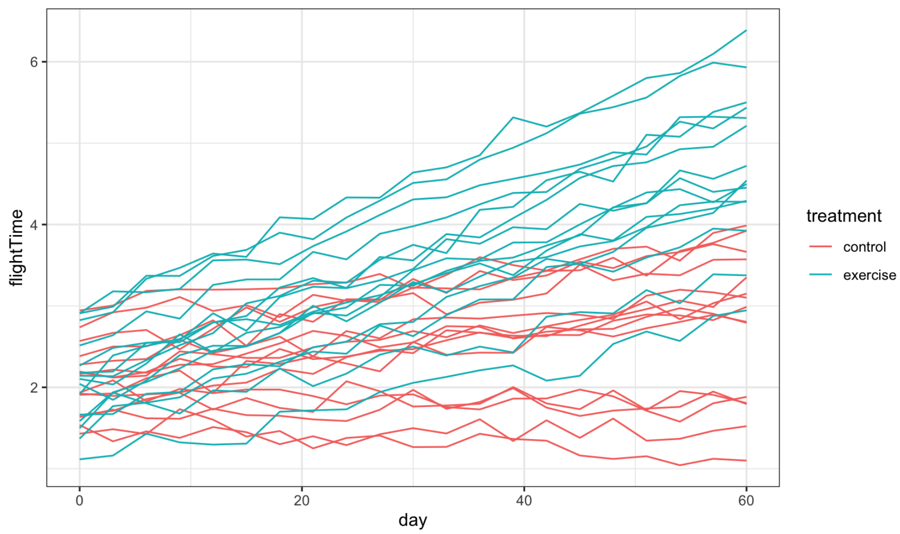

Figure 4. Flight time (seconds) simulated using set parameters, based on what we expect to see over the 60-day experiment. *Flight time increases for both groups, but the exercise group has a slightly larger increase than the control group. Figure created by the 'sim_flight_data.R' file, found in the Batflight repo.*

DATA ANALYSIS

Mass

We ran the simulation 2500 times, iteratively fitting the model, and extracting coefficient estimates (CEs) and confidence intervals (CIs) for each using the profile CI method (***Fig. 5***).

```
     sim                                 term           est                 lwr
 Length:7500        (Intercept)            :2500   Min.   :-0.06913   Min.   :-0.08014
 Class :character   day                    :2500   1st Qu.:-0.04599   1st Qu.:-0.05680
 Mode  :character   day:treatmentexercise:2500   Median :-0.02507   Median :-0.03341
                                                   Mean   : 9.97719   Mean   : 9.36821
                                                   3rd Qu.:29.41899   3rd Qu.:27.57154
                                                   Max.   :33.14796   Max.   :31.38195
      upr
 Min.   :-0.05928
 1st Qu.:-0.03492
 Median :-0.01671
 Mean   :10.58617
 3rd Qu.:31.18639
 Max.   :35.11870
```

Figure 5. Summary of 2500 bat mass simulations. *Found in 'use_sim_data.R' file in the Batflight repo.*

From these simulations, we were interested in the CEs and CIs for the effect of treatment across days. Specifically, we wanted to know how the CEs and CIs for the exercise group compared to the true simulated value we set (the average additional decrease in mass for the exercise group; -3/60). So, we filtered only the rows where the value in the term column equaled "day:treatmentexercise" (henceforth termed day:treatmentexercise values) and examined the proportion of CIs that did not contain the simulated true value. The proportion of CIs where the lower bound was greater than the simulated true value was 0.0392, and the proportion of CIs where the upper bound was lower than the simulated true value was 0.0308. We expect the proportion of CIs that are too high and too low to be ~0.025 (2.5%) for 95% confidence, so these seem somewhat high for our simulations. Also, the mean width of the CIs was 0.0217 and the proportion of CIs whose upper bounds were lower than 0 was one, indicating a negative effect (***Fig. 6***).

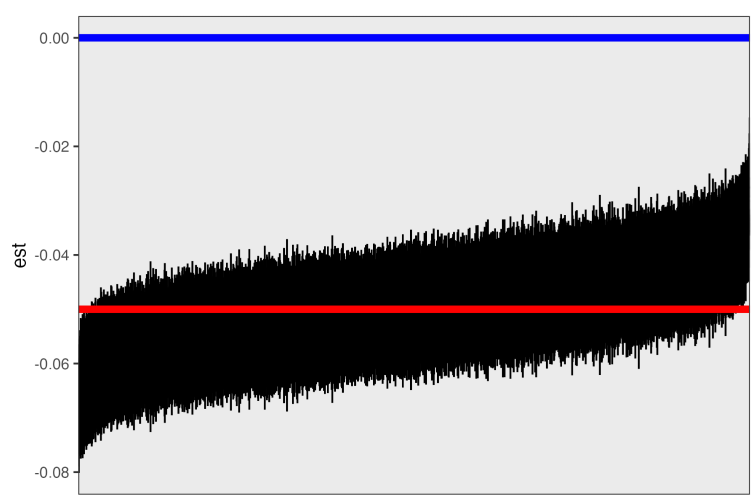

Figure 6. CEs and CIs for mass day:treatmentexercise values from 2500 simulations. *day:treatmentexercise CEs and their CIs (*black*) plotted in order of increasing estimate value.* Red line *represents the simulated true value of the parameter (-3/60).* Blue line *represents the reference line for effect direction (at 0 to check whether CIs are lower). Figure created by the 'use_sim_data.R' file, found in the Batflight repo.*

We also evaluated a linear model for mass that included the fixed effects without random effects. Following a very similar procedure, we ran 4000 simulations, iteratively fitting the new model and extracting the CEs and profile CIs (***Fig.*** *7*).

```
summary(cis)
  sim_mass                           term             est                lwr                upr
Length:12000       (Intercept)           :4000   Min.   :-0.05509   Min.   :-0.05732   Min.   :-0.05287
Class :character   day                   :4000   1st Qu.:-0.04920   1st Qu.:-0.05142   1st Qu.:-0.04698
Mode  :character   day:treatmentexercise:4000   Median :-0.02503   Median :-0.02746   Median :-0.02261
                                                 Mean   : 9.97502   Mean   : 9.94830   Mean   :10.00175
                                                 3rd Qu.:29.97331   3rd Qu.:29.89775   3rd Qu.:30.04881
                                                 Max.   :30.14408   Max.   :30.07219   Max.   :30.21597
```

Figure 7. Summary of 4000 mass simulations without random effects. *Found in 'mass_sims_without_random.R' file in the Batflight repo.*

We were interested in how the day:treatmentexercise CEs and CIs compared to the true simulated value of the additional effect of exercise on mass. With the linear model, the proportion of CIs where the lower bound was greater than the true simulated value was 0.0242, and the proportion where the upper bound was lower was 0.0255, closer to the 0.025 we expect. The mean CI width was 0.00446, which is smaller than previously, and the effect was still clearly negative (***Fig. 8***).

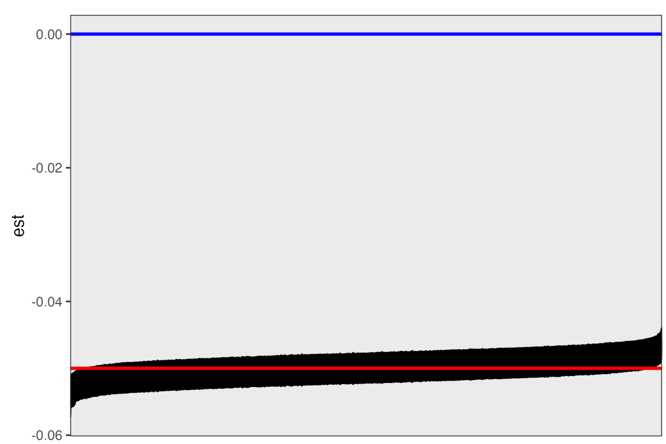

Figure 8. CEs and CIs for mass day:treatmentexercise values without random effects from 4000 simulations. *day:treatmentexercise CEs and their CIs (*black*) plotted in order of increasing estimate value.* Red line *represents the simulated true value of the parameter (-3/60).* Blue line *represents the reference line for effect direction (at 0 to check whether CIs are lower). Figure created by the 'mass_sims_without_random.R' file, found in the Batflight repo.*

Flight time

We ran the simulation 2500 times, iteratively fitting the model, and extracting the CEs and profile CIs for each (***Fig. 9***).

```
 sim_flight                          term           est
Length:7500       (Intercept)          :2500   Min.   :0.0004864
Class :character  day                  :2500   1st Qu.:0.0096751
Mode  :character  day:treatmentexercise:2500   Median :0.0333265
                                               Mean   :0.6800297
                                               3rd Qu.:1.9338699
                                               Max.   :2.3642061
     lwr                upr
Min.   :-0.003354   Min.   :0.00431
1st Qu.: 0.005750   1st Qu.:0.01370
Median : 0.028200   Median :0.03854
Mean   : 0.616446   Mean   :0.74361
3rd Qu.: 1.750916   3rd Qu.:2.11639
Max.   : 2.199871   Max.   :2.52854
```

Figure 9. Summary of 2500 flight time simulations. *Found in 'use_sim_data.R' file in the Batflight repo.*

For flight time, we were interested in how the day:treatmentexercise CEs and CIs compared to the true simulated value of the additional effect of exercise on flight time (2/60). The proportion of CIs where the lower bound was greater than simulated true value was 0.0332 and the proportion where the upper bound was lower was 0.0284. Again, we expect the proportion of CIs that are too high and too low to be ~0.25, so these values for flight time are also somewhat high. Furthermore, the mean width of the CIs was 0.0104 and the proportion of CIs whose lower bounds were higher than 0 was one, indicating a positive effect (***Fig. 10***).

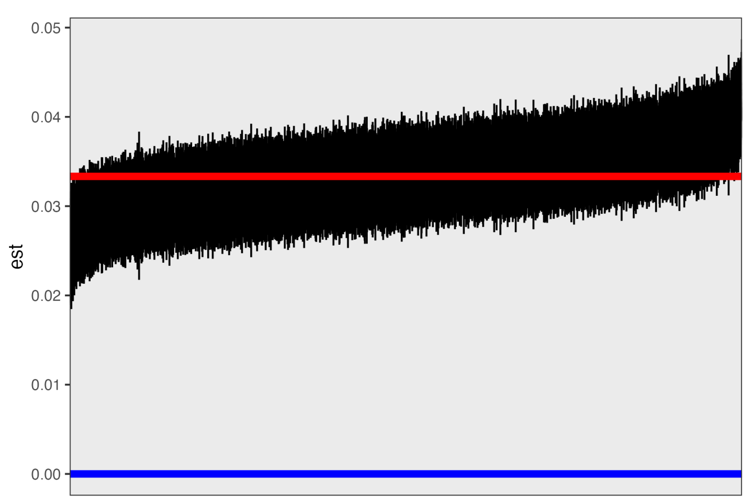

Figure 10. CEs and CIs for flight time day:treatmentexercise values from 2500 simulations. *day:treatmentexercise CEs and their CIs (*black*) plotted in order of increasing estimate value.* Red line *represents the simulated true value of the parameter (2/60).* Blue line *represents the reference line for effect direction (at 0 to check whether CIs are higher). Figure created by the 'use_sim_data.R' file, found in the Batflight repo.*

We also evaluated a linear model for flight time that included the fixed effects without random effects. Following a very similar procedure, we ran 4000 simulations, iteratively fitting the new model and extracting the CEs and profile CIs (***Fig. 11***).

```
> summary(cis)
 sim_flight                          term              est                lwr
Length:12000       (Intercept)          :4000   Min.   :0.007453   Min.   :0.006966
Class :character   day                  :4000   1st Qu.:0.008501   1st Qu.:0.008018
Mode  :character   day:treatmentexercise:4000   Median :0.033336   Median :0.032892
                                                Mean   :0.680501   Mean   :0.675163
                                                3rd Qu.:1.994524   3rd Qu.:1.979396
                                                Max.   :2.028136   Max.   :2.013197
      upr
Min.   :0.007941
1st Qu.:0.008989
Median :0.033780
Mean   :0.685839
3rd Qu.:2.009660
Max.   :2.043074
```

Figure 11. Summary of 4000 flight time simulations without random effects. *Found in 'flight_sims_without_random.R' file in the Batflight repo.*

Again, we were interested in how the day:treatmentexercise CEs and CIs compared to the true simulated value of the additional effect of exercise on flight time (2/60). With the linear model, the proportion of CIs where the lower bound was greater than the simulated true value was 0.0215 and the proportion where the upper bound was lower was 0.0242 (***Fig. 12***). These proportions are closer to the expected 0.025, but still not exact. Also, the mean CI width was 0.000891, much smaller than previously, and the effect was still positive.

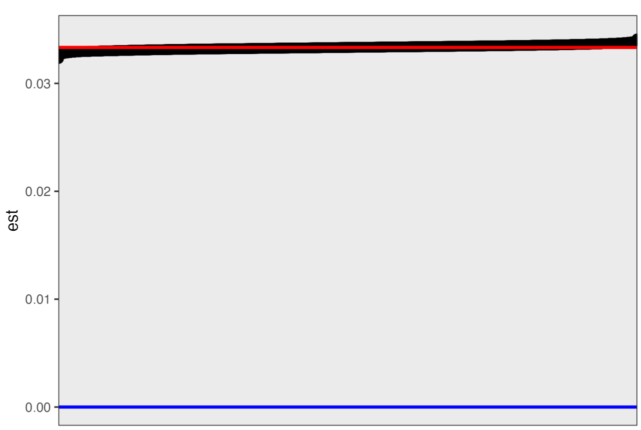

Figure 12. CEs and CIs for flight time day:treatmentexercise values without random effects from 4000 simulations. *day:treatmentexercise CEs and their CIs (*black*) plotted in order of increasing estimate value.* Red line *represents the simulated true value of the parameter (2/60).* Blue line *represents the reference line for effect direction (at 0 to check whether CIs are higher). Figure created by the 'flight_sims_without_random.R' file, found in the Batflight repo.*

DISCUSSION

We attempted to simulate the shake data but encountered warning messages and challenges with the parameters that we couldn't resolve. As the number of shakes are count data on the interval scale rather than continuous like the mass and flight time, we planned to run a generalized linear mixed model with the same fixed and random effects as our other models, but with a negative binomial distribution instead of Gaussian. However, the plot of a simulated shake data set did not look reasonable, and we were unsure how to correct it (***Supplementary Fig. 2***). For example, we set the intercept to 2, but the intercept of the plotted data set seems to average around 7.5. This shows that some aspects of the parameters were incorrect.

We also attempted to simulate flight time with simulated mass as a predictor variable but ran into some complications. Here, we needed to add extra parameters to the simulation to account for the effect of mass on flight time. However, we did not know how exactly to add these parameters to the code or what were reasonable values. Without these parameters, the main effect of mass and the effect of its interactions with day, treatment and day:treatment were essentially 0 for 1000 simulations (***Supplementary Fig. 3***).

Nonetheless, we simulated mass and flight time data, and observed that our models worked almost as expected. For the model including random effects, the proportion of CIs that were too high and too low were somewhat inflated and might be worth examining further. Still, we hope this simulation study will be comparable to the results from the real experiment, once completed.

References

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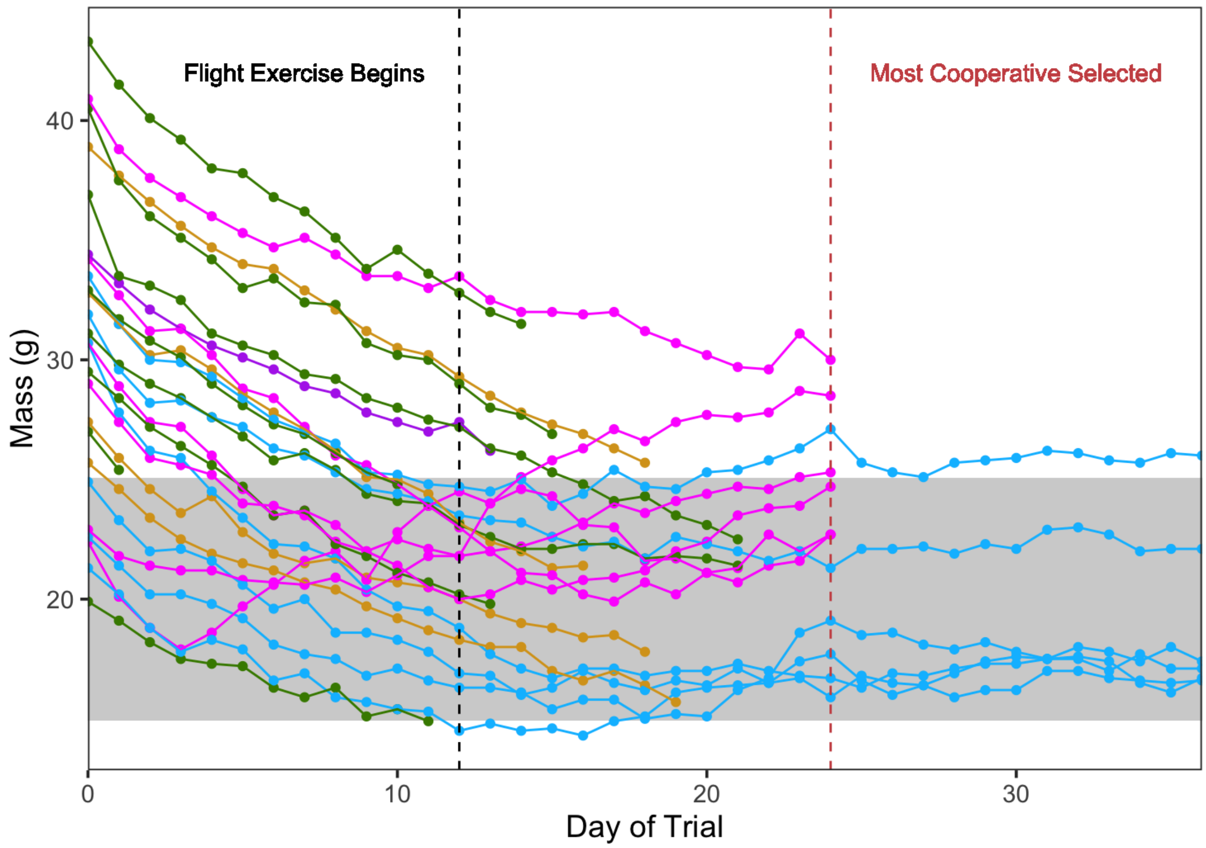
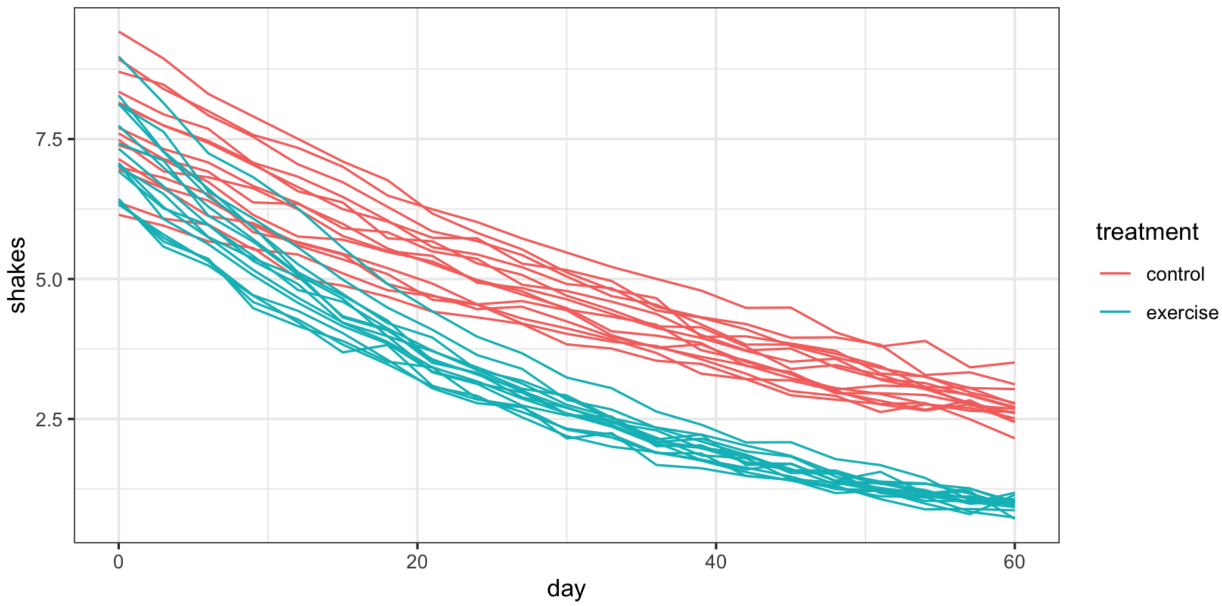
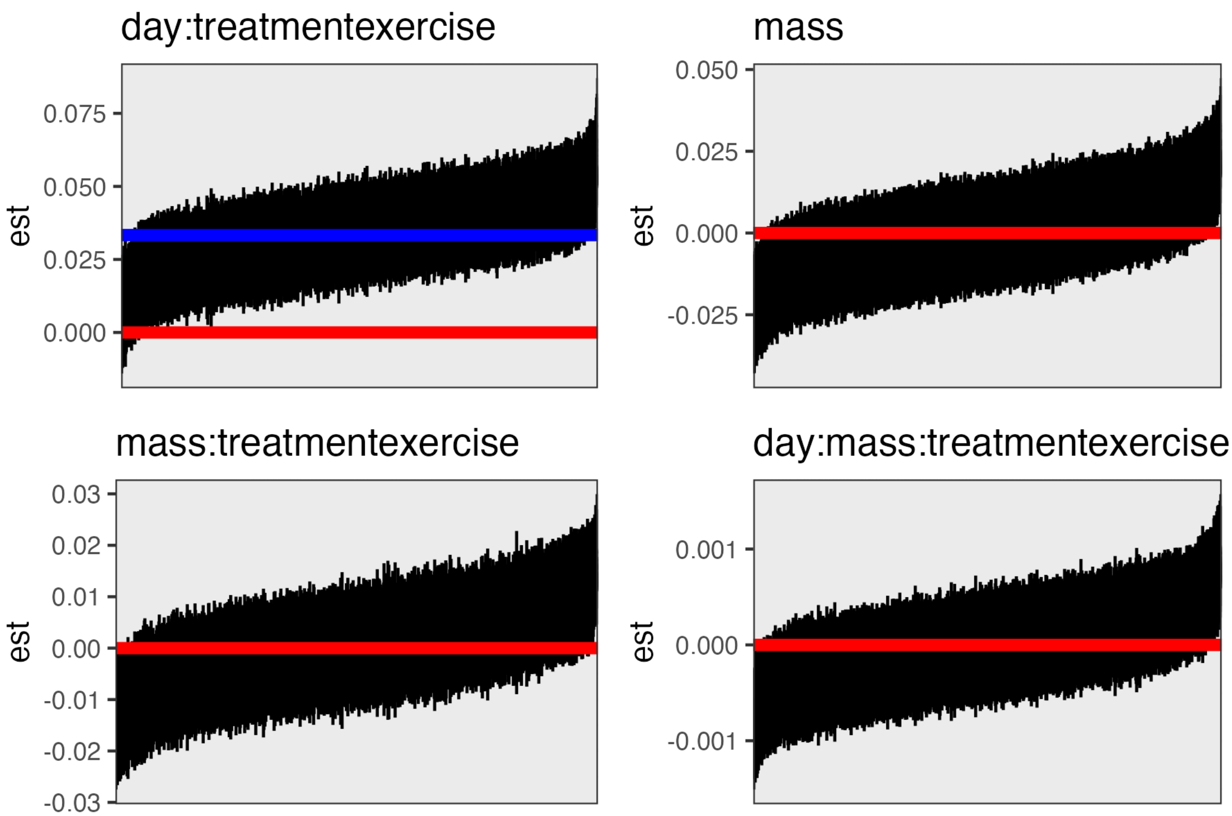
SUPPLEMENTAL MATERIAL



Supplementary Figure 1: Big brown bat masses during experiment attempt. *Day 0: bats were moved to the colony and food was placed in the room centre.* Black dashed line *represents when nightly 10 min flight exercise began;* red dashed line *represents when the six most cooperative bats (*blue*) were selected and fasted (fed during exercise) until study termination, while the rest of the bats were removed at this time (*pink*). Others were removed beforehand for various reasons: refusing to take-off from hand (*purple*), not eating (*yellow*), health concerns (*green*).* Grey area *is the average wild big brown bat mass. Figure not created by a file in the Batflight repo.*



Supplementary Figure 2. Attempted number of shakes simulation using set parameters, based on what we expect to see over the 60-day experiment. *We set the initial number of shakes (intercept) to 2, average shake decrease for the control group to -1/60, and the average additional shake loss for the exercise group to -2/60. For the random effects, we set the SD of the intercept, the slope, and the residual variance to 0.1, (0.1/60), and 0.1, respectively, and the correlation between the intercepts and slopes to 0.75. Figure created by the 'Shakes.R' file, found in the Batflight repo.*



Supplementary Figure 3. CEs and CIs of interest from attempting to including mass as a predictor variable on flight time in 1000 simulations. Top left *are the values for day:treatmentexercise, with* blue line *representing the true simulated value (2/60) and* red line *at 0.* Top right *are the CEs and CIs for mass;* bottom left *are for mass:treatmentexercise;* bottom right *are for day:mass:treatmentexercise.* Red line *represents a coefficient estimate of 0 in each figure. Figure created by the'flight_mass_predictor.R' file of the Batflight repo.*