CAPTIVE BIG BROWN BAT FLIGHT

EXPLORING METHODS TO MAINTAIN AND IMPROVE CAPTIVE BIG BROWN BAT (*EPTESICUS FUSCUS*) FLIGHT

By

RENATA SOLJMOSI, Hons. BSc

A Thesis Submitted in Partial Fulfillment of the Requirements for the

Degree of Master of Science

in the Department of Psychology, Neuroscience & Behaviour

Faculty of Science

McMaster University MASTER OF SCIENCE (2024) Hamilton, Ontario

Department of Psychology, Neuroscience & Behaviour

TITLE: Exploring methods to maintain and improve captive big brown bat (*Eptesicus fuscus*) flight

AUTHOR: Renata Soljmosi, Hons. BSc (McMaster University)

SUPERVISOR: Dr. Paul A. Faure

NUMBER OF PAGES: xvii, 125

Lay Abstract

Wild-caught big brown bats seem to gain mass and lose their flight ability in captivity. To show this, I recorded big brown bat masses and flight times about one week after they were caught, then again 11 weeks later. During this time, the bats were housed in small cages where they could not fly and had unrestricted food availability. I found the bats had higher masses and shorter flight times after 11 weeks. The second portion of this study explored methods to improve flight of big brown bats that had been in captivity for at least one year. Specifically, I examined whether diet and exercise could improve their flight ability. I flew the bats six times per week for 7.5 weeks. Limiting their food intake, and being housed in a large space improved flight times. In conclusion, to improve flight ability, bats can be put on a restricted diet, or can be housed in a space that allows flight while also being explicitly flown a few times per week. Also, to keep wild-caught bats healthy during their first few months after being captured, they should be housed in a larger space that allows flight, rather than in small cages where they cannot fly.

Abstract

Wild-caught big brown bats (*Eptesicus fuscus*) experience increased mass, diminished ability to sustain flight, and increased take-off latency after three months in captivity, making them ineligible for flight experiments. These animals had been housed in small cages with *ad libitum* food. I quantified these changes by weighing and flying newly-caught *E. fuscus*, housing them under the mentioned conditions, and re-recording them after 11 weeks in captivity. I used LMMs to analyze data. Surprisingly, take-off latency decreased somewhat, though the existence of a true effect was inconclusive. Conversely, the bats had increased mass and decreased flight duration, as expected. The second part of this study examined methods, namely food availability and crawling exercise, to improve *E. fuscus* flight. I housed some subjects in a large colony space that allowed free flight, and I did not explicitly exercise them. I housed other subjects in small cages that restrict exercise, and exercised some through crawling, which I hypothesized would improve flight. Finally, I restricted some subjects' food intake and gave *ad libitum* access to others. I hypothesized the restricted diet would improve flight, while *ad libitum* access would diminish it. I recorded flights three times per night, twice a week for 7.5 weeks. I analyzed the data using linear mixed-effects models. Restricting food intake had a positive effect on flight duration; *ad libitum* food access did not have a conclusive effect. Also, the crawling exercise did not positively affect flight; bats housed in the colony had increased flight duration. Apparently, having space for voluntary flight and being explicitly flown twice per week can improve flight. Take-off latency did not have conclusive results, though surprisingly, the bats on restricted diets had somewhat increased take-off latencies. In conclusion, restricting diet or explicitly flying bats housed

in the colony are two methods that can be employed to improve flight duration to allow for scientific studies requiring flight.

Acknowledgements

I would first like to thank my supervisor, Dr. Paul Faure, for introducing me to the bats, and for his continued support and guidance throughout my years in the McMaster Bat Lab. I would also like to thank my Supervisory committee, Dr. Sigal Balshine, Dr. Reuven Dukas, and Dr. David Shore for their guidance in completing this thesis.

Thank you to the hard-working undergraduate crew of the Bat Lab. I could not have completed my data collection without the help of undergraduates Shiao-Tang Guo, Tristan Gysling, Ella Keown, Riley Morgan, Joanna Zhu, and Swasthika Swaminathan.

My thanks also goes out to the Welch Lab at the University of Toronto Scarborough for lending me their mouse treadmill to use for this thesis. Putting McMaster's big brown bats on a treadmill was a truly unique experience.

Thank you to Dawn Graham, Kathleen Delaney, and the staff of the Central Animal Facility for their continued care of McMaster's big brown bats.

I thank my cohort friends in the Free Support Group—Dania, Grace, Jackie, Maya and Reshna—who have supported and encouraged me from the moment I met them.

I also thank my family: Rita, Arnold, Adrianne, and Tom. They have encouraged and empowered my love of bats and science, and I could not have made it this far in my academic career without their support.

Finally, I thank my partner, Carl Lyons, for always taking the time to help me throughout my completion of this thesis. Thank you for motivating and inspiring me every day.

Actually, finally, thank you to the bats. You're cool.

Table of Contents

List of Figures

List of Tables

List of Appendix Figures

List of Appendix Tables

List of Abbreviations and Symbols

Standard metric units were used unless otherwise specified

95% CIs	95% confidence intervals
β	beta (i.e. estimate) coefficient
E. fuscus	*Eptesicus fuscus*; big brown bat
EMM	estimated marginal mean
df	degrees of freedom
D. rotundus	*Desmodus rotundus*; common vampire bat
LMM	linear mixed-effects model
ln()	variable transformed using the natural logarithm function
n	sample size
No tread./rest. diet	no-treadmill/restricted-diet group
No tread./unrest. diet	no-treadmill/unrestricted-diet group
p	p-value
P. livingstonii	*Pteropus livingstonii*; Livingstone's fruit bat
SD	standard deviation
SE	standard error
t	t statistic
T. molitor	*Tenebrio molitor*; mealworm
Tread./rest. diet	treadmill/restricted-diet group

Declaration of Academic Achievement

Exploring methods to maintain and improve captive big brown bat (*Eptesicus fuscus*) flight

Authors: Renata Soljmosi

The design of this study was conceived primarily by R.S., under the supervision of P.A.F. R.S. performed experiments with help from undergraduate students in the McMaster Bat Lab. R.S. analyzed data, prepared figures, and wrote manuscript. P.A.F. revised manuscript.

Introduction

Animals in captivity rely on their human caretakers for survival. The animals must be provided their basic needs, including food, water, habitat, and hygiene (Jordan, 2005). However, measures should be taken beyond providing basic needs to maintain captive animal welfare. For example, the animals should have opportunities to experience infrastructural and behavioural enrichment, interact with others (for social species), and escape from fear and distress. In essence, caretakers should allow captive animals to express their natural instincts and behaviours (Jordan, 2005). Captive animals used in scientific research should also be encouraged to exhibit natural behaviours to improve external validity.

A natural behaviour of wild animals is movement; they exercise because their survival may depend on their ability to forage, escape predation, migrate, and more. However, captive animal counterparts often face excess weight gain because they do not or cannot express the same movement when not housed properly. Firstly, caretakers tend to overfeed captive animals. For example, captive lemurs of different species have greater weight when overfed calorie-rich foods than when fed a balanced diet (Mellor et al., 2020). Secondly, the captive environment generally restricts activity, which can lead to lack of exercise. Broom (2002) has suggested that captive animals should be able to express normal locomotion for at least five seconds. However, the type of physical enclosure still affects activity and weight. For example, male captive lemurs experience weight gain when their enclosures contain fixed structures, rather than flexible structures requiring more energy expenditure to climb (Mellor et al., 2020). Finally, once overweight, animals such as cynomolgus macaques (*Macaca fascicularis*) are more likely to be inactive than normal-weight counterparts, further compounding the problem of

weight gain (Bauer et al., 2012). Overall, overeating and/or lack of exercise in captivity cause an imbalance between energy intake and energy expenditure, contributing to excess, unnatural weight gain (McMillan, 2013).

The present study examines captive bat (Order: Chiroptera) flight, which is a natural behaviour for wild bats, but seems to be negatively affected by overeating and lack of exercise in captivity. Specifically, I explore whether controlled diet and exercise can improve captive big brown bat (*Eptesicus fuscus*) flight.

Project background

McMaster University houses Canada's only captive research colony of insectivorous bats available for year-round research. I planned to study the species housed, *E. fuscus* (family: Vespertilionidae), in a flight behaviour task. I began the research using wild-caught bats that had been in captivity for approximately three months before project onset. However, when I attempted to fly these animals, most did not maintain powered flight and immediately landed on the floor. Some also displayed a relatively long latency to initiate flight from my hand.

As per the protocol for housing newly-captive bats, these animals were quarantined in small metal cages (28.0 × 21.0 × 20.5 cm; l × w × h) with *ad libitum* access to food (mealworms; *Tenebrio molitor*). Like other mammalian species, bats tend to overeat when they become accustomed to *ad libitum* food access (Orr, 1958). Also, compared to wild *E. fuscus*, whose flight times average 100 minutes per night (Kurta & Baker, 1990), the captive bats had no room to fly and thus experienced greatly decreased physical activity during the three months.

Upon capture, the bats' masses were 9.7–16.6 g, which were on the lower end, or under, the average wild *E. fuscus* mass of 11–25 g (Brigham, 1987; Kurta & Baker, 1990; Pearce et al., 2008). However, at my study's onset, only six of the 25 bats were in the average mass range, while the rest were above—the heaviest of which weighed 43.3 g. I hypothesized the bats did not fly well due to increased food intake from a consistently available food supply and decreased physical activity in captivity, which resulted in them becoming overweight.

Similar observations have been recorded in wild-caught Livingstone's fruit bats (*Pteropus livingstonii*). Upon capture, 10 adult male *P. livingstonii* experienced weight gain due to lack of exercise and year-round good quality food. Within 1.5–2 years of captivity, the bats' masses had increased 16% on average (Wormell et al., 2018)

From my observations, I pivoted my research toward bat husbandry; I focused on methods, namely restricted food availability and exercise, that may improve captive *E. fuscus* flight. In the wild, bats rely on flight as their primary method for locomotion. So, it is important for bat welfare for them to be able to continue to fly in captivity. It is also important for scientific research requiring bat flight.

Bats in captivity

Bats may be held in captivity for different purposes, including public education (e.g., zoos), wildlife rehabilitation, or research. Regardless of the purpose, a fundamental goal of maintaining captive bat welfare is to create an environment that best mimics features encountered in the wild. This is so the captive bats can continue to display their natural behaviours.

Firstly, bat captivity requires a proper enclosure. Ideally, long-term enclosures should be large enough for free flight (Barnard, 2011). However, at minimum, all enclosures must be large enough for the bats to spread their wings without touching the sides of the enclosure. To allow for some movement and exercise, non-flight enclosures should have heights at least: (1) 60 cm for bats with mass up to 50 g, (2) 90 cm for bats between 50–150 g, or (3) 120 cm for bats over 150 g (Barnard, 2011; Skrinyer et al., 2017).

Captive bats also require adequate diet and nutrition. However, caretakers tend to over-supplement captive bat diets with vitamins, minerals (especially calcium), and other supplemental products. Bats have adapted to handle limited deficiencies in their diet, so over-supplementing them can sometimes be toxic (Barnard et al., 2011). McMaster's captive bats receive live *T. molitor* to mimic their wild diet of mostly Coleoptera. Though, the *T. molitor* are fortified with supplements such as wheat semolina and powdered milk to increase nutritional content (Skrinyer et al. 2014). It is important to know which foods captive bats require, but also how and to what extent they should be supplemented.

Furthermore, the quantity of insects that *E. fuscus* and other insectivorous species should consume nightly depends on several factors, including the season, ambient temperature, genetic disposition, daily activity levels, body size, and overall health; there is no definitive rule (Barnard et al., 2011). The two approaches to feeding captive insectivorous bats involve either feeding the number of insects necessary to maintain a normal mass for the species, or allowing *ad libitum* access. The latter works well for most insectivorous species, though species like *E. fuscus* are known to overeat and become overweight, especially when not provided with enough space to fly and exercise (Barnard

et al., 2011; Skrinyer et al. 2014). So, the level of food consumption should be relative to physical activity.

Bat flight

Wild bats complete most of their exercise through flight. They achieve flight with thin, flexible wings, and two major flight muscles; the pectoralis muscle, which drives the wing's downstrokes during flight, and the biceps brachii muscle, which folds the wing at the elbow during downstroke (Cao & Jin, 2020; Cheney et al., 2014; Von Busse et al., 2012). Wild bats rely on flight for many behaviours crucial to their survival.

Firstly, bats access an array of roost types through flight, including caves, trees, foliage, and buildings. They spend over half their lives roosting. As most bats are nocturnal, they use day roosts to sleep and night roosts to rest between feeding bouts, ingest food transported from nearby foraging areas, or for some species, to perch in wait of prey (Kunz, 1982). Roosts provide a place to interact with other individuals, secure mates, avoid predation, and rear young. Also, sheltered roosts provide a stable microclimate against ambient temperature, and protection from sunlight and inclement weather (Kunz, 1982). Secondly, bats use flight to forage and meet their energy intake requirements. Bats must fly the commute to their foraging site, then return to a roost. Some bats, such as *E. fuscus* and European free-tailed bats (*Tadarida teniotis*), forage within 5 km of their roost (Kurta & Baker, 1990; Marques et al., 2004). Others, such as the greater noctule bat (*Nyctalus lasiopterus*), can fly over 100 km a night to meet both foraging and roosting requirements (Popa-Lisseanu et al., 2009). Finally, a considerable number of bat species migrate to survive seasonal changes. Though some temperate bat species in North America and Europe do not migrate—rather, they hibernate within a ≤ 50 km radius of their summer roost—others display 100–500 km regional migration, or

≥1000 km long-distance migration (Fleming, 2019). Conversely, tropical and subtropical bats do not hibernate, and can migrate up to 1500 km along seasonal food resource gradients (Fleming, 2019; Thomas, 1983). Because bats must fly to roost, forage, and migrate, it evidently follows that wild bat survival depends on their sustained ability.

Bat crawling

While all bats fly, many bat species can also crawl. Vampire bat species likely have the best crawling performance (Hermanson et al., 1993; Lawrence, 1969). Of the vampire bats, the common vampire bat's (*Desmodus rotundus*) crawling has been tested using motorized treadmills, revealing that it uses its wings to raise its body far off the ground and that it has exceptional crawling capability—moving forwards, backwards, and sideways, as well as running with a stereotyped gait distinct from walking (Riskin & Hermanson, 2005). A recent study capitalized on their unique running ability using respiratory treadmill equipment, and discovered that *D. rotundus* oxidize amino acids from recently ingested blood to power crawling exercise. Interestingly, no study has investigated the contribution of recently ingested blood in powering their flight (Rossi & Welch, 2024).

For most other bat species**,** the evolution of flight has resulted in diminished crawling ability, and relatively limited terrestrial locomotion compared to other mammals (Lawrence, 1969; Riskin & Hermanson, 2005). Some species, such as the greater horseshoe bat (*Rhinolophus ferrumequinum*), cannot crawl due to having short extensor thigh muscles (i.e. the quadriceps femores) which prevent full extension of the knee joints. Most other bat species have longer quadriceps femores muscles that exert longer and stronger knee joint extensions to support crawling (Kobayashi, 2018). Crawling also involves using forelimb muscles, like the pectoralis muscles used in flight (Hermanson et

al., 1993). Several forelimb muscles are larger in species that crawl well, such as *D. rotundus*, compared to species with moderate crawling ability. These include the pectoralis muscles, as well as the subscapularis, supraspinatus, rhomboideus, and triceps brachii muscles (Strickler, 1978). Also, vampire bats' pectoralis muscles contain type I fibers, which drive postural activities and slow, repetitive movements, likely supporting their upright postural crawling. Species with diminished crawling ability do not contain these fibers in their pectoralis muscles (Hermanson et al., 1993). Overall, most species will not prefer to crawl, but can when required to navigate the terrestrial environment.

E. fuscus has moderate crawling ability. *E. fuscus* crawl with a lateral gait (i.e. the same side forelimb and hindlimb move together), which is common in quadrupedal vertebrates (Jones & Hasiotis, 2023). Though, diagonal gait (i.e. opposite side forelimb and hindlimb move together) may be more common for bats (Lawrence, 1969). *E. fuscus* also has intermediate size pelvic and hindlimb morphology, but interestingly, has better crawling ability than non-vespertilionid bats with similar morphology (Jones & Hasiotis, 2023). These include the greater sac-winged bat (*Saccopteryx bilineata*) of the Emballonuridae family, which can only perform uncoordinated diagonal gait, and the Trinidadian funnel-eared bat (*Natalus tumidirostris*) of the Natalidae family, which cannot crawl (Jones & Hasiotis, 2018; Riskin et al., 2005). The present study aimed to capitalize on *E. fuscus*' moderate crawling ability.

Cross-training exercise

Specifically, the present study examined whether motivating *E. fuscus* to crawl can affect their flight. Interestingly, human research has shown that training in one modality can benefit other modalities. For example, resistance/strength training on its

own, or in addition to endurance exercises like running and cycling, can increase endurance capacity in sedentary and trained individuals. Resistance training can also improve running and cycling performance (Tanaka & Swensen, 1998). Furthermore, swim training is muscularly dissimilar to running, but can improve running performance in well-trained recreational runners (Foster et al., 1995). Improving aerobic health (e.g., increasing maximum energy uptake) through either running, swimming, or cycling can benefit performance in the other two modalities (Tanaka, 1994). Cross-training different exercises also benefits injured athletes by offering an alternative exercise to avoid reinjury during recovery (Tanaka, 1994).

Similarly, crawling exercise may benefit captive bat flight. Captive bats seem to maintain their crawling ability; I have observed that captive bats should be able to crawl, even if they have poor flight ability. As both crawling and flight require pectoralis muscle activation, crawling exercise may strengthen the muscle, which could translate to improved flight. Increased exercise in general should benefit bats' health by increasing energy expenditure, which may improve physical performance for flight. So, this study examined the effect of crawling on flight in *E. fuscus*, a species with moderate crawling ability.

The current study

As outlined in the *Project Background*, bats demonstrated poor flight after about three months in captivity. Bats typically landed on the floor, and some were hesitant to initiate flight. I hypothesized that inactivity and overeating in captivity, compared to the wild, caused this poor flight performance. My current study aims to test this hypothesis

by: (1) quantifying the decline in *E. fuscus* flight performance upon entering captivity, and (2) examining if non-flight exercise and controlling food availability can improve bat flight ability in individuals that have been in captivity for at least one year. Flight measures of interest include flight duration and take-off latency.

Experiment 1: Quantifying decline in flight performance. The purpose of this experiment was to test the effects of the typical quarantine housing conditions (see *Animals* under *Experiment 1 Methods* for details) on newly-captive bat mass and flight, in order to quantify my observations outlined in the *Project Background*. Newly captive bats were housed in small cages with *ad libitum* access to food and water. I recorded their mass and flight performance (i.e. flight duration and take-off latency) approximately one week after their capture, then restricted them in these housing conditions until I re-recorded them approximately 11 weeks later. I aimed to examine how these measures are maintained after the 11 weeks, and I hypothesized that the quarantine housing conditions would negatively affect mass and flight. So, I predict bats housed in these conditions for 11 weeks will have increased mass, decreased flight duration, and increased take-off latency compared to newly-captive bats.

Half of the bats in this experiment were pregnant at the time of capture and during the initial testing period, then gave birth and weaned pups before the second testing period. So, I was able to test whether pregnant versus non-reproductive individuals' differed in mass and flight performance, as well as whether females that were pregnant for the initial test differed when they were no longer pregnant or lactating.

Experiment 2: Methods to improve flight in captive bats. The second part of my study was an experiment on whether diet and crawling exercise could improve the

flight performance (i.e. flight duration and take-off latency) of bats that have been in captivity for at least one year.

Bats in this experiment were housed in the McMaster colony prior to study onset. The colony provides space for free flight, as is recommended for long-term captivity (Barnard et al., 2011). It also provides *ad libitum* food access. As these are the typical long-term housing conditions, I continued to house one experimental group in the colony for the study.

Conversely, McMaster's bats are sometimes housed in small cages for purposes such as observation and veterinary care. For this experiment, I moved bats from the colony into three small cages. The cages allowed me to manipulate their food access and level of exercise.

Firstly, the cages allowed me to examine whether restricted food access affects captive bat flight performance. Both the literature (Barnard et al., 2011; Orr, 1958; Skrinyer et al. 2014) and my observations suggested that bats with *ad libitum* access to food tend to overeat and become overweight. Though the bats in the McMaster colony have *ad libitum* food access, I have observed that bats can increase their food consumption when housed in smaller cages. In these cases, bats may eat more because it is easier to access food. So, I tested the effects on mass and flight of giving *ad libitum* food or limiting food intake, and instead hand-feeding bats a specific number of *T. molitor*.

Secondly, the cages allowed me to mostly restrict bats' physical activity, and increase exercise through crawling. Although flying seems like the most direct way to improve flight performance, I wanted to test whether a non-direct method could improve

captive bat flight. Pilot work suggested that a food motivator improved bat crawling performance. So, to not overfeed bats, I decided to exercise food-restricted bats by motivating them with food to crawl on a motorized treadmill. Research on bat crawling remains generally limited, and to my knowledge, there are no previous studies examining the effect of crawling exercise on bat flight. Therefore, this research is novel.

Overall, I compared mass and flight performance of four bat groups:

- colony group, a group housed in the colony with no-treadmill exercise and unrestricted diet;
- no-treadmill/unrestricted-diet group, a group housed in a small cage with no treadmill exercise and unrestricted diet;
- no-treadmill/restricted-diet group, a group housed in a small cage with no treadmill exercise and restricted diet and;
- treadmill/restricted-diet group, a group housed in a small cage with treadmill exercise and restricted diet.

I hypothesized that the colony conditions would not affect the group's mass, flight duration, or take-off latency because they remained in their typical housing conditions. I also hypothesized that moving bats into small cages with *ad libitum* food and no exercise would affect mass, so I predict the bats in the no-treadmill/unrestricted-diet group should experience an increase in mass compared to the colony group. I also hypothesized that the *ad libitum* diet affects flight performance, and I predict the bats in the no-treadmill/unrestricted-diet group should have decreased flight duration and increased take-off latency. Conversely, I ensured that the no-treadmill/restricted-diet and treadmill/restricted-diet bats decreased in mass, while remaining within the average wild

E. fuscus mass range. I hypothesized that the restricted diet would improve flight performance, so I predict these bats will have increased flight duration and decreased take-off latency. Finally, if crawling exercise improves flight, then I predict the treadmill/restricted-diet group, relative to the no-treadmill/restricted-diet group, will have a greater increase in flight duration and greater decrease in take-off latency.

I also recorded the four groups' masses over 34 days after completing the experiment and returning all the bats to the colony. This was to observe how the cage group bat masses responded to returning to a larger space with *ad libitum* food access.

Overall, *Experiment 2* examined methods to improve captive bat flight. If I am able to improve their flight performance, these methods could be implemented prior to future research projects requiring flight. Also, if crawling can improve bat flight, it can be implemented in rehabilitating injured or sick animals that have temporarily lost their flight ability. Overall, factors that improve animal health and allow bats to exhibit natural behaviours in captivity positively affect their welfare. As wild *E. fuscus* fly frequently, improving and maintaining their flight ability in captivity would positively affect their wellbeing.

Methods

Ethics statement

All experimental procedures were approved by the Animal Research Ethics Board of McMaster University and conformed to the *Guide to the Care and Use of Experimental Animals* published by the Canadian Council of Animal Care.

Animals

37 *E. fuscus* were used in this study (33 females, 4 males; Appendix Table 1). All bats were wild-caught as adults and held in captivity at McMaster University. Each bat was individually identified with a coloured, numbered, plastic split-ring forearm band and a passive integrated transponder tag injected subcutaneously between the shoulder blades. Bats were monitored for health changes throughout the study.

Before *Pilot Studies* and during *Experiment 2*, bats were housed in a husbandry facility at McMaster University (i.e. a colony). Bats in *Experiment 1* were not housed in the colony (see *Experiment 1 Methods* below for details). The colony consists of a quarantine and an established living area (each 2.5 × 1.5 × 2.3 m), but only the established side was used for this study (henceforth termed the colony). Bats in the colony could crawl through a hole in the wall to freely access a roofed semi-outdoor flying area (2.5 × 3.8 × 2.7 m). Food (*T. molitor*) and water were provided *ad libitum*. Temperature and light varied seasonally following ambient conditions (Skrinyer et al. 2014). The outdoor portion's far wall is made of 3.6 cm stainless steel mesh, so the bats were exposed to ambient weather and noise but mostly not directly to rain or snow. The indoor portion is insulated, with a central window, so bats were exposed to changes in photoperiod.

Experiment 1: Quantifying decline in flight performance

Animals. I used 10 bats (6 females, 4 males) that were caught from the wild in May 2024 (Appendix Table 1). From the time of capture and throughout this experiment, the bats were housed in either a cloth mesh (40 × 40 × 75 cm) or metal (28.0 × 21.0 × 20.5 cm) cage with *ad libitum* access to *T. molitor* and water. The sizes of these cages do not provide space for bats to fly. Throughout this thesis, I refer to these specific housing conditions as "quarantine housing conditions". Quarantine refers to bats that have not yet completed a disease-free 3-month quarantine period after being wild-caught. These housing conditions are typically how *E. fuscus* are housed at McMaster during their quarantine period.

I flew the bats approximately one week after they were caught, on May 31, June 1, and June 3 (termed the first phase), and again approximately 11 weeks later on August 13, August 14, and August 16, 2024 (termed the second phase). Five of the female bats were pregnant during the first phase, but gave birth and weaned pups before they were flown again in the second phase.

Flight trials. Flight trials were conducted beginning right after sunset in an outdoor A-frame wooden gazebo with an epoxy floor (4.5 × 4.5 × 2.5 m) at McMaster University (Fig. 1A). A Hero5 GoPro camera was attached to one wall of the gazebo to record bat flight trials (Fig. 1B).

Fig. 1. Outdoor screened gazebo used for flight trials in Experiments 1 and 2. (**A**) Outside view of gazebo with wooden walls and window screening. (**B**) Inside view of gazebo from Hero5 GoPro camera showing walls lined with polypropylene vapour barrier and red lighting.

The sides of the gazebo have window screening, but the four walls and ceiling were lined with polypropylene vapour barrier to deter bats from landing on them. The bats were protected from rain and wind, but were exposed to ambient temperature and sound. The top edge of the barrier was adhered to the gazebo with Sheathing Tape (Tuck Tape) to prevent bats from getting behind it, but this caused small creases along the tape where bats could attempt to land. The gazebo was illuminated with an overhead red light and a second red light bulb positioned near one corner.

On nights with flight trials, bats were weighed in the laboratory and then transported to the gazebo in a metal cage (28.0 × 21.0 × 20.5 cm) placed inside a cooler. In a randomized order, each bat was flown three consecutive times and the trials were video recorded.

At the beginning of each flight trial, I held the bat in my hand about 1.65 m above the gazebo floor. A timer was started when I opened my hand. If the bat did not immediately take flight, I gently prodded the bat's back to encourage them to fly. If the bat did not take off after 30 seconds, I then shook my hand with the bat downwards to further encourage the bat to fly. I continued doing this approximately every five seconds, until the bat took flight. I decided *a priori* to terminate flight trials if a bat did not take flight after 3 minutes, but this never happened. I also ended flight trials after 30 minutes of sustained flight. Afterwards, I extracted flight duration and take-off latency times from the video recordings.

Pilot study 1: Methods to improve flight in captive bats

Animals. I used six female bats that had been in captivity since May 2023 in this pilot study (Appendix Table 1). In January 2024 these bats were removed from the

colony to participate in a separate study and were housed at room temperature in a cloth mesh cage (40 × 40 × 75 cm) for about 3.5 months. After the study concluded, I continued to house these bats in the same cloth mesh cage for this pilot study,

Treadmill trials. *Pilot Study 1* was the first effort to exercise bats through crawling. Bats were encouraged to crawl with an automated treadmill (Promethion Core Mouse Metabolic Treadmill, Sable Systems International) designed for animal exercise research. The treadmill's running chamber (48.5 × 25.4 × 31.8 cm) was set to a 15-degree incline, such that when the running belt was turned on, the bats would crawl up the incline against the movement. The back end of the treadmill has a shock grid and manual push brush that encourage animals to keep moving forward; however, I placed the push brush over the shock grid and turned the shock grid off so the bats would not receive any shocks. Treadmill trials were completed during the day with the room lighting off, but with a red light near the treadmill's back end (Fig. 2A).

I began treadmill trials by placing individual bats into the treadmill's running chamber, facing upward. I then closed the chamber's lid and turned the treadmill belt on to a speed of 0.5 m/min. I observed the bat's behaviour throughout the trial and scored its overall crawling performance. The categorical scale ranged from 0 to 4 and scores were based on my perception of the amount of time a bat spent crawling *versus* not crawling (Table 1).

Fig. 2. Treadmill used to encourage bats to perform crawling exercise set at 15-degree incline. (**A**) Lateral view of setup for *Pilot Studies* with red light positioned near the treadmill's back end. (**B**) Lateral view of setup for *Experiment* 2 with overhead white lighting. A Hero5 GoPro camera positioned above the treadmill was used to record trials.

Table 1. Treadmill scores for Pilot Studies 1 and 2. Subjective scores were assigned based on the general amount of time the bat spent crawling in a given treadmill trial.

Score	Description
4	Bat crawled forward for the entire or almost entire trial (Entirety)
3	Bat spent >50% of the trial crawling forward (Majority)
2	Bat spent <50% of the trial crawling forward (Minority)
1	Bat did not crawl forward at all during trial (Didn't crawl)
0	Bat did not complete the trial (Didn't complete)

Each bat completed a single 10-minute treadmill trial per day over five days. Treadmill trials 1–4 were on consecutive days, but trial 5 took place two days after trial 4. One bat (Sky 106) in trial 4 and four bats (Sky 101, Sky 106, Sky 122, Sky 126) in trial 5 did not complete the full 10-minutes of crawling exercise.

I terminated *Pilot Study 1* after treadmill trial day 5, because it was obvious the bats were not motivated to crawl forward on the treadmill and therefore I would not be able to test my study predictions with this experimental design.

Pilot study 2: Methods to improve flight in captive bats

Animals. In February 2024, I moved three new female bats (Appendix Table 1) from the colony to a cloth mesh cage (40 × 40 × 75 cm), housed at room temperature. The bats were provided *ad libitum* access to water but did not have free access to *T. molitor*. Instead, I fed the bats a limited number of *T. molitor* per day to restrict their diet, and fed them on the treadmill to encourage them to crawl throughout this pilot study. Also, I lengthened this study's period, compared to *Pilot Study 1*.

After moving the bats, I allowed them to habituate for three days before beginning treadmill training. I did not feed them on day 1, then hand-fed them five *T. molitor* on day 2, and six on day 3. For reference, the mass of a single *T. molitor* is about 0.1 g. After day 3, the bats were fed a specific number of *T. molitor* in the treadmill's crawling chamber during training and exercise trials (see below). If a bat's mass after feeding was <75% of its original mass, I fed it additional *T. molitor* until its mass was ≥75% of the original mass.

Treadmill training and trials. On day 4, I began training the bats to associate the treadmill's top with food (i.e. training day 1). Every day for six days, I placed individual

bats in the treadmill's chamber but did not run or cover it. The bats were placed facing forward at the top of the chamber and were repositioned to this location if they moved. Using forceps, I fed the bats individual *T. molitor*, placed either directly in their mouth or a few centimeters away so they were encouraged to reach for it. Bats were fed 10 *T. molitor* on training days 1 and 2. To prevent bats from losing excessive mass, I increased the number of *T. molitor* to 13 on training days 3–5 and to 14 on training day 6.

The day after training day 6 (i.e. on day 9 of the study), bats were given treadmill trials every morning for 31 days. The treadmill was set to a speed of 1.5 m/min, and each trial lasted 3 minutes. Trials were repeated three times per day for each bat, for a total of 9 minutes. During each trial, I fed the bat 2–4 *T. molitor*, which I held in front of them with forceps to encourage forward crawling. At the conclusion of each trial, I fed them more *T. molitor* (six total for every trial) on the treadmill. So, in total they received 18 *T. molitor* per day. I scored crawling performance using the same scoring system as in *Pilot Study 1* (Table 1).

Experiment 2: Methods to improve flight in captive bats

Animals. I used 24 female bats that had been in captivity since May 2023 or earlier (Appendix Table 1). Prior to the study, all bats were housed in the colony.

Treatment groups. On June 10, 2024 (day 1), the 24 bats were randomly assigned to one of four groups (n = 6 per group). One group (i.e. colony group) remained housed in the colony with *ad libitum* access to *T. molitor* and water. The other three groups were housed separately in three cloth mesh cages (40 × 40 × 75 cm) in the colony's outdoor flying area and were provided *ad libitum* access to water. Bats in the colony had space to fly voluntarily, whereas bats in the cages did not. Bats in one of the

three cages had *ad libitum* access to *T. molitor* (i.e. no-treadmill/unrestricted-diet group), while bats in the two remaining cages (i.e. no-treadmill/restricted-diet and treadmill/restricted-diet groups) were on a restricted diet and fed a specific number of *T. molitor* per day. Finally, bats in the treadmill/restricted-diet group were exercised daily throughout the study through crawling on a treadmill, while the other three groups were not systematically exercised.

Feeding procedure. Bats in the colony and no-treadmill/unrestricted-diet groups ate *T. molitor* on their own without experimenter assistance. Bats in the no-treadmill/restricted-diet and treadmill/restricted-diet groups were hand-fed *T. molitor*. I fed the no-treadmill/restricted-diet group while holding them in my hand, and fed the treadmill/restricted-diet group with forceps during training and exercise trials on the treadmill.

The restricted diet bats from both groups were given 18 *T. molitor* per day until day 23, after which, I decreased their food intake to 14 *T. molitor* until day 38, and then to 12 *T. molitor* until day 59. On day 60, bats were returned to the colony and were no longer hand-fed.

The mass of each bat was recorded daily throughout the 59 trial days. Bats in the no-treadmill/restricted-diet and treadmill/restricted-diet groups were weighed before and after feeding. Bats in the colony and no-treadmill/unrestricted-diet groups were weighed once per day, immediately after the other restricted diet groups. All exercise, feeding, and weighing procedures were completed by 5:00 PM daily.

After feeding the restricted diet bats, I noted if any bat's mass remained below a specific threshold relative to its initial mass (i.e. 60% if initial mass was ≥ 25.0 g, 70% if

initial mass was 20.0–24.9 g, or 75% if initial mass was <20.0 g). I decided *a priori* that if any bat's mass after feeding was below its respective threshold, I would feed them additional *T. molitor* until their mass was above threshold. In the end, no bats required additional food because all were above threshold after feeding.

During the first 16 days of the study, some bats ate less than 18 *T. molitor* because they refused to eat. Their masses did not fall below their thresholds and eventually they began eating the correct number of *T. molitor*. One bat (Grey 100) in the treadmill/restricted-diet group refused to eat 18 *T. molitor* until day 17. However, her mass began increasing when she did eat 18 *T. molitor* per day. To prevent this, I continually decreased her food intake until reaching 6 *T. molitor* per day.

Treadmill training and trials. Bats in the treadmill/restricted-diet group were encouraged to crawl in the same Promethion Core Mouse Metabolic Treadmill (Sable Systems International) with a similar setup to my *Pilot Studies* (i.e. with a 15-degree incline and the push brush covering the shock grid). Differences in methodology between this and my *Pilot Studies* include: not using red light and instead dimming the overhead white lights in the room to 2% of maximum, attaching cardboard to the treadmill's front and far side walls to prevent bats from climbing on them, and positioning a Hero5 GoPro camera above the treadmill to record crawling trials in case video footage needed to be reviewed (Fig. 2B).

From day 3 to day 7, I trained bats in the treadmill/restricted-diet group to associate the treadmill's top with food. I followed the same procedure as in *Pilot Study 2*, wherein I placed individual bats on the treadmill, facing forward at the top. I fed them 18 individual *T. molitor* with forceps, either directly to their mouth or a few centimeters away so they had to crawl forward to reach it.

After completing treadmill training (i.e. on day 8), I exercised these bats on the treadmill daily for 50 days. Each bat crawled on the treadmill, set to 3 m/min speed, twice for five minutes, for a total of 10 minutes of crawling exercise per day. In a randomized order, I weighed three bats at a time and ran their trials in interchanging order (i.e. while one was on the treadmill, the other two bats remained in a cooler). I reweighed the three bats after completing their crawling trials, then continued the procedure with the next three bats. For each trial, I fed bats half of their total daily *T. molitor* (e.g., 9 *T. molitor* each when bats ate 18 *T. molitor* per day).

Flight trials. The 24 bats were randomly divided into two groups (n = 12; Appendix Table 2) that were flown on alternating nights. Flight trials were conducted following the same flight trial procedure as in *Experiment 1*, except the bats were transported in two coolers (six per cooler).

I recorded baseline flights on day 1 for Group 1 and on day 2 for Group 2. Then, beginning on day 8 and continuing until day 59, the bats were flown twice a week: Group 1 on Mondays and Thursdays, and Group 2 on Tuesdays and Fridays. Owing to a severe heatwave and thunderstorm warnings, bats were not flown on days 11 or 12. In total, each bat was tested on 15 nights for a total of 45 flight trials. The same data were extracted from the GoPro videos as in *Experiment 1*.

Returning bats to colony. On day 60, I returned all bats from the no-treadmill/unrestricted-diet, no-treadmill/restricted-diet, and treadmill/restricted-diet groups to the colony. To monitor post-trial mass changes, I continued to record the mass of the 24 bats twice per week until day 94 (i.e. for 34 days).

Analyses

Experiment 1: Quantifying decline in captive bat flight. Bat mass (g), flight duration (s), and take-off latency (s) were analyzed with linear mixed-effects models (LMM) and estimated marginal means (EMMs). For all analyses, the bats were split into two groups: non-reproductive individuals (i.e. the non-pregnant female and males) or pregnant-at-start females (n = 5 per group). Data collected on May 31, June 1, and June 3 were grouped into "first phase", and data from August 13, August 14, and August 16 were grouped into "second phase". Due to the small sample size, all flight trials were included in flight duration and take-off latency analyses, rather than the total values per bat on a given day.

Mass LMM. Mass was log-transformed using the natural logarithm function to improve linearity and homoscedasticity. The LMM used to analyze the coefficient estimates (β) and 95% profile confidence intervals (95% CIs) included the intercept, trial phase (i.e. first or second phase), group (i.e. non-reproductive individuals or pregnant-at-start females), the interaction between trial phase and group, and a random effect term for individual bat intercept, trial phase, and correlation between intercept and trial phase.

Flight duration LMM. Flight duration was log-transformed using the natural logarithm function because on the original scale, model-predicted data for the response variable did not match the observed data. The LMM used to analyze the β estimates and 95% CIs was similar to the mass analysis, but also included mass as a predictor variable and a random effect term for individual bat intercept.

Take-off latency LMM. An offset of 0.1 s was added to all take-off latency data points because some were originally 0 s. I then log-transformed them with the natural logarithm function because on the original scale, model-predicted data for the response

variable did not match the observed data. The LMM used to analyze β estimates and 95% CIs was the same as the flight duration analysis.

EMMs. I computed EMMs using the mass, flight duration, and take-off latency models to evaluate group differences. The EMMs account for captivity duration (i.e. first versus second phase) and pregnancy status covariates, providing adjusted means that represent the response variable at the mean covariate levels. Pairwise comparisons of EMMs were conducted using Tukey's method for multiple comparisons, both within groups across time of trial (i.e. first phase minus second phase), as well as between the groups (i.e. non-reproductive individuals minus pregnant-at-start females) during the first and second phases.

Experiment 2: Methods to improve flight in captive bats. Owing to the small sample sizes, the pilot study data were not statistically analysed; instead, qualitative observations were described from them. For *Experiment 2*, bat mass, flight duration, and take-off latency for the four groups (n = 6 per group) were analyzed with LMMs and EMMs. For flight duration and take-off latency analysis, the three flight trials of a given bat on a given trial day were summed to calculate totals, and the 15 data points per bat were used for analyses. Also, outliers in mass, flight duration, and take-off latency were identified visually from data plots (i.e. individuals that visually appeared to differ from all other bats). Analyses were completed including all bats, then with the visualized outliers removed.

Mass LMM. Mass data were separated into two timeframes—the 59 days when the animals were used in the experiment and the 34 days post-experiment phase when the bats were returned to the colony—and were analyzed separately. Reported masses during the 59-day experiment for no-treadmill/restricted-diet and treadmill/restricted-diet bats

are before daily feeding. The LMM used to analyze β estimates and 95% CIs for the response variable mass included terms for the intercept, bat group (i.e. colony, no-treadmill/unrestricted-diet, no-treadmill/restricted-diet, treadmill/restricted-diet), trial day (mean-centered), the interaction between trial day and bat group, and a random effect term for individual bat intercept, slope, and correlation between intercept and slope.

Flight duration LMM. Total flight duration was log-transformed using the natural logarithm function because on the original scale, model-predicted data for the response variable did not match the observed data. The LMM used to analyze the β estimates and 95% CIs included the same terms as the mass model, as well as mass (mean-centered), and an interaction term between mass and bat group. Mass data for flight analyses were recorded directly before flight trials.

Take-off latency LMM. An offset of 0.1 s was added to all take-off latency data points because some were originally 0 s. I then log-transformed them using the natural logarithm function because on the original scale, model-predicted data for the response variable did not match the observed data. The LMM used to analyze the β estimates and 95% CIs included the same terms as the mass model, as well as mass (mean-centered). Including an interaction between mass and bat group, as in the flight duration analysis, caused high multicollinearity between the predictor variables, so it was removed for a better model fit. Also, including days in captivity as a predictor variable did not produce a better fit, so it was excluded.

EMMs. I computed EMMs using the mass, flight duration, and take-off latency LMM models to evaluate group differences accounting for the covariates in the models. Pairwise comparisons of EMMs were conducted using Tukey's method for multiple

comparisons, both within groups across trial day (i.e. first trial day minus final trial day), as well as between groups on the first and final trial days.

Result interpretations. LMM results (i.e. β estimates apart from the intercept) are the effects of the predictor variables in reference to the intercept. EMMs are estimated group means calculated after adjusting for predictor variable effects. That is, EMMs analyze model predictions, not observed data directly.

For *Experiment 1*, the LMM intercept is the expected value of the response variable for the non-reproductive individuals group during the first phase. In *Experiment 2*, the LMM intercept is the colony group when the expected variables are zero (or the mean-centered value for centered continuous predictor variables).

The β estimate for a categorical predictor variable (e.g., a group other than the intercept) signifies the group's deviation from the intercept. The group's own β estimate can be calculated by adding its β estimate deviation to the intercept group's β estimate. The β estimate for a continuous predictor variable (e.g., trial day) signifies its effect on the intercept group per unit increase. The β estimate for an interaction between a categorical and a continuous predictor variable (e.g., group and trial day interaction) signifies the group's slope deviation from the intercept group's slope. The group's own slope can be calculated by adding its β estimate to the trial day β estimate.

The β estimates for a response variable on the original scale are additive (e.g., $\beta = 0.5$ for a categorical predictor signifies a positive deviation by a factor of 0.5, and $\beta = 0.5$ for a continuous variable signifies an increase in the response variable by the same factor per unit increase of the predictor variable). In contrast, the β estimates for a response variable on a natural log scale are multiplicative (e.g., $\beta = 0.5$ for a categorical predictor

signifies a positive deviation by a factor of $e^{0.5} \approx 1.65$ or 65%, and $\beta = 0.5$ for a continuous predictor signifies an increase in the response variable by the same factor per unit increase of the predictor variable).

The LMM models also include either a random effect term to account for individual bat intercept variance and residual variance, or a random effect term to account for individual bat intercept variance, individual slope across trial phase or day variance, the correlation between intercepts and slopes, and residual variance.

The 95% CIs for LMM results provide a range within which the true β parameter is likely to lie with 95% confidence. Similarly, the 95% CIs for EMM contrasts provide a range within which the true estimated mean difference is likely to lie with 95% confidence. Thus, if 95% CIs include zero, we cannot confidently conclude that the true effect (for a LMM) or contrast (for an EMM contrast) is non-zero. It also implies uncertainty about the direction of the effect or contrast.

Circle symbols for fixed effects in LMM figures indicate the 95% CIs do not contain zero; square symbols for fixed effects in LMM figures indicate the 95% CIs do contain zero. Asterisks (*) in EMM figures indicate significant p-values; * is $p < .05$, ** is $p < .01$, *** is $p < .001$, and **** is $p < .0001$.

Results

Experiment 1: Quantifying decline in captive bat flight

Mass. I compared bat masses between the first and second time phases of the study (Fig. 3). Average mass in the non-reproductive individuals group showed a large increase from 16.3 g to 26.9 g from when bats were first introduced into captivity compared to 11 weeks later. Conversely, the pregnant females' average mass stayed the same at 25.7 g. Appendix Tables 3–5 list relevant β estimates or EMM contrasts, standard errors (SE), degrees of freedom (df), t statistics (t), p-values (p), and 95% CIs for *Experiment 1* mass analyses.

LMM results. Overall, during the first phase, the pregnant females had larger mass than the non-reproductive individuals. Then, there was a large positive effect of the 11-week captivity on the non-reproductive individuals group mass, but not on the pregnant-at-start females group mass (Fig. 4 and Appendix Table 3).

More specifically, the LMM results show that during the first phase, compared to the non-reproductive individuals, there was a 58% positive deviation in the pregnant-at-start females group mass (β = 0.46, 95% CI [0.34, 0.57]). Then, for the non-reproductive individuals, there was a 65% positive effect of being in the second phase compared to the first phase (β = 0.50, 95% CI [0.31, 0.70]). Finally, the effect of the second phase on the pregnant-at-start females differed from its effect on the non-reproductive individuals, wherein the change in mass from the first to second phase was much less for the pregnant-at-start females than the non-reproductive individuals (β = -0.51, 95% CI [-0.79, -0.24]).

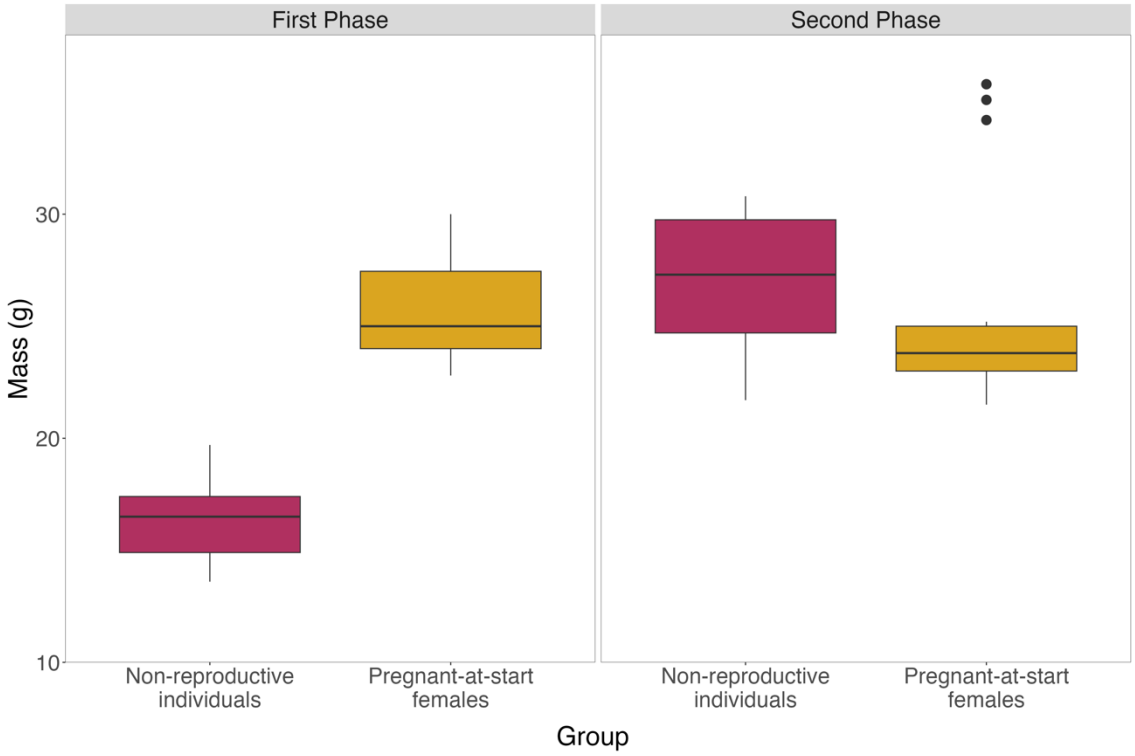


Fig. 3. Experiment 1. Comparison of *E. fuscus* (*n* = 5 per box) mass (g) between the two study phases. Maroon boxes represent non-reproductive individuals group; yellow boxes represent pregnant-at-start females group. Boxes illustrate median bat masses (line within box) and ± interquartile range (edge of box). Whiskers represent the smallest and largest values within 1.5 x interquartile range.

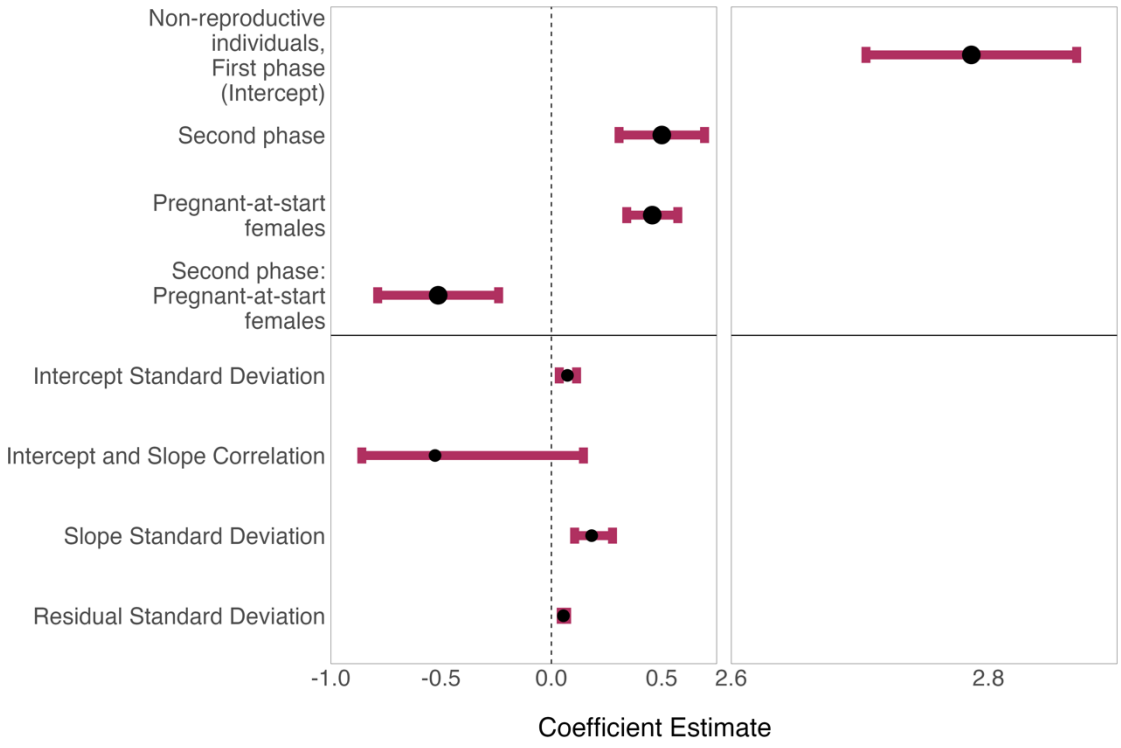


Fig. 4. Experiment 1. *E. fuscus* (*n* = 10) mass (ln(g)) LMM results. Points represent β estimates; maroon lines represent 95% CIs. Fixed effects (above horizontal line) include: non-reproductive individuals group during the first phase as intercept, the second phase, pregnant-at-start females group, and the second phase and pregnant-at-start females interaction; random effects (below horizontal line) include: intercept SD, intercept and slope correlation, slope SD, and residual SD.

EMM contrasts. The EMM for bat mass in the non-reproductive individuals group increased significantly from the first to the second phase (EMM contrast = -0.50, 95% CI [-0.70, -0.31]; Fig. 5 and Appendix Table 4). Conversely, the EMM for the pregnant-at-start females group decreased slightly, but the difference was non-significant (EMM contrast = 0.01, 95% CI [-0.18, 0.21]).

During the first phase, the EMM for bat mass in the pregnant-at-start females group was significantly larger than the non-reproductive individuals group (EMM contrast = -0.46, 95% CI [-0.57, -0.34]; Fig. 5 and Appendix Table 5). Conversely, during the second phase, the EMM for the pregnant-at-start females group was slightly smaller, but the difference was non-significant and the two EMMs were similar (EMM contrast = 0.06, 95% CI [-0.18, 0.29]).

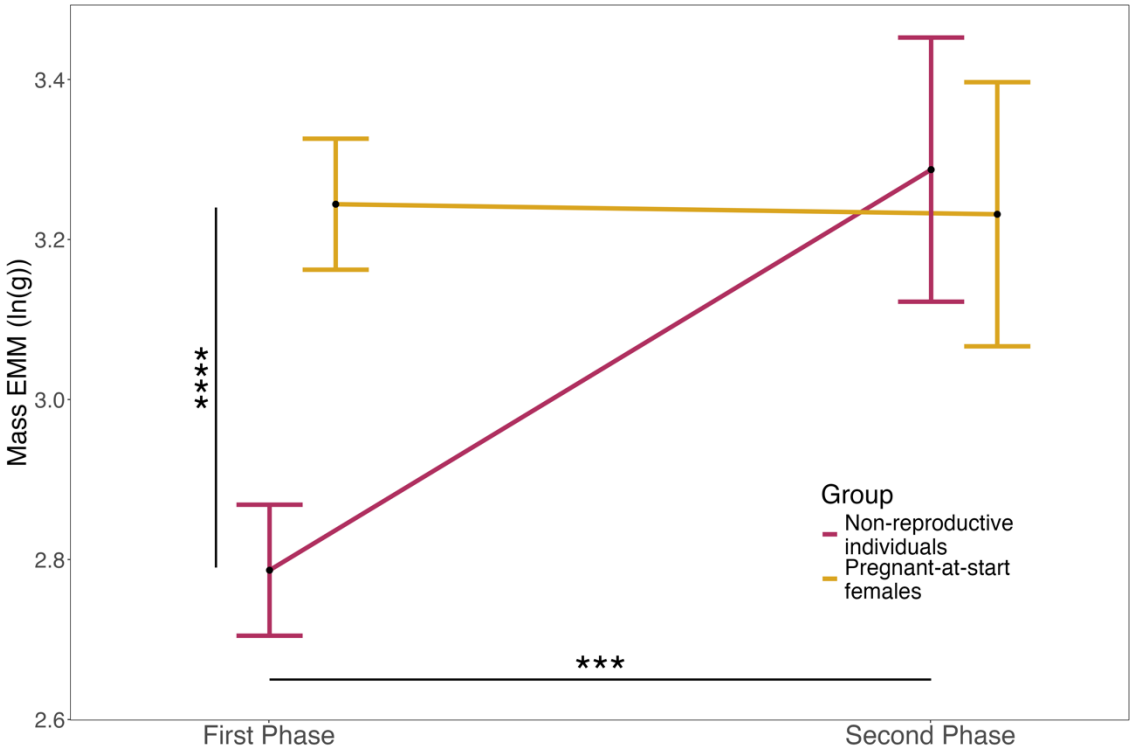


Fig. 5. Experiment 1. *E. fuscus* (*n* = 10) mass (ln(g)) EMMs during first and second phase. Significant contrasts are labeled; horizontal label represents within-group contrast from the first to the second phase; leftside vertical label represents between-group contrast during the first phase.

Flight duration. I also compared flight duration between the two time phases (Fig. 6). During the first phase, the average flight duration for the non-reproductive individuals group was 87.1 s, which was greater than the average of 15.8 s for the pregnant-at-start group's. Then, the average for the non-reproductive individuals showed a large decrease from 87.1 s to 2.6 s between when bats were first introduced into captivity compared to 11 weeks later. The pregnant-at-start group's average also decreased, but to a lesser extent, from 15.8 s to 5.5 s. Appendix Tables 6–8 list relevant β estimates or EMM contrasts, SE, df, t statistics, p-values (p), and 95% CIs for flight duration analyses.

LMM results. Overall, during the first phase, the two groups' flight durations were similar. For the non-reproductive individuals, there was a negative effect of weight gain on their flight duration, and the effect of being in the quarantine housing conditions was not conclusive, though trended downwards. The negative trend of being in the housing conditions was similar on the pregnant-at-start females' flight duration (Fig. 7 and Appendix Table 6).

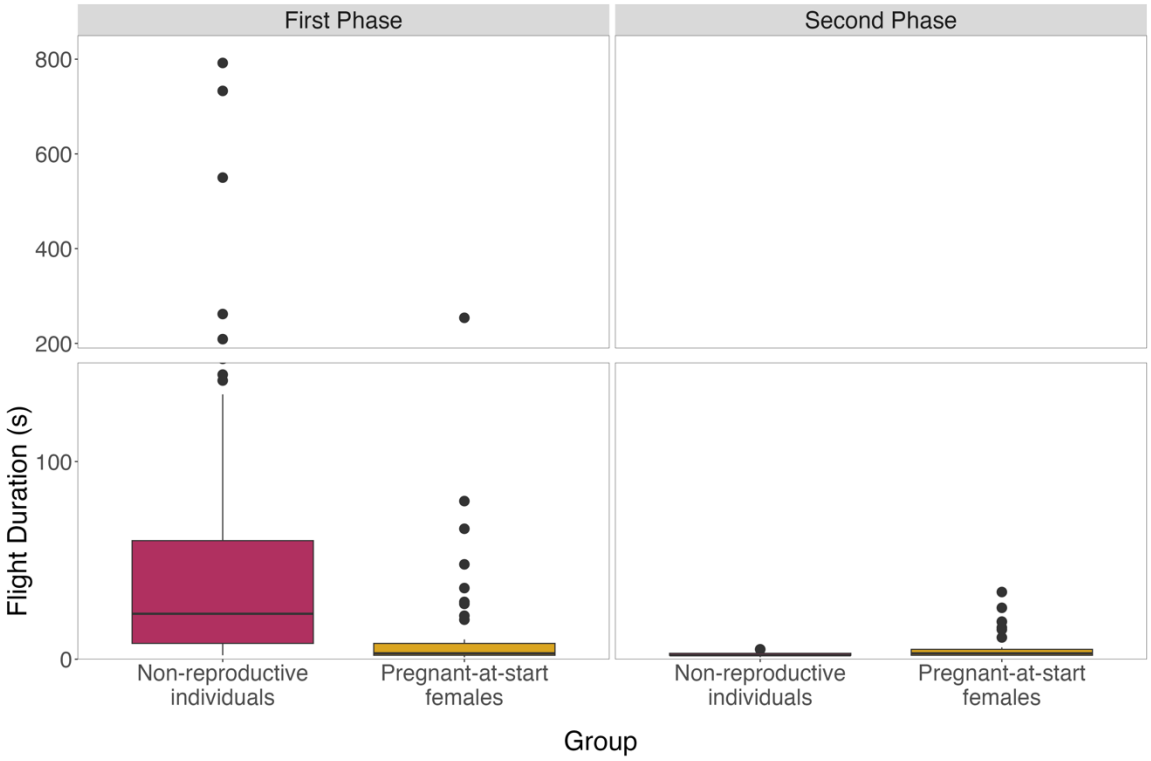


Fig. 6. Experiment 1. Comparison of *E. fuscus* (*n* = 5 per box) flight duration (s) between the two study phases. Maroon boxes represent non-reproductive individuals group; yellow boxes represent pregnant-at-start females group. Boxes illustrate median bat masses (line within box) and ± interquartile range (edge of box). Whiskers represent the smallest and largest values within 1.5 x interquartile range.

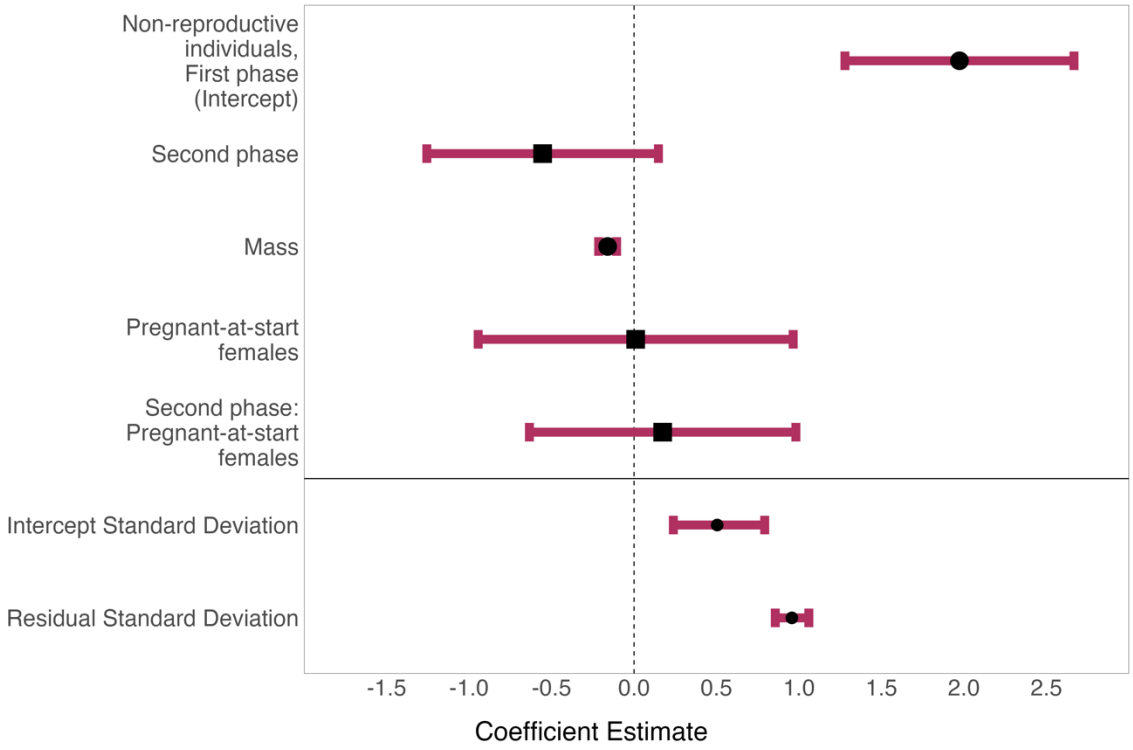


Fig. 7. Experiment 1. *E. fuscus* (*n* = 10) flight duration (ln(s)) LMM results. Points represent β estimates; maroon lines represent 95% CIs. Fixed effects (above horizontal line) include: non-reproductive individuals group during the first phase as intercept, the second phase, mass, pregnant-at-start females group, and the second phase and pregnant-at-start females interaction; random effects (below horizontal line) include: intercept SD, and residual SD.

More specifically, the LMM results show that during the first phase, the pregnant-at-start females did not conclusively differ from the non-reproductive individuals (β = 0.01, 95% CI [-0.96, 0.97]). There was evidence of a 15% negative effect per unit increase in mass on the non-reproductive individuals group (β = -0.16, 95% CI [-0.21, -0.11]). However, the effect of being in the second phase on the non-reproductive individuals was negative, but not conclusive (β = -0.55, 95% CI [-1.26, 0.15]). Finally, the interaction between the pregnant-at-start females group and the second phase was positive, but the 95% CIs include zero (β = 0.17, 95% CI [-0.63, 0.98]). This indicates that there may have been a smaller negative effect of the 11-week captivity on the pregnant-at-start females' flight duration than on the non-reproductive individuals, but again, there was insufficient evidence to conclude the effect was non-zero.

EMM contrasts. The EMM for flight duration of both groups decreased, but not significantly (EMM contrast = 0.55, 95% CI [-0.16, 1.27] for non-reproductive individuals and EMM contrast = 0.38, 95% CI [-0.02, 0.78] for pregnant-at-start females; Fig. 8 and Appendix Table 7).

During both phase, the EMM for the pregnant-at-start females' flight duration did not significantly differ from the non-reproductive individuals (EMM contrast = -0.01, 95% CI [-0.97, 0.95] during first phase and EMM contrast = -0.18, 95% CI [-1.02, 0.65]; Fig. 8 and Appendix Table 8). Thus, the group's EMMs were similar in both phases.

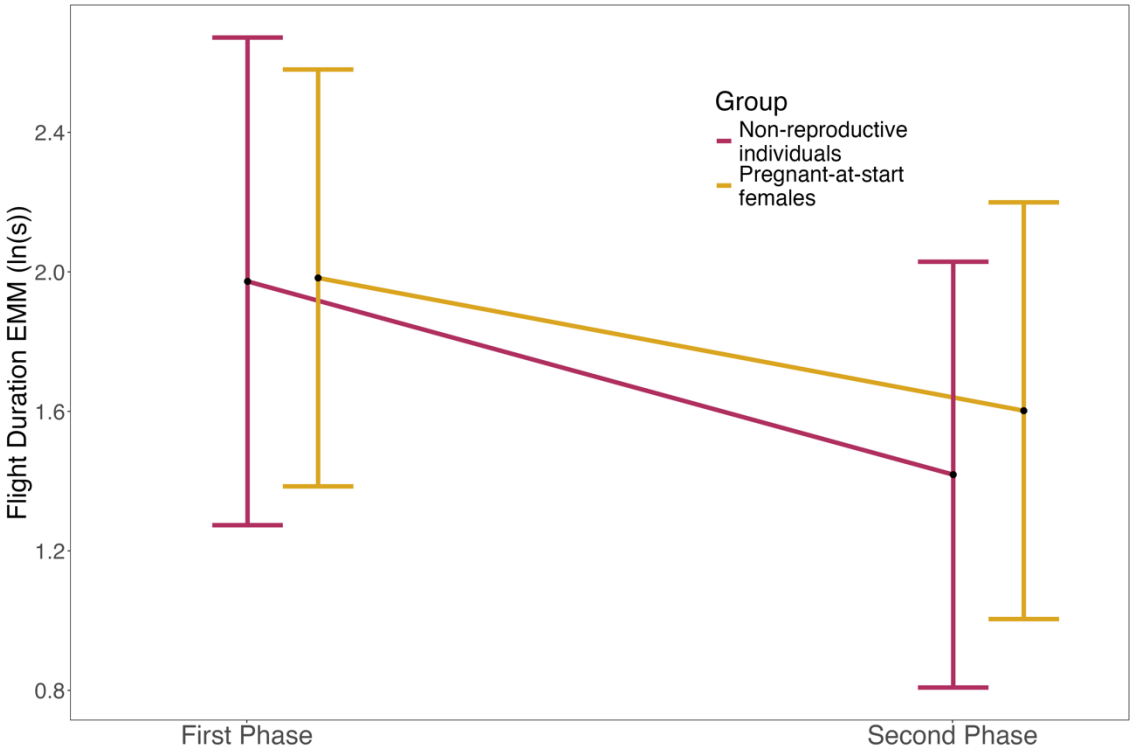


Fig. 8. Experiment 1. *E. fuscus* (*n* = 10) flight duration (ln(s)) EMMs during first and second phase. Significant contrast is labeled; horizontal label represents within-group contrast from the first to the second phase.

Take-off latency. Finally, I compared take-off latency between the two time phases (Fig. 9). Surprisingly, both groups' take-off latency averages decreased somewhat between early captivity and 11 weeks later. For the non-reproductive individuals group, the average decreased from 2.91 s to 1.44 s, and for the pregnant-at-start group, the average decreased from 4.58 s to 1.64 s. Appendix Tables 9–11 list relevant β estimates or EMM contrasts, SE, df, t statistics, p-values (p), and 95% CIs for take-off latency analyses.

LMM results. Overall, the groups' take-off latencies trended downwards, but there may not have been a true effect of the being in captivity for 11 weeks on take-off latency (Fig. 10 and Appendix Table 9).

More specifically, the LMM results show that during the first phase, the pregnant-at-start females had somewhat longer take-off latency, but the 95% CIs contain zero, indicating there was insufficient evidence to conclude that the groups differed (β = 0.42, 95% CI [-0.24, 1.08]). Also, the non-reproductive individuals' take-off latency decreased somewhat, but again there was insufficient evidence to conclude that the 11-week captivity had a true negative effect (β = -0.61, 95% CI [-1.26, 0.05]). Though, it is worth noting that the 95% CIs only marginally cross zero. Finally, the interaction between the second phase and the pregnant-at-start females was somewhat negative, though the 95% CIs include zero (β = -0.30, 95% CI [-1.07, 0.47]). This indicates that there may have been a larger negative effect of the 11-week captivity on the pregnant-at-start females' take-off latency than on the non-reproductive individuals, but there is insufficient evidence to confidently conclude this.

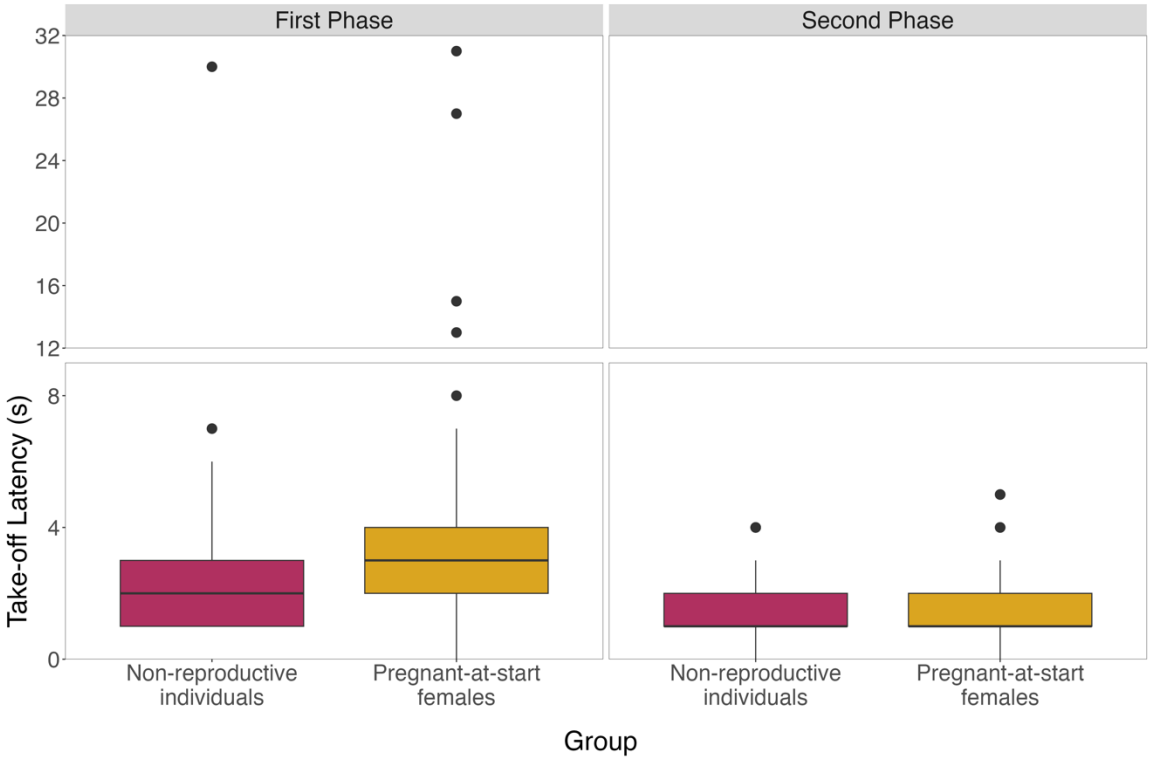


Fig. 9. Experiment 1. Comparison of *E. fuscus* (*n* = 5 per box) take-off latency (s) between the two study phases. Maroon boxes represent non-reproductive individuals group; yellow boxes represent pregnant-at-start females group. Boxes illustrate median bat masses (line within box) and ± interquartile range (edge of box). Whiskers represent the smallest and largest values within 1.5 x interquartile range.

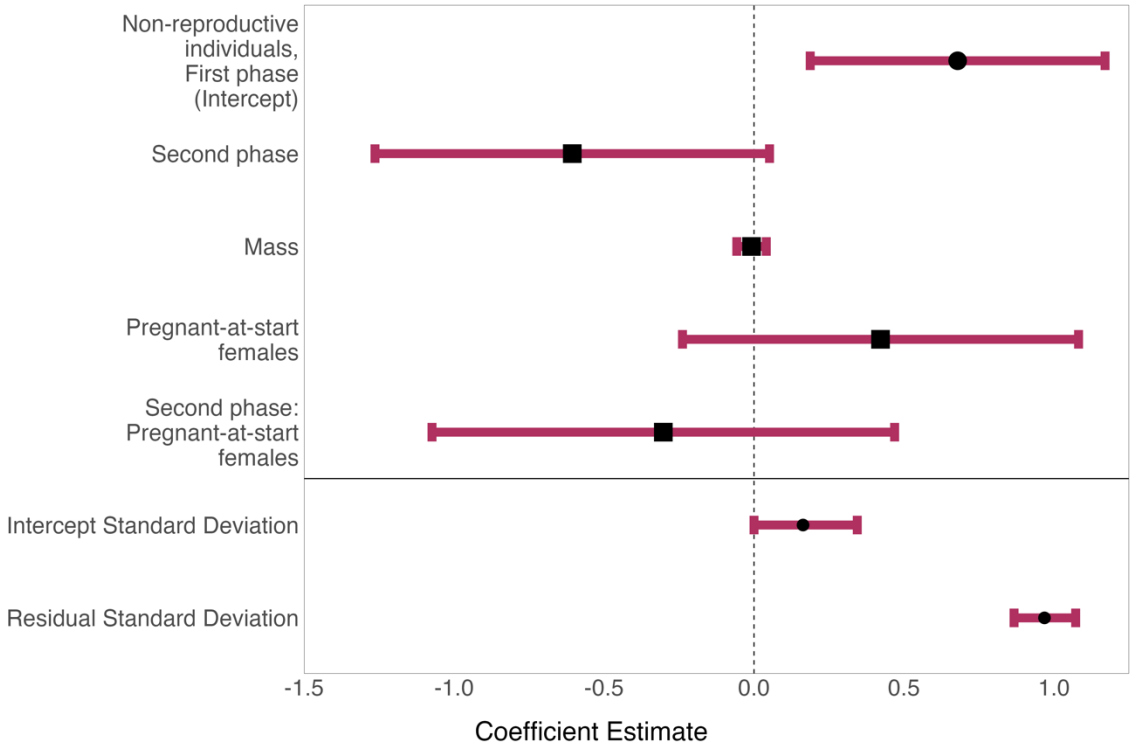


Fig. 10. Experiment 1. *E. fuscus* (*n* = 10) take-off latency (ln(s)) LMM results. Points represent β estimates; maroon lines represent 95% CIs. Fixed effects (above horizontal line) include: non-reproductive individuals group during the first phase as intercept, the second phase, mass, pregnant-at-start females group, and the second phase and pregnant-at-start females interaction; random effects (below horizontal line) include: intercept SD, and residual SD.

EMM contrasts. The EMM for the non-reproductive individuals group decreased non-significantly from the first to the second phase (EMM contrast = 0.61, 95% CI [-0.08, 1.29]), and the EMM for the pregnant-at-start females group decreased significantly (EMM contrast = 0.91, 95% CI [0.51, 1.31]; Fig. 11 and Appendix Table 10).

During both the first and the second phase, the pregnant-at-start females group's take-off latency EMM was non-significantly longer than the non-reproductive individuals group (EMM contrast = -0.42, 95% CI [-1.10, 0.26], and EMM contrast = -0.12, 95% CI [-0.60, 0.36], respectively; Appendix Table 11).

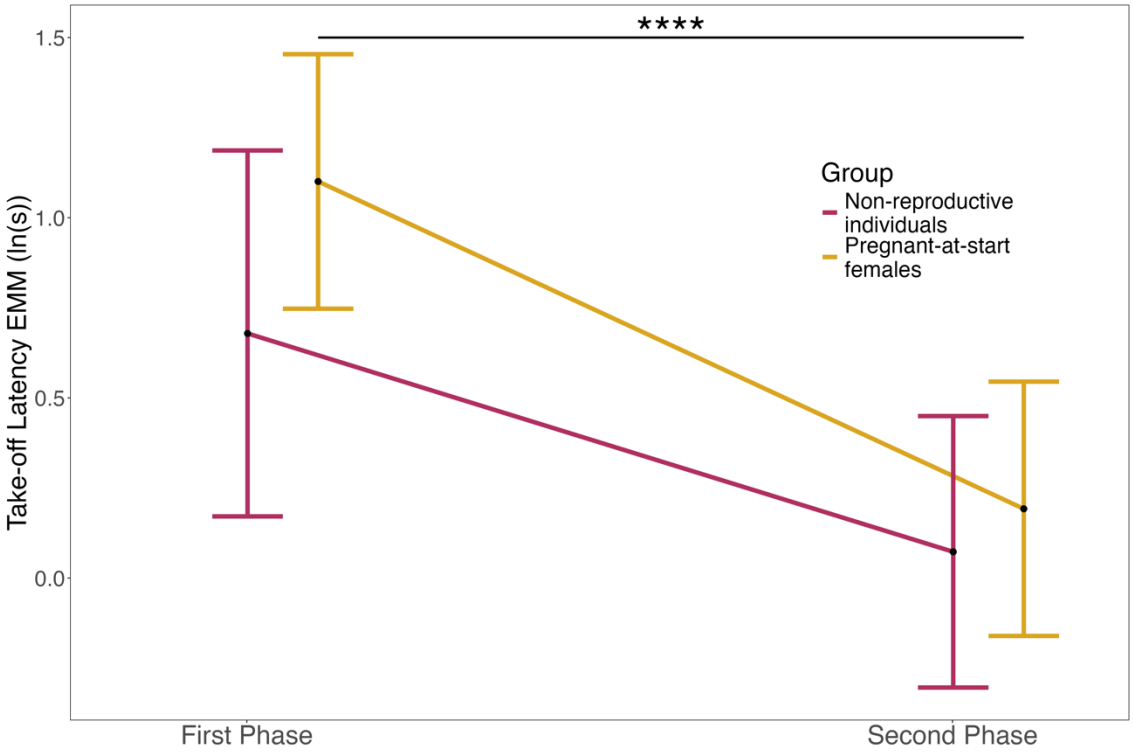


Fig. 11. Experiment 1. *E. fuscus* (*n* = 10) take-off latency (ln(s)) EMMs during first and second phase. Significant contrast is labeled; horizontal label represents within-group contrast from the first to the second phase.

Qualitative observations from pilot studies

During trial 1in *Pilot Study 1*, the bats usually crawled to the front (i.e. the highest point) of the sloped treadmill and then turned around to face the opposite direction. They also attempted to climb the treadmill chamber's walls and the push brush, but could not stay up. After a few minutes of trial 1, each bat began spending more time against the push brush. Four of the bats still attempted to crawl, but for less than 50% of the time, and the other two sat against the push brush for majority of the trial. Across the remaining trials, their treadmill crawling mostly decreased (Fig. 12).

I did not complete the full 10-minute treadmill trial for one bat (Sky 106) on day 4 and for four bats (Sky 101, Sky 106, Sky 122, Sky 126) on day 5 because these bats tended to stay against the push brush without attempting to crawl. When this occurred, the treadmill belt's motion caused their wings to fold underneath the bat's body. To avoid the risk of injury to these bats, I decided to end these trials early.

In *Pilot Study* 2, bats displayed better crawling performance compared to animals tested in *Pilot Study 1* (Fig. 13). For example, the bats often crawled for over 50% of the trial or the entire trial. From trial day 18 to the study's end, the bats crawled for over 50% of the trial or the entire trial. Before trial day 18, some bats crawled for under 50%, but they all crawled at least somewhat. In general, motivating food-restricted bats with food improved their crawling performance.

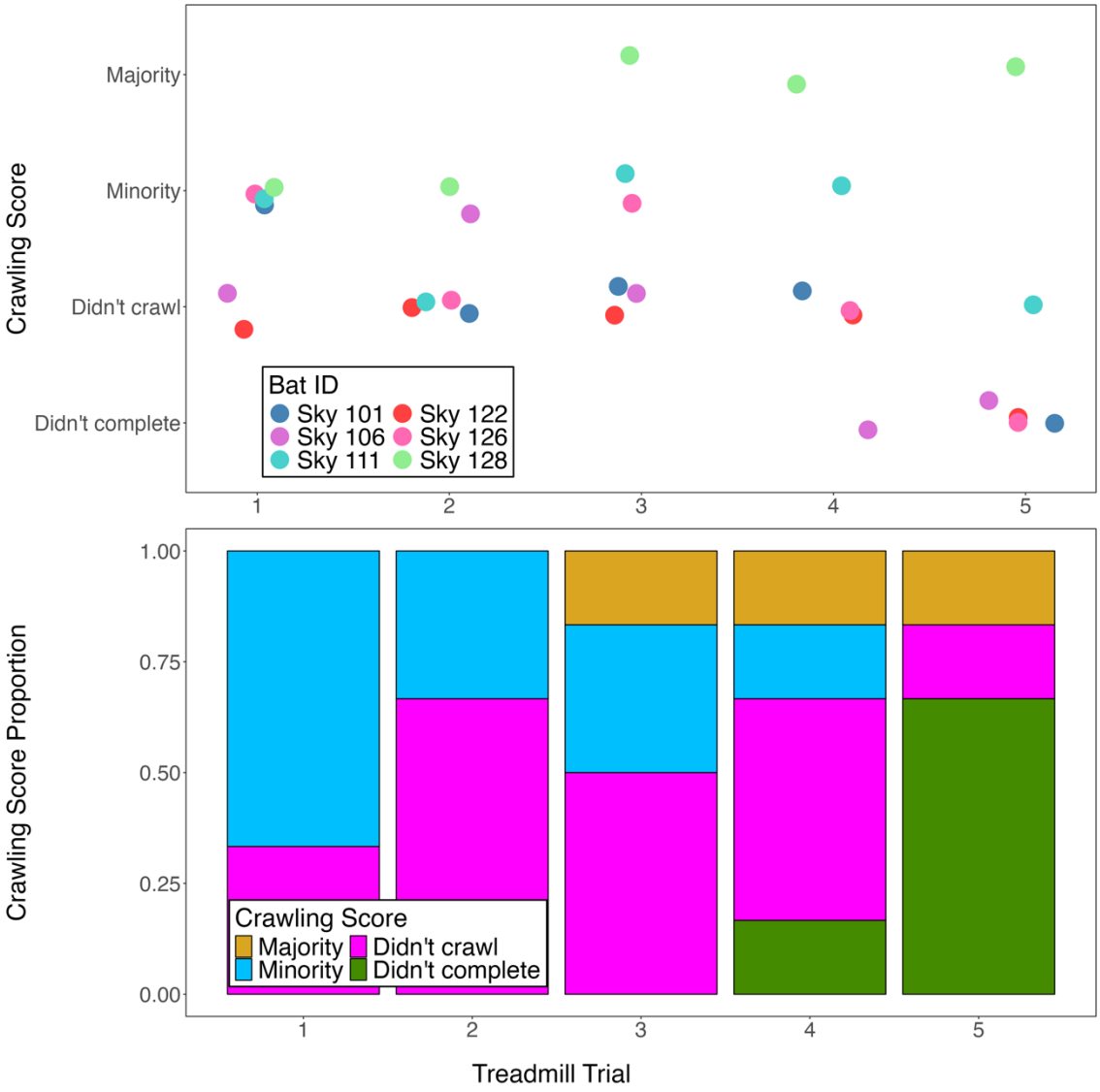


Fig. 12. Pilot study 1. *E. fuscus* (*n* = 6) treadmill crawling scores. (**A**) Individual bat crawling scores. (**B**) Proportion of crawling scores. Scores were assigned based on the general amount of time bats spent crawling during three daily trials across 5 days. See *Methods* for definitions of subject scores.

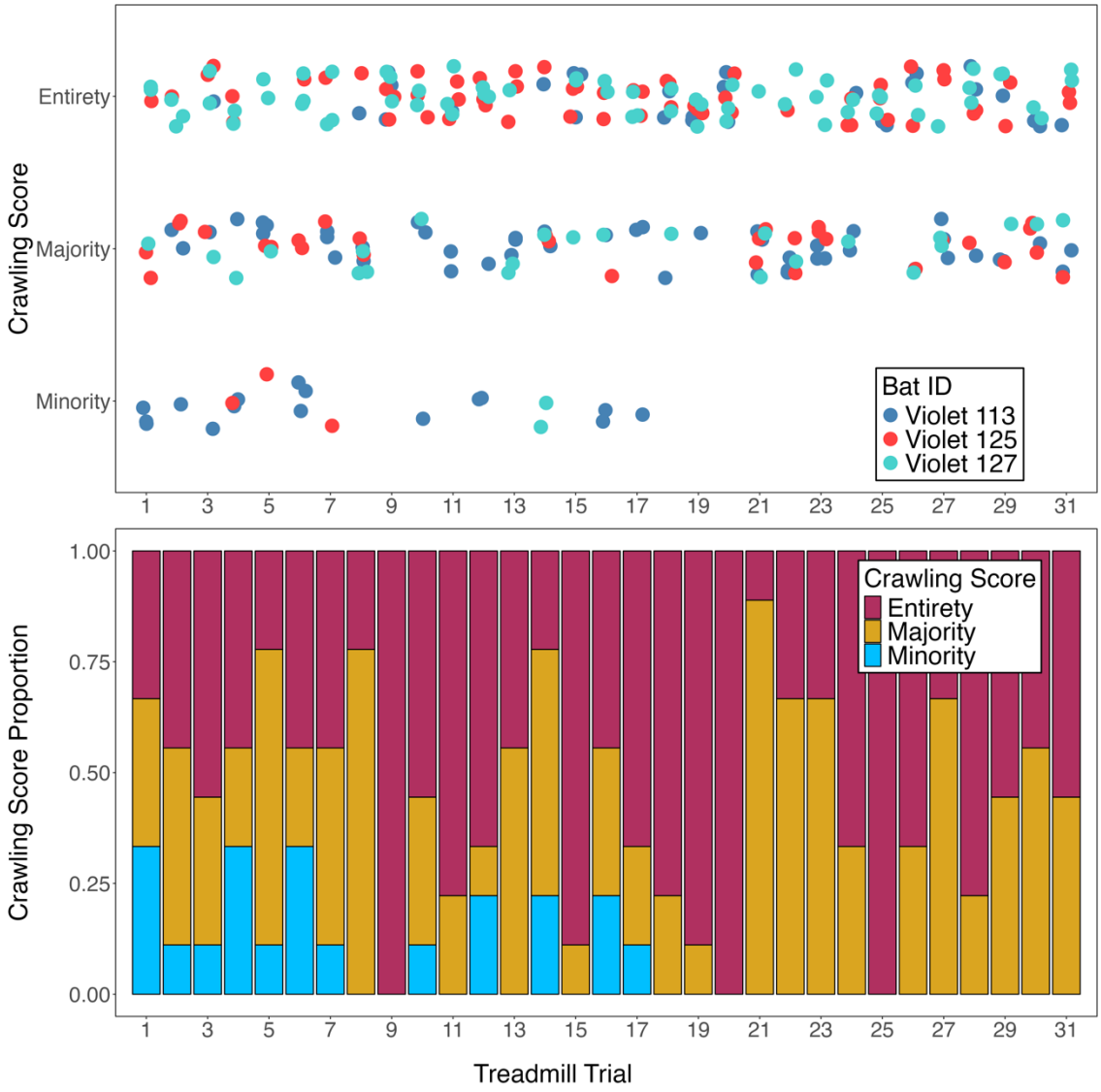


Fig. 13. Pilot study 2. *E. fuscus* (*n* = 3) treadmill crawling scores. (**A**) Individual bat crawling scores. (**B**) Proportion of crawling scores. Scores were assigned based on the general amount of time bats spent crawling during three daily trials across 31 days. See *Methods* for definitions of subject scores.

Experiment 2: Methods to improve flight in captive bats

Due to a technical error, there are no flight duration and take-off latency data available for three flight trials: Sky 101 trial 3 and White 166 trial 3 on flight trial day 1, and Yellow 84 trial 2 on flight trial day 10. Total flight duration and take-off latency data are recorded from the two available flight trials for these bats on these nights. For analyses excluding outliers (see below), changes in significance level and/or the direction of effects are reported.

Mass. I compared how masses changed under the different treatments over the 59-day study (Fig. 14). One treadmill/restricted-diet bat (Grey 100; see *Feeding Procedure* in *Experiment 2 Methods* for details) was 15.5 g at the study's onset, and 15.3 g on day 59. So, I was able to restrict her diet, and her mass somewhat, but to a lesser extent than other restricted diet bats. Also, one colony group bat (Violet 111) continuously increased in mass throughout the study, and more so than any other bats. Mass, flight duration, and take-off latency analyses are first reported with data from Violet 111 included, and then again with data from Violet 111 excluded as an outlier. Appendix Tables 12–14 list relevant β estimates or EMM contrasts, SE, df, t statistics, p-values, and 95% CIs for mass analyses.

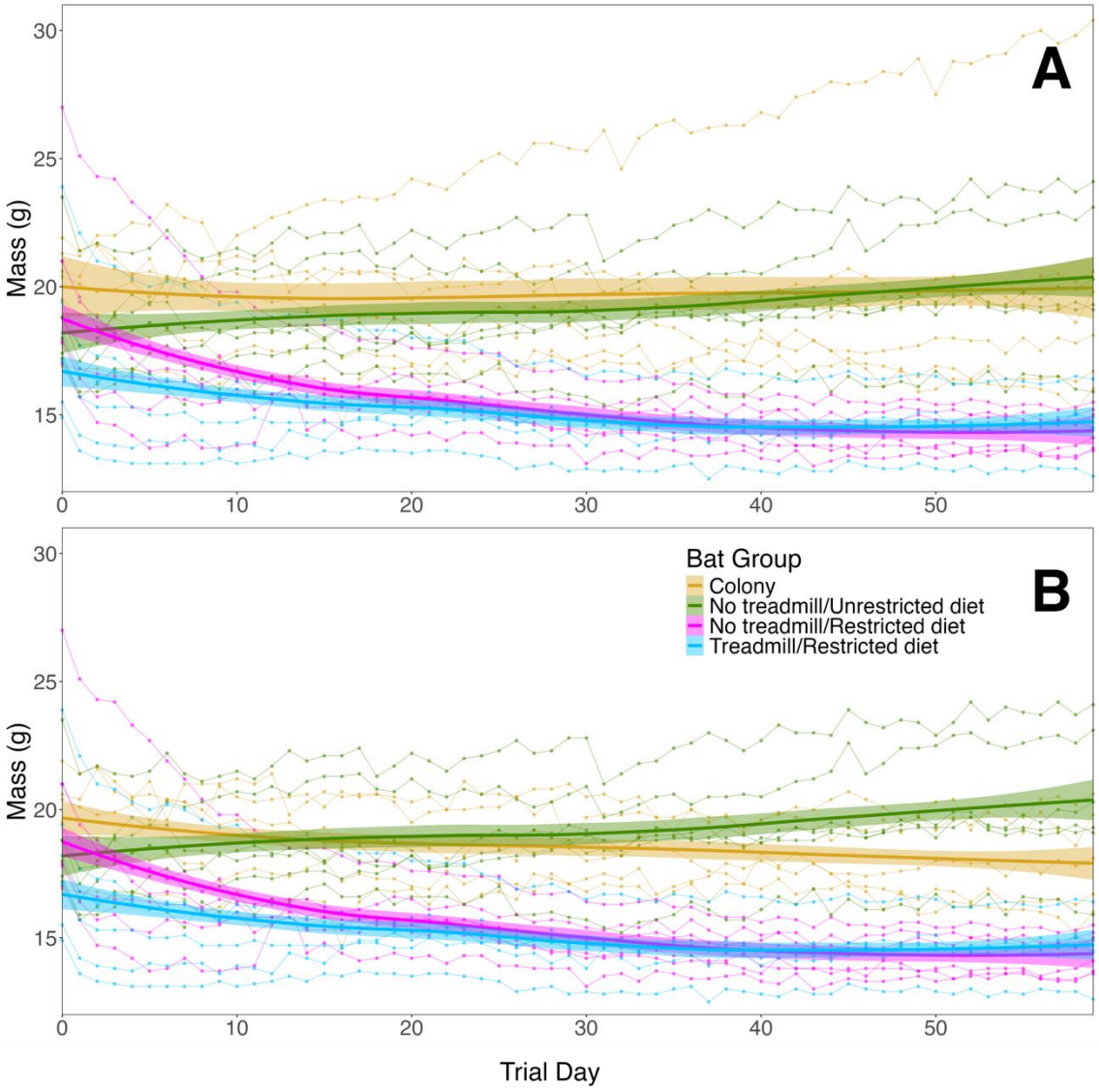


Fig. 14. Experiment 2. Change in mass (g) for *E. fuscus* in the different treatment groups. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of mass outlier (Violet 111) excluded (see text; *n* = 23). Points and connecting lines represent individual bats; thicker lines represent group averages fitted with local regression smoothing curves and shaded 95% CIs (calculated based on standard error of fitted values).

Including data from Violet 111, the colony group's average remained similar from the first to the final trial day (20.1 g and 20.3 g, respectively). Surprisingly, the no-treadmill/unrestricted-diet group's average only increased slightly from 19.0 g on the first day to 20.3 g on the final day. Conversely, average masses for the restricted diet groups showed larger changes. The no-treadmill/restricted-diet group's average decreased from 20.3 g to 14.5 g, and the treadmill/restricted-diet group's decreased from 18.2 g to 14.6 g. When data from Violet 111 are excluded from the colony group, the group's average masses were smaller on the first and final days, decreasing slightly from 19.9 g to 18.2 g.

LMM results including all bats. Overall, there was a large negative effect of restricting diets on the no-treadmill/restricted-diet and treadmill/restricted-diet group masses. Conversely, there were not strong effects of being housed in the colony or in a small cage with *ad libitum* food on the colony or no-treadmill/unrestricted-diet group masses, respectively (Fig. 15A and Appendix Table 12).

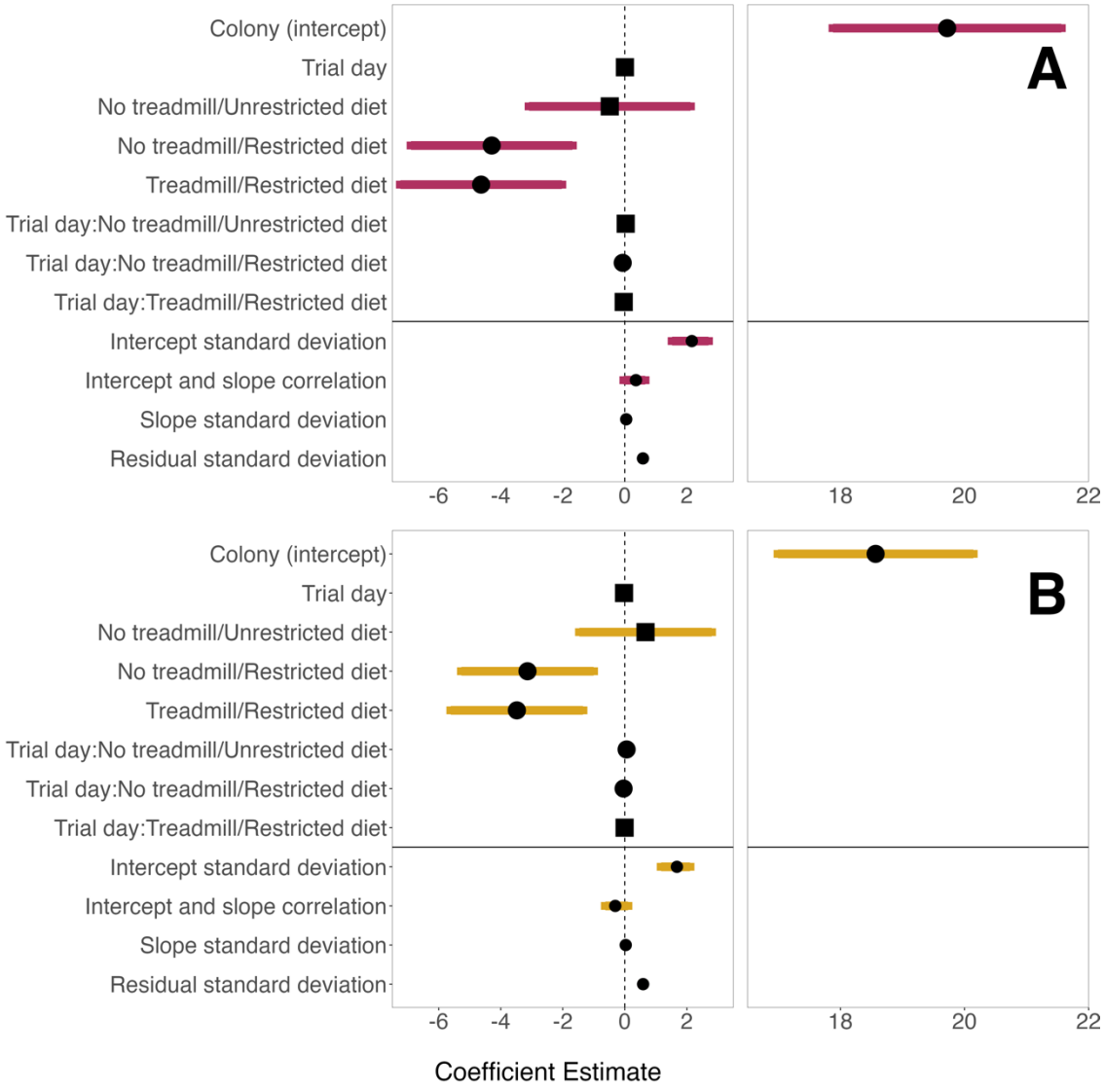


Fig. 15. Experiment 2. Mass (g) LMM results. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of mass outlier (Violet 111) excluded (see text; *n* = 23). Points represent β estimates; maroon and yellow lines represent 95% CIs. Fixed effects (above horizontal line) include: colony group as intercept, centered trial day, three cage group deviations, and centered trial day and three cage group interactions; random effects (below horizontal line) include: intercept SD, intercept and slope correlation, slope SD, and residual SD.

More specifically, the LMM results show that the colony group's mass increased very slightly over trial days, but the 95% CIs cross zero, so there was not sufficient evidence that there was an effect of trial day on the group's mass, as predicted (β = 0.004, 95% CI [-0.03, 0.04]). Also, there was a small negative deviation of the no-treadmill/unrestricted-diet group, but again, there was insufficient evidence to conclude the no-treadmill/unrestricted-diet and colony groups' masses differed (β = -0.49, 95% CI [-3.09, 2.12]). Conversely, there was sufficient evidence that there were large negative deviations in mass from the colony group for the no-treadmill/restricted-diet group (β = -4.29, 95% CI [-6.89, -1.69]), and the treadmill/restricted-diet group (β = -4.63, 95% CI [-7.23, -2.03]). Finally, the effect of trial day only conclusively differed from its effect on the colony group for the no-treadmill/restricted-diet group, which had a slope of -0.066 (β = -0.07, 95% CI [-0.12, -0.01]).

LMM results excluding mass outlier. When data from the mass outlier (Violet 111) were removed, the colony group's mass slightly decreased, though there still wasn't conclusive evidence of a true effect (β = -0.02, 95% CI [-0.05, 0.003]; Fig. 15B and Appendix Table 12). The no-treadmill/unrestricted-diet group had greater mass than the colony group, rather than smaller, but still, there was not conclusive evidence of a true difference (β = 0.67, 95% CI [-1.46, 2.80]). However, there was evidence of a larger effect of trial day on the no-treadmill/unrestricted-diet group's mass than on the colony group's mass, and the no-treadmill/unrestricted-diet group had a slope of 0.04 (β = 0.06, 95% CI [0.02, 0.10]).

EMM contrasts including all bats. The EMMs for mass in both the colony and no-treadmill/unrestricted-diet groups increased from the start of the study to the end, though not significantly (EMM contrast = -0.23, 95% CI [-2.52, 2.05], and EMM contrast

= -1.94, 95% CI [-4.22, 0.35], respectively; Fig. 16A and Appendix Table 13). Conversely, the EMM for the no-treadmill/restricted-diet group decreased (EMM contrast = 3.78, 95% CI [1.50, 6.07]). Finally, the EMM for the treadmill/restricted-diet group also decreased, but not significantly (EMM contrast = 1.86, 95% CI [-0.42, 4.14]).

On the first trial day, the EMM for mass in the treadmill/restricted-diet group was significantly lower than the colony group's (EMM contrast = 3.59, 95% CI [0.20, 6.97]; Fig. 16A and Appendix Table 14). On the final trial day, the EMM for the no-treadmill/restricted-diet EMM was smaller than the no-treadmill/unrestricted-diet group's (EMM contrast = 6.67, 95% CI [1.94, 11.339]) and the colony group's (EMM contrast = 6.30, 95% CI [1.58, 11.02]). Similarly, the EMM for the treadmill/restricted-diet group was smaller than the no-treadmill/unrestricted-diet group's (EMM contrast = 6.05, 95% CI [1.32, 10.77]) and the colony group's (EMM contrast = 5.68, 95% CI [0.96, 10.40]; Appendix Table 14).

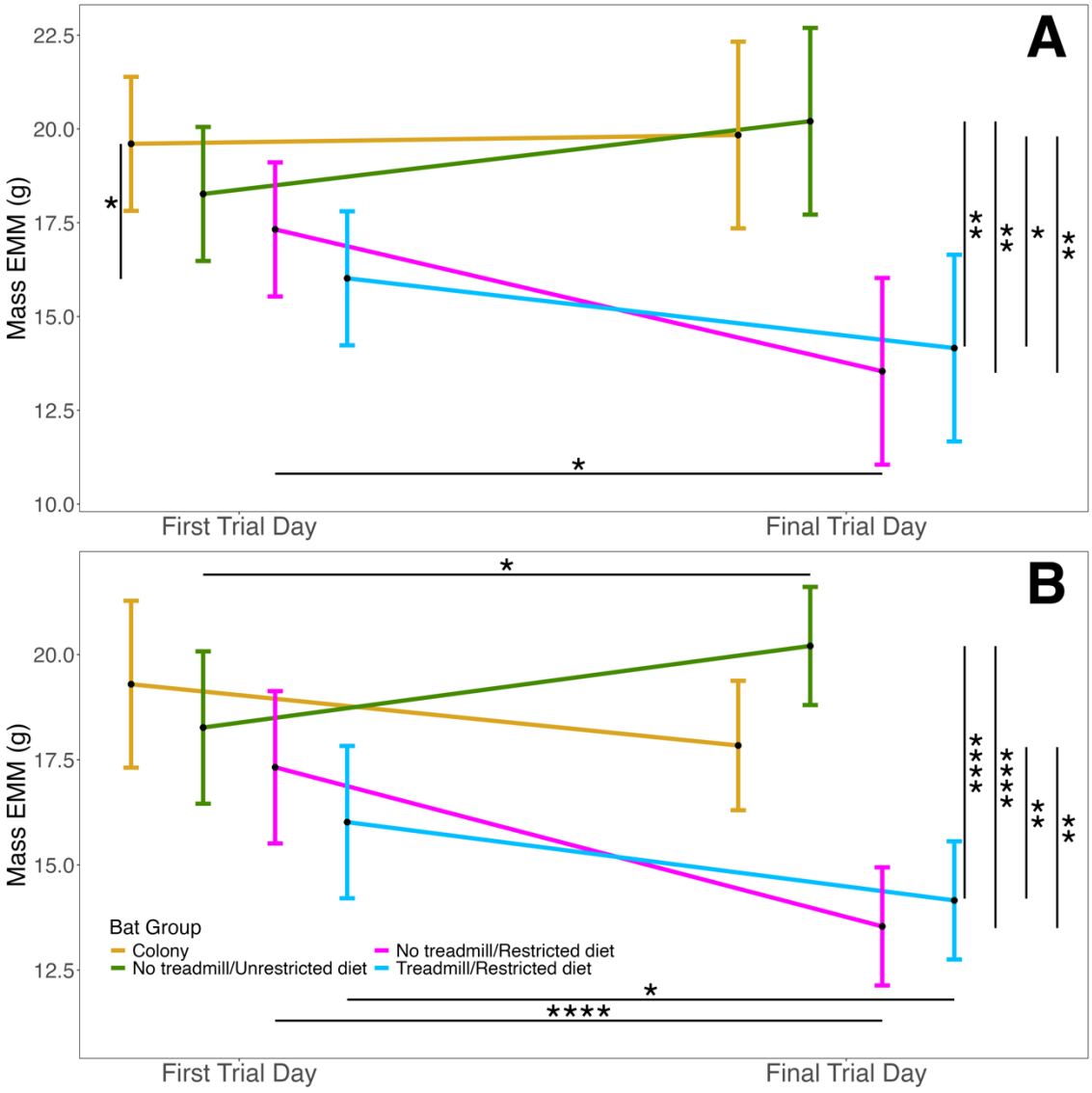


Fig. 16. Experiment 2. Mass (g) group EMMs during first and final trial days. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of mass outlier (Violet 111) excluded (see text; *n* = 23). Significant contrasts are labeled; horizontal labels represent within-group contrasts from first to final trial day; leftside vertical labels represent between-group contrasts on first trial day and rightside represent final trial day.

EMM contrasts excluding mass outlier. When data from Violet 111 were removed, the EMM for mass in the colony group decreased, rather than increased, though still not significantly (EMM contrast = 1.46, 95% CI [-0.18, 3.09]; Fig. 16B and Appendix Table 13). The EMM for the no-treadmill/unrestricted-diet group increased significantly (EMM contrast = -1.94, 95% CI [-3.43, -0.44]). Finally, the EMM for the treadmill/restricted-diet group decreased significantly (EMM contrast = 1.86, 95% CI [0.37, 3.36]).

The pairwise group contrasts were similar, except on the first trial day, the EMM for the no-treadmill/unrestricted-diet group was similar to the colony group (EMM contrast = 3.28, 95% CI [-0.33, 6.88]; Appendix Table 14).

Flight duration. I also compared how flight duration changed under the different treatments over the 59-day study (Fig. 17). One colony bat (Sky 151) began to fly for longer than all other bats from flight trial 8 until the study's end. Also, one no-treadmill/unrestricted-diet bat (Violet 170) flew for much longer than all other bats during the first two flight trials. Analyses are completed first including data from all bats, then excluding data from the large mass colony bat (Violet 111; termed mass outlier), and finally excluding data from the two long flight bats (Sky 151 and Violet 170; termed visualized outliers). The mass and visualized outliers were excluded separately because Violet 111 was an outlier in a predictor variable, whereas Sky 151 and Violet 170 were outliers in the response variable, and the two analyses could compare whether these factors impacted the results. Appendix Tables 15–17 list relevant β estimates or EMM contrasts, SE, df, t statistics, p-values, and 95% CIs for flight duration analyses.

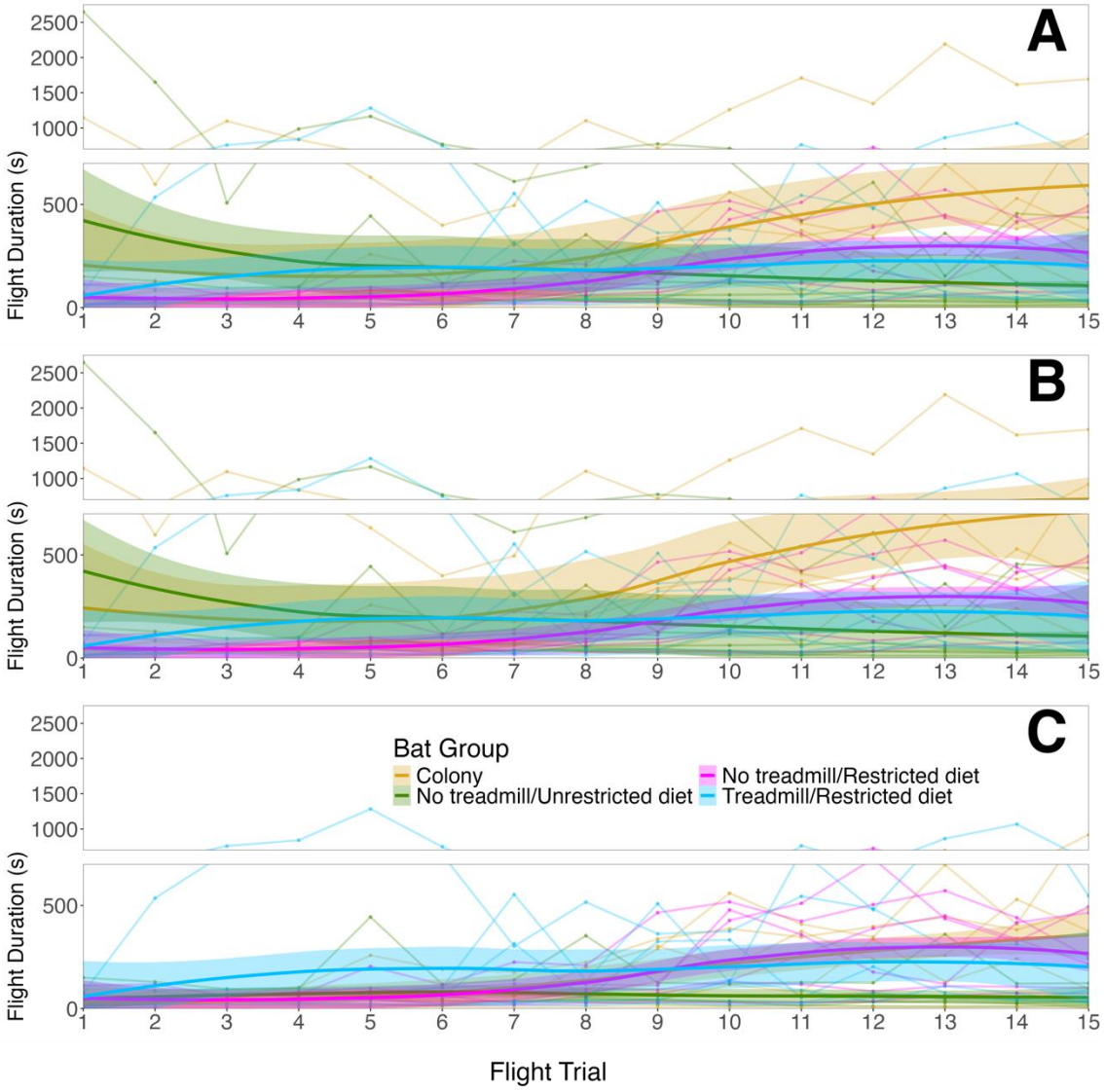


Fig. 17. Experiment 2. Change in flight duration (s) for *E. fuscus* in the different treatment groups. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of mass outlier (Violet 111) excluded (see text; *n* = 23). (**C**) Same data but with values of visualized outliers (Sky 151 and Violet 170) excluded (see text; *n* = 22). Points and connecting lines represent individual bats; thicker lines represent group averages fitted with local regression smoothing curves and shaded 95% CIs.

Including data from Violet 111, Sky 151, and Violet 170, the colony group's average flight duration surprisingly showed a large increase from the first to final trial day, from 208 s to 596 s. The no-treadmill/restricted-diet group also increased, but to a relatively less extent, from 43.5 s to 266 s. The treadmill/restricted-diet group also increased, but to an even lesser extent, from 39.3 s to 162 s. Finally, the no-treadmill/unrestricted-diet group's average flight duration decreased from 489 s to 112 s. When data from Violet 111 are excluded from the colony group, the group's average flight duration showed an even greater increase from 246 s on the first trial day to 713 s on the final trial day. Conversely, when data from Sky 151 are excluded from the colony group, the group's average flight duration increased to a lesser extent, from 20 s to 376 s. Finally, when data from Violet 170 are excluded from the no-treadmill/unrestricted-diet group, the group's average flight duration decreased to a lesser extent, from 57.2 s to 47.6 s.

LMM results including all bats. Surprisingly, all three cage groups had somewhat shorter flight durations than the colony group, though not conclusively (Fig. 18A and Appendix Table 12). There was a moderate positive effect of trial day on the colony group's flight duration. The effect of trial day differed from its effect on the colony group for the no-treadmill/unrestricted-diet and treadmill/restricted-diet groups, but not for the no-treadmill/restricted-diet group.

More specifically, the LMM results show that there was a 17% positive effect of trial day on the colony group's flight duration ($\beta = 0.16$, 95% CI [0.10, 0.22]) and a 16% negative effect of mass ($\beta = -0.30$, 95% CI [-0.42, -0.17]). The cage group deviations had large 95% CIs. Still, unexpectedly, the treadmill/restricted-diet group had the greatest negative deviation from the colony group, though the 95% CIs marginally include zero,

so there was insufficient evidence to conclude this (β = -1.29, 95% CI [-2.59, 0.01]). The other two cage groups also had negative deviations, but again, there was insufficient evidence to conclude that there were true differences (β = -0.42, 95% CI [-1.69, 0.86] for the no-treadmill/unrestricted-diet group, and β = -0.80, 95% CI [-2.02, 0.43] for the no-treadmill/restricted-diet group). There was sufficient evidence that the effect of trial day on the colony group was larger than the effect of trial day on the no-treadmill/unrestricted-diet group (β = -0.16, 95% CI [-0.25, -0.08]) and the treadmill/restricted-diet group (β = -0.11, 95% CI [-0.20, -0.02]). Conversely, the effect of trial day was similar on the colony group and no-treadmill/restricted-diet group (β = -0.01, 95% CI [-0.09, 0.08]). Finally, the effect of mass on the colony group was larger than its effect on the no-treadmill/restricted-diet group (β = 0.22, 95% CI [0.06, 0.37]), but was similar for the no-treadmill/unrestricted-diet (β = 0.06, 95% CI [-0.14, 0.26]) and the treadmill/restricted-diet groups (β = -0.06, 95% CI [-0.26, 0.15]).

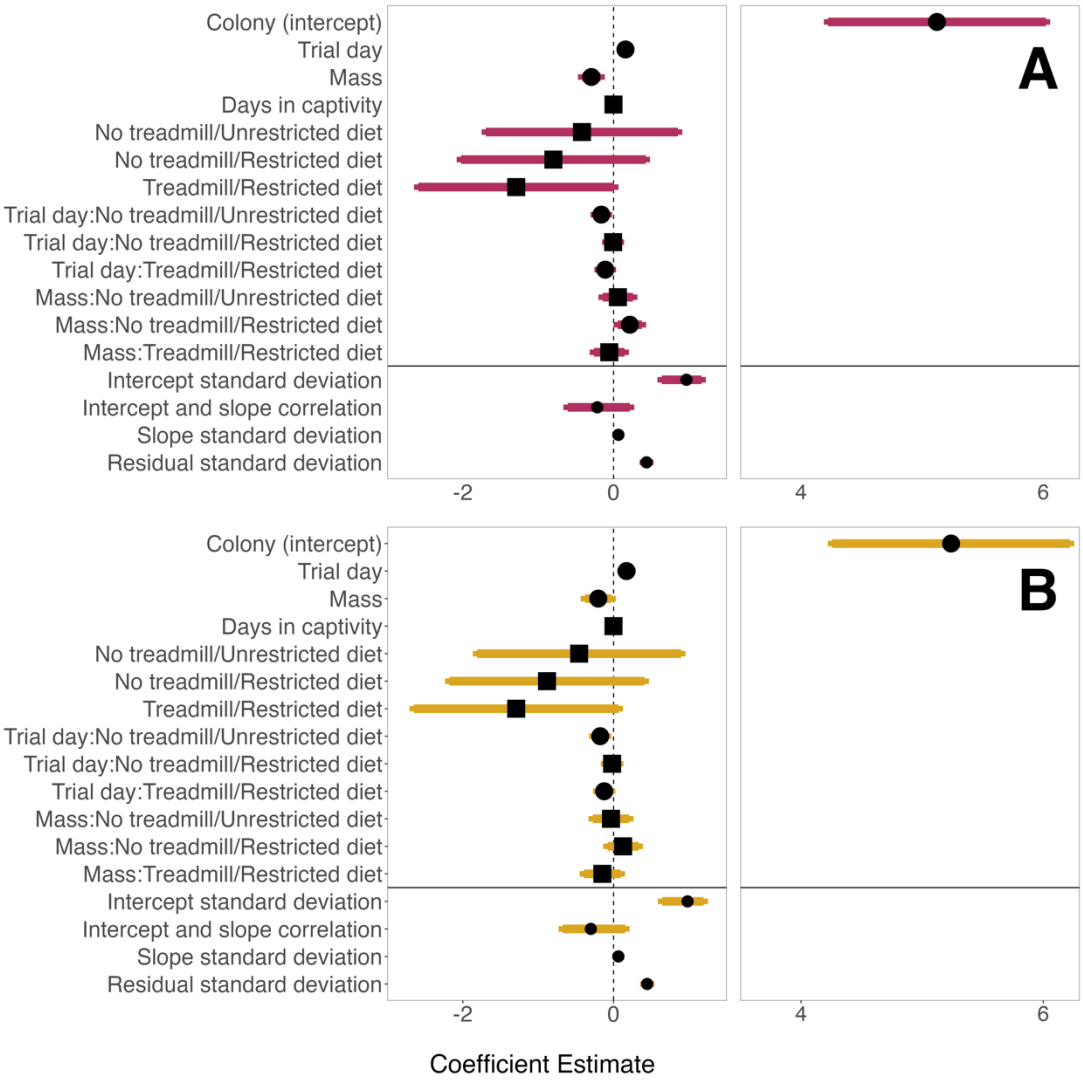


Fig. 18. Experiment 2. Flight duration (ln(s)) LMM results including and excluding mass outlier. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of mass outlier (Violet 111) excluded (see text; *n* = 23). Points represent β estimates; maroon and yellow lines represent 95% CIs. Fixed effects (above horizontal line) include: colony group as intercept, centered trial day, centered mass, centered days in captivity, three cage group deviations, centered trial day and three cage group interactions, and centered mass and three cage group interactions; random effects (below horizontal line) include: intercept SD, intercept and slope correlation, slope SD, and residual SD).

LMM results excluding outliers. When data from the mass outlier (Violet 111) were removed, the effect of mass on all three of the cage groups was similar to its effect on the colony group (Fig. 18B and Appendix Table 15).

When data from the visualized outliers (Sky 151 and Violet 170) were removed, the β estimates and 95% CIs remained similar (Appendix Table 15).

EMM contrasts including all bats. The EMMs for flight duration for the colony, no-treadmill/restricted-diet, and treadmill/restricted-diet groups increased from the first to final flight trial, though the treadmill/restricted-diet group contrast was non-significant (EMM contrast = -2.21, 95% CI [-3.05, -1.38], EMM contrast = -2.13, 95% CI [-3.03, -1.23], and EMM contrast = -0.67, 95% CI [-1.54, 0.20], respectively; Fig. 19A and Appendix Table 16). Conversely, the EMM for the no-treadmill/unrestricted-diet group decreased somewhat, but the contrast was non-significant (EMM contrast = 0.07, 95% CI [-0.80, 0.94]).

On the final flight trial day, the EMM for the treadmill/restricted-diet group's flight duration was smaller than the colony group's (EMM contrast = 2.06, 95% CI [0.23, 3.90]; Appendix Table 17).

EMM contrasts excluding outliers. When data from the mass outlier (Violet 111) were removed, the EMM contrasts remain similar (Appendix Table 17).

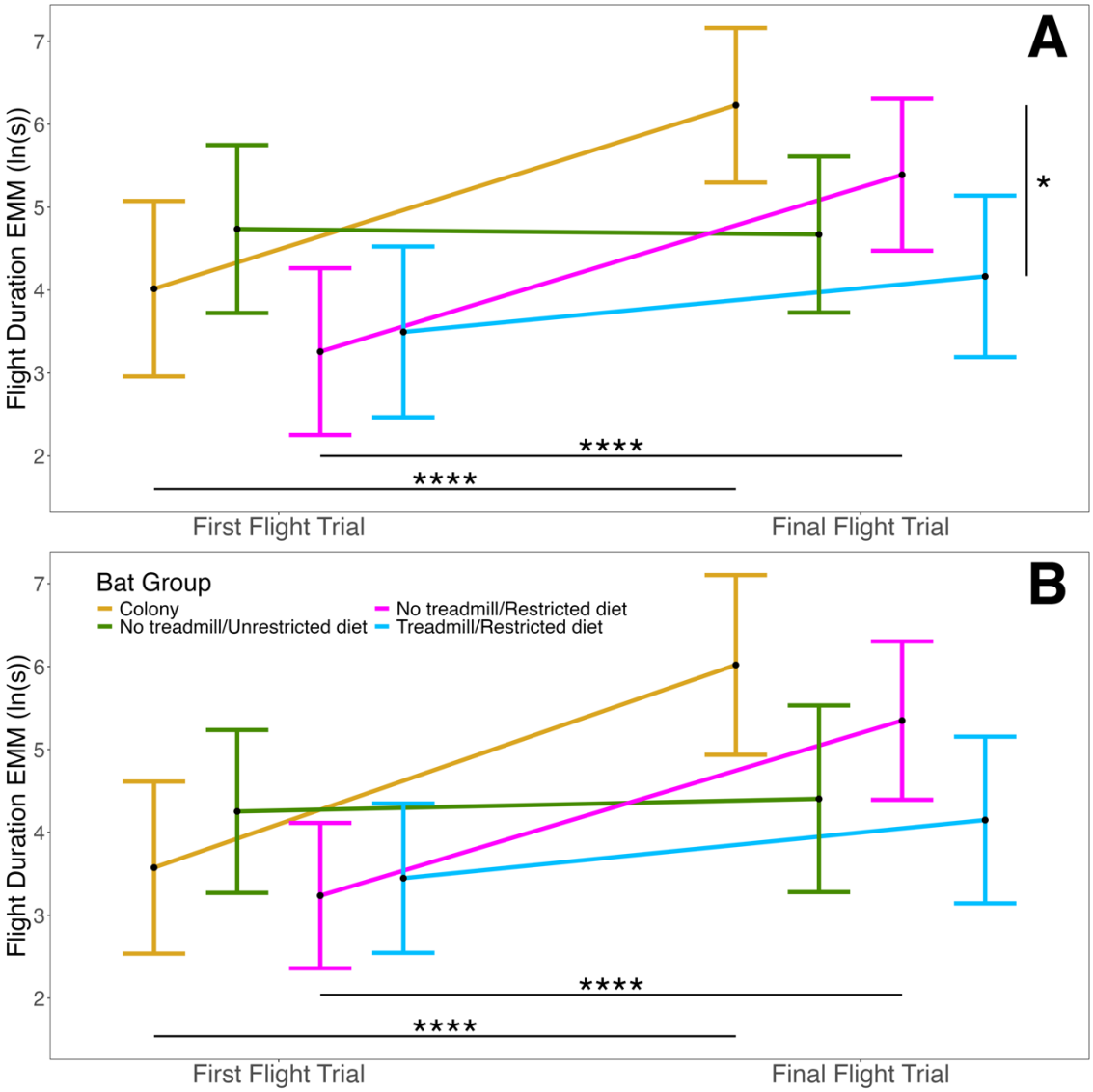


Fig. 19. Experiment 2. Flight duration (ln(s)) group EMMs during first and final trial days including and excluding visualized outliers. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of visualized outliers (Sky 151 and Violet 170) excluded (see text; *n* = 22). Significant contrasts are labeled; horizontal labels represent within-group contrasts from first to final trial day; rightside vertical label represents between-group contrast on final trial day.

When data from the visualized outliers (Sky 151 and Violet 170) were removed, the EMM for the no-treadmill/unrestricted-diet group slightly increased, but the contrast was non-significant (EMM contrast = -0.15, 95% CI [-1.07, 0.76]; Fig. 19B). Also, the EMM for the treadmill/restricted-diet group on the final trial day was not significantly smalller than the colony group's (EMM contrast = 1.87, 95% CI [-0.14, 3.88]).

Take-off latency. Next, I compared how take-off latency changed under the different treatments over the 59-day study (Fig. 20). One no-treadmill/unrestricted-diet bat (Sky 101) had longer take-off latencies than other bats during most flight trials. Analyses are completed including data from all bats, then excluding data from the large mass colony bat (Violet 111; termed mass outlier), and finally excluding data from the long take-off latency no-treadmill/unrestricted-diet bat (Sky 101; termed visualized outlier). Appendix Tables 18–20 list relevant β estimates or EMM contrasts, SE, df, t statistics, p-values, and 95% CIs for take-off latency analyses.

Including data from Violet 111 and Sky 101, the average take-off latency interestingly increased from the first to final trial day for the treadmill/restricted-diet group, from 4.27 s to 55.1 s. The average also increased for the no-treadmill/unrestricted-diet group from 8.93 s to 26.9 s. Conversely, averages decreased slightly for the colony group from 18.1 s to 16.6 s, and for the no-treadmill/restricted-diet group from 19.6 s to 15.1 s. When data from Violet 111 are excluded from the colony group, the group's take-off latency decreased to a greater extent, from 19.9 s to 12.7 s. Finally, when data from Sky 101 are excluded from the no-treadmill/unrestricted-diet group, the group average surprisingly decreased somewhat from 9.1 s to 4.3 s.

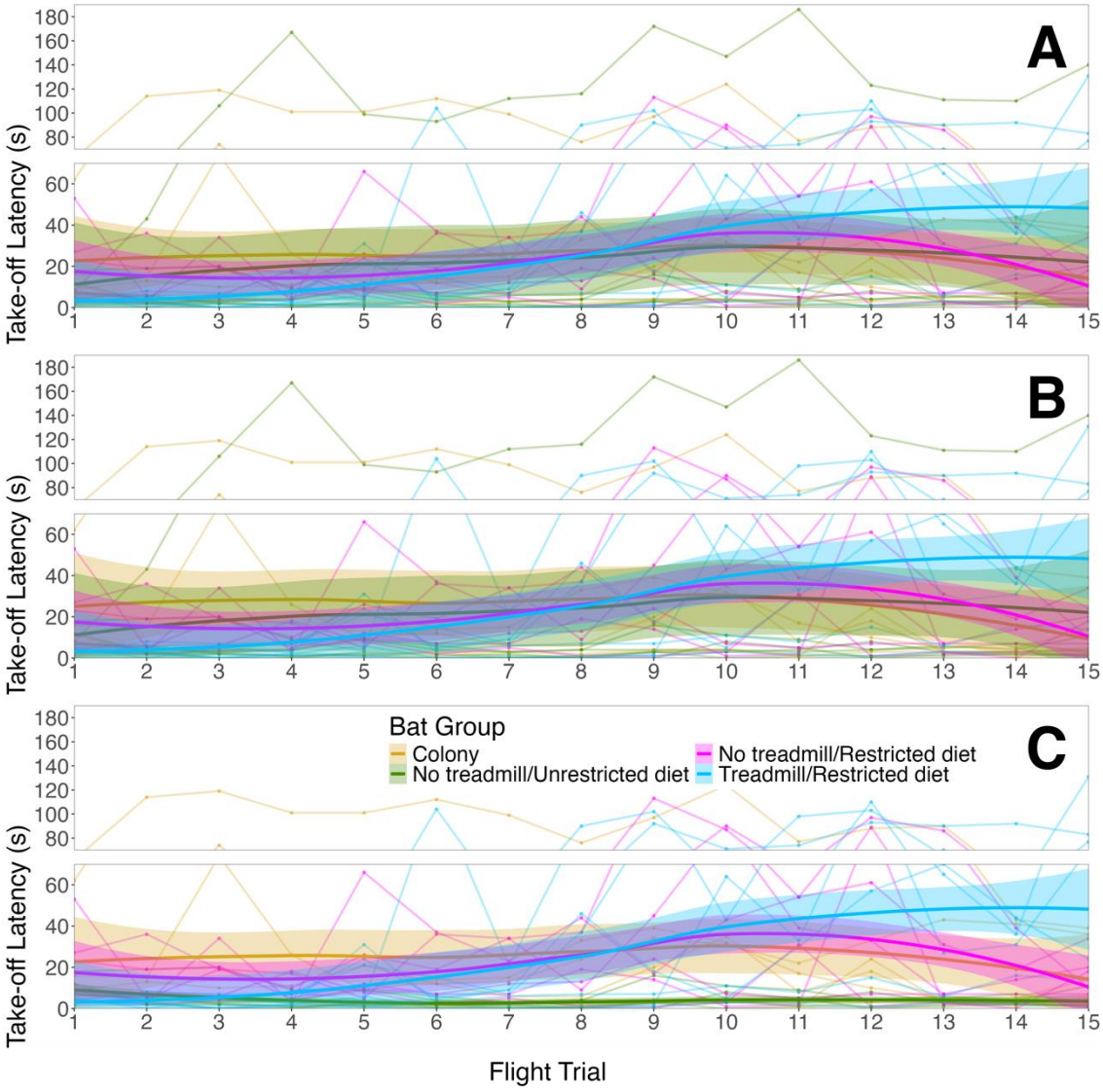


Fig. 20. Experiment 2. Change in take-off latency (s) for *E. fuscus* in the different treatment groups. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of mass outlier (Violet 111) excluded (see text; *n* = 23). (**C**) Same data but with values of visualized outlier (Sky 101) excluded (see text; *n* = 23). Points and connecting lines represent individual bats; thicker lines represent group averages fitted with local regression smoothing curves and shaded 95% CIs.

LMM results including all bats. Overall, there was not a strong effect of trial day on the colony group's take-off latency, and the cage groups' take-off latencies were similar to the colony group's. Though, there was a larger effect of trial day on the treadmill/restricted-diet group than on the colony group (Fig. 21A and Appendix Table 18).

More specifically, the LMM results show that the colony group's take-off latency decreased very slightly across trial days, but the 95% CIs include zero, so there was insufficient evidence to conclude that there was an effect (β = -0.01, 95% CI [-0.07, 0.06]). However, there was sufficient evidence that there was 16% increase in take-off latency per unit increase in mass for the colony group (β = 0.15, 95% CI [0.03, 0.27]). The cage group deviations all had large 95% CIs that include zero, so we cannot conclude there were true differences. Though, unexpectedly, the no-treadmill/unrestricted-diet group deviated somewhat negatively (β = -0.82, 95% CI [-2.37 0.72]), while both the restricted diet groups deviated somewhat positively (β = 0.65, 95% CI [-0.95, 2.25] for the no-treadmill/restricted-diet group, and β = 0.36, 95% CI [-1.25, 1.97] for the treadmill/restricted-diet group). Finally, there was a larger effect of trial day on the treadmill/restricted-diet group than on the colony group, and the treadmill/restricted-diet group's slope was positive 0.22 (β = 0.23, 95% CI [0.13, 0.32]). Conversely, the effect of trial day on the colony group was similar to its effect on the no-treadmill/unrestricted-diet (β = -0.001, 95% CI [-0.10, 0.09]) and no-treadmill/restricted-diet groups (β = 0.05, 95% CI [-0.05, 0.15]).

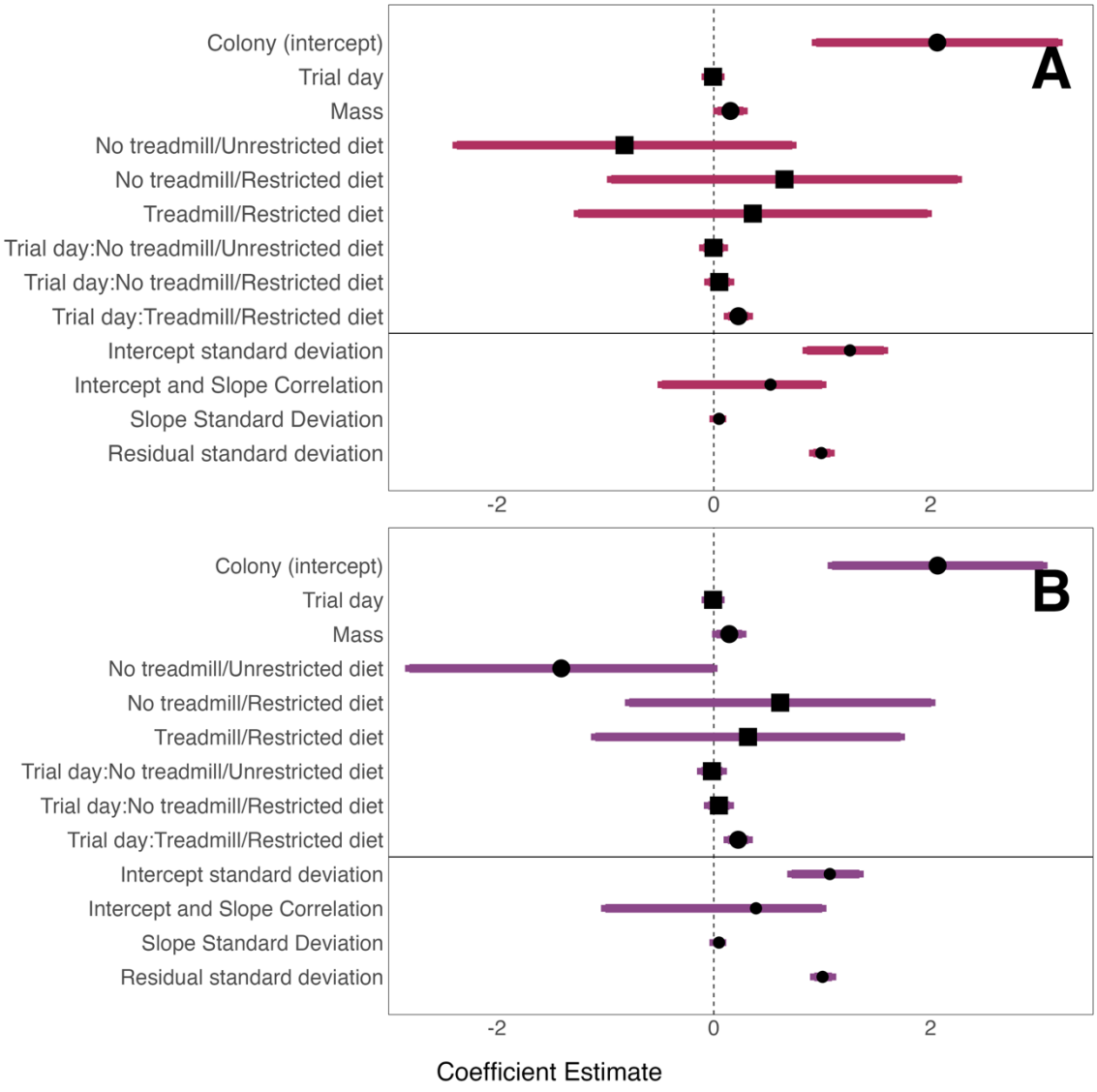


Fig. 21. Experiment 2. Take-off latency (ln(s)) LMM results including and excluding visualized outlier. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of visualized outlier (Sky 101) excluded (see text; *n* = 23). Points represent β estimates; maroon and purple lines represent 95% CIs. Fixed effects (above horizontal line) include: colony group as intercept, centered trial day, centered mass, centered days in captivity, three cage group deviations, and centered trial day and three cage group interactions; random effects (below horizontal line) include: intercept SD, intercept and slope correlation, slope SD, and residual SD.

LMM results excluding outliers. When data from the mass outlier (Violet 111) were removed, the β estimates and 95% CIs remained similar (Appendix Table 18).

When data from the visualized outlier (Sky 101) were removed, the no-treadmill/unrestricted-diet group's take-off latency was shorter than the colony group's, though with wide 95% CIs (β = -1.41, 95% CI [-2.81, -0.01]; Fig. 21B and Appendix Table 18).

EMM contrasts including all bats. The EMM for the treadmill/restricted-diet group's take-off latency increased significantly from the first to final trial day (EMM contrast = -3.09, 95% CI [-4.03, -2.15]; Fig. 22A and Appendix Table 19). The EMM for the no-treadmill/restricted-diet group also increased, but the contrast was non-significant (EMM contrast = -0.62, 95% CI [-1.64, 0.40]). Finally, the EMMs for colony and no-treadmill/unrestricted-diet groups decreased, but not significantly (EMM contrast = 0.09, 95% CI [-0.83, 1.01], and EMM contrast = 0.12, 95% CI [-0.83, 1.06], respectively).

On the final flight trial day, the EMM for the treadmill/restricted-diet group's was significantly larger than the no-treadmill/unrestricted-diet group's (EMM contrast = -2.79, 95% CI [-5.42, -0.61]; Appendix Table 20).

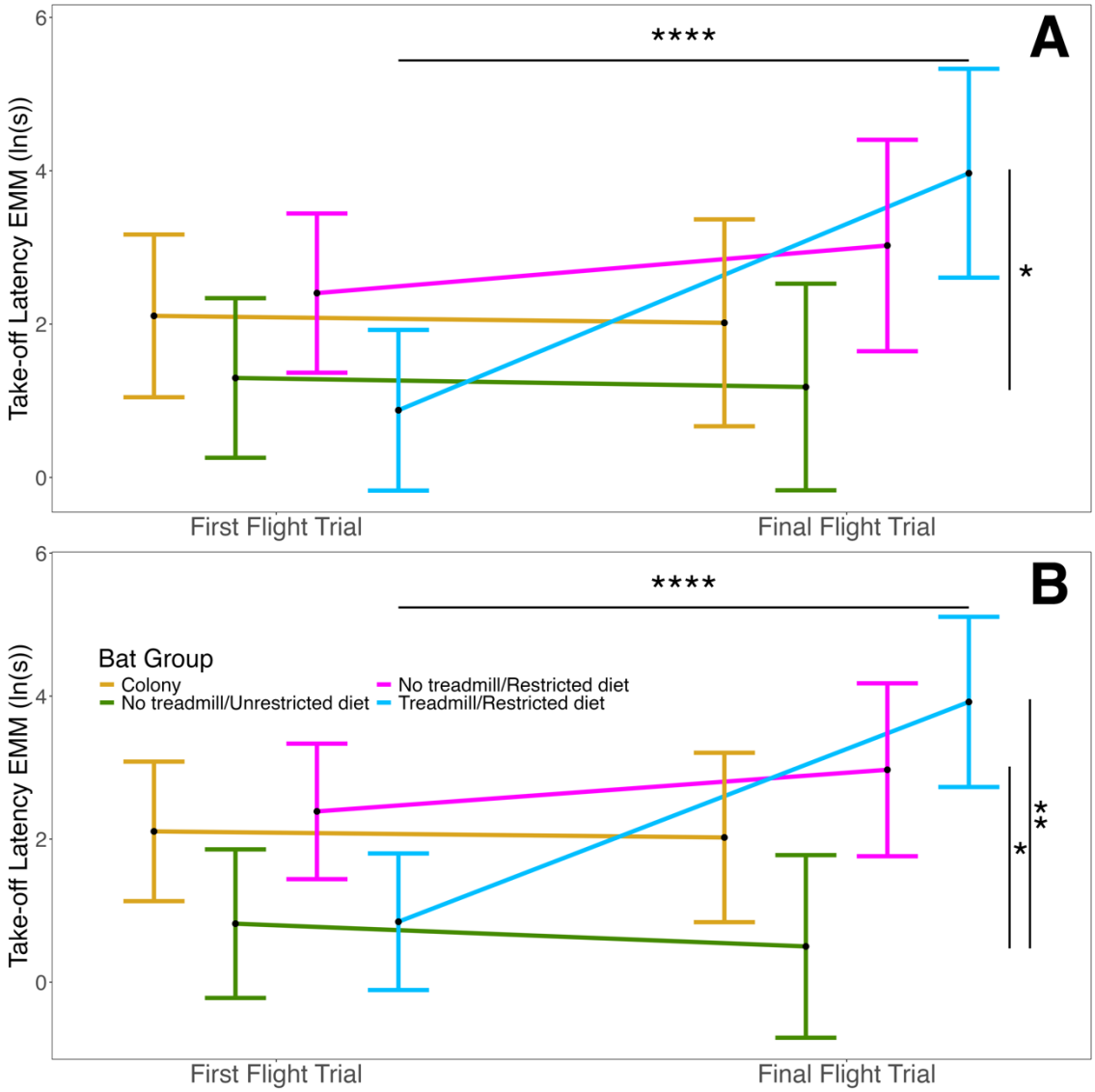


Fig. 22. Experiment 2. Take-off latency (ln(s)) group EMMs during first and final trial days including and excluding visualized outlier. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of visualized outlier (Sky 101) excluded (see text; *n* = 23). Significant contrasts are labeled; horizontal labels represent within-group contrasts from first to final trial day; rightside vertical labels represent between-group contrasts on final trial day.

EMM contrasts excluding outliers. When data from the mass outlier (Violet 111) were excluded, the EMM contrasts remain similar (Appendix Table 19)

When data from the visualized outlier (Sky 101) were removed, on the final trial day, the EMM for the no-treadmill/restricted-diet group's take-off latency was also significantly larger than the no-treadmill/unrestricted-diet group (EMM contrast = -2.47, 95% CI [-4.89, -0.05]; Fig. 22B).

Post-experiment mass. Finally, I compared how masses changed after returning all bats to the colony, over an approximately one-month period (Fig. 23). Overall, group mass averages trended upwards. However, two bats increased to a greater extent. These were Violet 111, the same colony group mass outlier, and Grey 82, a prior no-treadmill/unrestricted-diet bat. Appendix Fig. 1 compared post-experiment change in mass including data from all bats and excluding data from Violet 111 and Grey 82. Appendix Tables 21–23 list relevant β estimates or EMM contrasts, SE, df, t statistics, p-values, and 95% CIs for post-experiment mass analyses.

LMM results. Overall, there was a small 0.19 g positive effect of trial day on the prior colony group's mass. The masses for the groups previously housed in cages were similar to the prior colony group's, and the effect of trial day on the three groups was similar to its effect on the prior colony group (Appendix Fig. 24 and Appendix Table 21).

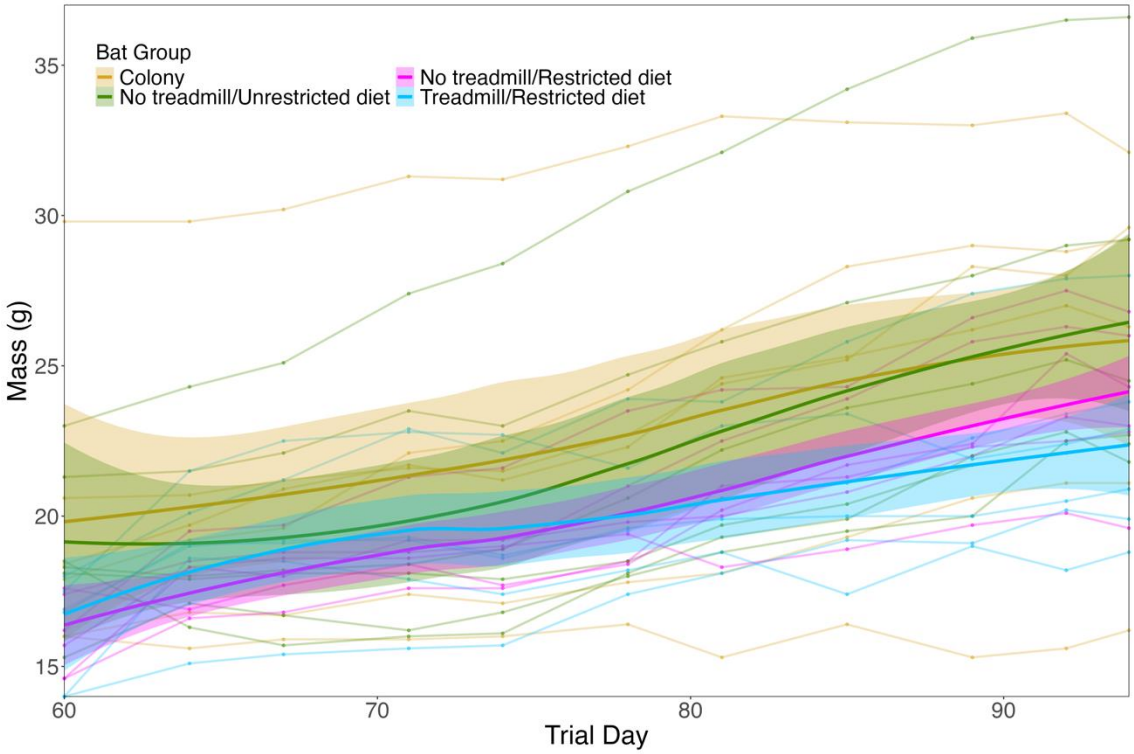


Fig. 23. Post-Experiment 2. Change in mass (g) for *E. fuscus* (*n* = 24) after returning all bats to colony. Points and connecting lines represent individual bats; thicker lines represent group averages fitted with local regression smoothing curves and shaded 95% CIs.

EMM contrasts. All EMMs increased from the first to final day they were weighed (EMM contrast = -6.51, 95% CI [-9.53, -3.84] for colony group, EMM contrast = -8.31, 95% CI [-11.33, -5.28] for no-treadmill/unrestricted-diet group, EMM contrast = -7.63, 95% CI [-10.65, -4.60] for no-treadmill/restricted-diet group, and EMM contrast = -4.93, 95% CI [-7.95, -1.90] for treadmill/restricted-diet group; Appendix Fig. 25 and Appendix Table 22).

Results excluding outliers. When data from the two visualized outliers (Violet 111 and Grey 82) were excluded, the β estimates and 95% CIs, as well as the EMM contrasts remained similar (Appendix Tables 21–23).

Discussion

The goal of the present study was to examine how to maintain and improve captive bat flight, with the goal of informing caretakers on possible ways to advance captive *E. fuscus* housing and care.

Experiment 1: Quantifying decline in flight performance

This first experiment quantified how *E. fuscus* maintain—or do not maintain—mass and flight after being brought into captivity and restrained in the typical quarantine housing conditions.

Firstly, mass results for the non-reproductive individuals were as predicted; there was a large positive effect of being in the quarantine housing conditions on their mass. Conversely, the quarantine housing conditions did not have the same effect on the pregnant-at-start females. The pregnant females had larger mass than the non-reproductive individuals when they were newly-caught, which is expected due to their pregnancy. Interestingly, the two groups' masses converged during the second phase because the pregnant-at-start females' mass remained the same. This suggests the pregnant-at-start females did not lose their pregnancy-related mass after parturition, which would be abnormal in the wild. For example, wild postpartum females and adult males do not differ in body mass, and their masses are smaller than pregnant females (Yacoe, 1983). In general, wild *E. fuscus* do not typically increase in mass during this seasonal period as they are active (i.e. not in torpor) in ambient temperatures above 10 degrees Celsius, and do not begin to accumulate body fat for hibernation until at least August (Beer & Richards, 1956; Kurta & Baker, 1990). Therefore, these results support the hypothesis that the quarantine housing conditions affect mass.

Second, flight durations decreased somewhat while being in the quarantine housing conditions. LMM results suggest that there may have been a negative effect of being in the second phase on the non-reproductive individuals' flight duration, though it was not conclusive. The effect also did not conclusively differ for the pregnant-at-start females, suggesting that they may have experienced a similar downward trend in flight duration. There was a negative effect of mass on the non-reproductive individuals' flight duration. This indicates that the increased mass these individuals experience in the quarantine housing conditions can impact their flight ability.

The flight duration results suggest that the quarantine housing conditions may have a negative effect on flight ability. However, it is possible that flight duration was shorter during the second phase for reasons unrelated to decreased flight ability. That is, the bats may have been able to fly for the same duration, but were not motivated to. The motivating factors were controlled through the housing and flight trial conditions for both phases, but a future study could explore other possible covariate motivating factors for bat flight duration.

Third, take-off latency results showed that the quarantine housing conditions may have actually decreased take-off latency somewhat, though the results did not support a definitive conclusion about the effect. Still, take-off latency for both groups trended downwards, which was opposite of my prediction that take-off latency would increase. This may have been because the bats were more eager to initiate flight after not having the opportunity to fly for 11 weeks, but future work would need to examine whether this is a motivating factor for bats to initiate flight.

Finally, during the first phase, the pregnant individuals had somewhat longer take-off latency and shorter flight duration than the non-reproductive individuals. Though

these effects were not conclusive, they suggest that their pregnancy may have negatively affected their flight ability to some degree. However, it is worth noting that the differences may not have been related to flight ability. Rather, the pregnant individuals may have opted to conserve their energy by being less willing to initiate flight, and by flying for a shorter duration than the non-reproductive individuals.

The EMM contrast predicted that the pregnant-at-start group's take-off latency would decrease more than the non-reproductive individuals from the first to second phase. After parturition and lactation, the need to conserve energy would have decreased (Oftedal, 1985), which could be a reason why their take-off latency decreased more than the other group. However, this does not explain why their flight duration decreased somewhat from the first to the second phase, suggesting they still may have experienced some decrease in flight ability.

Overall, the mass result support my hypothesis, and flight duration results trended in the predicted direction, while the take-off latency results did not. The quarantine housing conditions have a positive effect on mass and may have a negative effect on flight duration. Such changes in mass and flight duration are not natural for *E. fuscus*, so the quarantine housing conditions likely do not provide the quality of care required in captivity.

Experiment 2: Methods to improve flight in captive bats

This second experiment aimed to explore methods, namely restricted diet and crawling exercise, to improve captive bat flight in individuals that had been in captivity for over a year.

Firstly, mass results showed, as predicted, that the restricted diet had a large negative effect on the two groups' (i.e. no-treadmill/restricted-diet and treadmill/restricted-diet) masses; however, the EMMs for the two groups' masses did not differ on the final trial day, so the crawling exercise was not estimated to decrease mass to a greater extent. Next, there was not a strong effect of being housed in the colony on the group's mass, as predicted. Finally, there was not conclusive evidence that there were differing effects between being in the no-treadmill/unrestricted-diet or the colony group. EMM analysis showed that when data from the colony group's large mass outlier were removed, the no-treadmill/unrestricted-diet group's mass was estimated to increase significantly from the first to final trial day, but not to a point where it differed from the colony group. These EMM predictions suggest that moving the bats from the colony to a smaller cage with *ad libitum* food has some positive effect on mass, but not to the extent I predicted.

Second, unexpectedly, the LMM results showed that there was a positive effect of being housed in the colony on the group's flight duration. This was unexpected because I predicted that their flight duration would not change throughout the study. Based on the EMM analysis, the colony, no-treadmill/restricted-diet, and treadmill/restricted-diet groups' flight durations were estimated to increase from the first to final trial day, but the treadmill/restricted-diet group's increase was not significant. Interestingly, the colony group and no-treadmill-restricted-diet group flight durations were estimated to increase with similar slopes, whereas the treadmill/restricted-diet increase had a shallower slope.

This suggests that crawling exercise may actually have had a detractive effect on flight duration, rather than the positive effect I predicted. Also, the no-treadmill/unrestricted group results did not support my prediction that the conditions would have a negative effect on flight. Instead, the EMM analysis estimated that the group's flight duration wouldn't change much from the first to final trial day. This suggests that moving the bats into a smaller cage with *ad libitum* food access may not have a negative effect on their flight. Though, it may have prevented a positive effect, as the group's flight duration did not trend upwards like the other three groups.

Third, the LMM results showed that, overall, there were not conclusive effects of the housing conditions on take-off latency. However, when data from the visualized outlier in the no-treadmill/unrestricted-diet group were removed, the group had shorter take-off latency than the colony group, which was not expected. Another unexpected result was that the treadmill/restricted-diet group had a positive slope across trial day, which was reflected in the EMM analysis, wherein the group's take-off latency was estimated to increase significantly from the first to final trial day. It was also estimated to be significantly greater than the no-treadmill/unrestricted-diet group on the final trial day. Furthermore, the EMM analysis showed that the no-treadmill/restricted-diet group's take-off latency was also predicted to increase, though not significantly. When data from the visualized outlier were removed, the no-treadmill/restricted-diet group's take-off latency was also predicted to be significantly greater than the no-treadmill/unrestricted-diet group on the final trial day. These EMM results do not support my predictions for take-off latency, which were: (1) the small cage with *ad libitum* food access would cause an increase, (2) the restricted diet would cause a decrease, and (3) the crawling exercise would cause a relatively larger decrease. Instead, the small cage with *ad libitum* food

access may have decreased take-off latency, whereas the restricted diets, and especially the crawling exercise, may have increased it.

Overall, the diets and housing conditions had the predicted effects on group mass, but not the predicted effects on flight ability. Moreover, crawling exercise did not have the predicted effects in any of the measures. The treadmill/restricted-diet group's predicted changes in the flight ability measures were surprising, and suggested there were additional factors to consider.

One possible factor could have been the available energy levels. The treadmill/restricted-diet bats had limited energy intake due to the restricted diet, and increased energy expenditure due to the crawling exercise. This may have caused them to opt to conserve their energy, which is supported by their EMM increase in take-off latency, and smaller EMM increase in flight duration compared to the colony and no-treadmill/restricted-diet groups. Though, the shorter flight duration could have been due to not having enough energy to expend on longer flights, rather than an intentional conservation of energy. Conversely, the no-treadmill/restricted-diet and colony groups would likely have had greater energy levels, allowing them to perform longer flights.

Finally, regarding outliers, there were no apparent patterns in how results changed when either data from the large mass outlier, or data from the respective response variable outliers were removed. Therefore, to avoid data selection bias, the main results including all bats were:

- a negative effect of restricting diet on mass, though only the no-treadmill-restricted-diet group EMM decreased significantly;

- a positive effect of trial day on the colony group's flight duration, and smaller effects of trial day on the no-treadmill/unrestricted-diet and treadmill/restricted-diet group flight durations, which were reflected in the colony group and no-treadmill/restricted-diet EMM flight durations increasing significantly, and;
- a positive slope for the treadmill/restricted-diet group's take-off latency, with a significant EMM increase for the group.

Implications

The present study was novel in designing a procedure to train captive *E. fuscus* to crawl on a motorized treadmill with a food motivator. Though crawling exercise did not have the predicted effects in this study, it may have benefits in other avenues. For example, it could be a way to increase physical activity for bats that have lost their flight ability. Crawling exercise may also have differing effects on different bat species. For example, it may not largely benefit species with moderate crawling ability, like *E. fuscus*, but could have greater effects on species with great crawling ability, like *D. rotundus*. Future studies could examine the degree to which crawling affects different species, as well as possible uses for the crawling procedure.

Furthermore, the present study showed that the colony group's conditions improved flight duration, which I had not predicted. The finding suggests that explicitly flying the bats on two nights per week, in addition to any voluntary flight they may have had in the colony, could improve flight ability. Future research should examine the effect of systematic flight exercise on captive bat flight ability.

Along with the colony group's increased flight duration, the no-treadmill/restricted-diet group's conditions were also estimated to improve flight

duration. Future studies that require flight could employ either of these conditions to improve flight duration prior to study onset. Additionally, restricting the animals' diet effectively decreased their mass, which can be employed for future studies requiring bats with smaller mass.

However, the extent to which I restricted their diets is not ideal for long-term studies as the bats would likely have eventually become underweight. Chronically undernourished animals face negative effects such as increased susceptibility to stressors like cold stimuli and having offspring with arrested development (Campbell & Richardson, 1988; Martins et al., 2011; Roeder & Chow, 1972). Furthermore, quantitative food restriction can be associated with signs of hunger such as increased food motivation, leading to overeating when food is available (D'Eath et al., 2009). My post-experiment mass analysis suggested that this did not occur when the bats were returned to the colony with *ad libitum* food access (i.e. all bats increased similarly in mass), but this may not be the case after longer-term food restriction. Quantitative food restriction can also cause redirected oral behaviours (e.g., chewing on the enclosure) which can develop into stereotypies (D'Eath et al., 2009). Additionally, as discussed, the restricted diet may have increased their take-off latency somewhat, so prolonging the restriction may further increase their hesitancy to initiate flight. Finally, for general long-term housing, restricting diets and hand-feeding bats daily is not feasible. Therefore, long-term quantitative food restriction may not provide the quality of care needed for captive animals.

Furthermore, while the no-treadmill/restricted-diet group's flight duration increased throughout my study, the small cage housing restricts their exercise, which may eventually counteract the effect. This would detract from longer-duration flight studies.

In summation, the colony conditions are more ideal for long-term studies and housing, and explicitly flying colony-housed bats can improve flight duration for future studies.

Finally, my results show that the quarantine housing conditions do not allow bats to maintain mass and flight duration, so bats should not be housed under these conditions for an extended period of time (i.e. months). Though not statistically analyzed, *Experiment 1* bat masses during the second phase appeared to be greater than *Experiment 2* masses of bats housed in the colony prior to study onset. Similarly, *Experiment 1* flight durations during the second phase appeared to be shorter than *Experiment 2* flight durations at study onset. Taking the two experiments' results together, to maintain mass and flight duration upon capture, newly-caught bats should be housed in a larger enclosure that allows for voluntary free flight. Because the colony group's flight duration increased in *Experiment 2,* caretakers could also aim to systematically fly newly-caught bats along with their voluntary flight. Future research should examine how systematic and voluntary flight exercise helps maintain newly-captive *E. fuscus* flight.

Limitations

The non-reproductive individuals group in *Experiment 1* had one non-pregnant female and four males due to limited bat availability. Therefore, it is unknown whether there were differences between non-reproductive female and male mass and flight ability.

Also, due to limited space availability, the colony group in *Experiment 2* was housed with other captive bats not included in the study. Conversely, the cage groups were only housed with the individuals in their groups. It is unknown whether the differences in social composition had an effect on behaviours like feeding, or their performance.

Additionally, *Experiment 2* did not include a group that was exercised through crawling and had *ad libitum* food access, in either a small cage or the colony. This was to not overfeed bats while motivating them to crawl on the treadmill. However, I have speculated that the treadmill/restricted-diet group had limited available energy, which may have negatively affected their flight performance. Therefore, a study including a treadmill exercise group with *ad libitum* food access could examine whether crawling exercise can improve flight when the animal has unrestricted energy intake.

Experiment 2 also did not include a group housed in an enclosure large enough for free flight, but on a restricted diet. Again, this was due to limited space. Based on my results, I would predict that a colony group on a restricted diet would decrease in mass, and possibly have an even greater increase in flight duration than the colony and no-treadmill/restricted-diet group conditions alone.

Furthermore, *Experiment 2* only included female captive bats because there were not enough males available. I am not aware of any studies that compare female and male bat flight performance, so a future experiment examining this would be novel.

Finally, I was not blind to the bats' treatment conditions during flight trials for both experiments. Though I controlled factors such as the starting point, and how each trial began and ended, I may have subconsciously modified my behaviour across different bats.

Conclusion

This study offers novel research on maintaining and improving captive *E. fuscus* flight. My results show that the quarantine housing conditions for newly-captive bats may not maintain proper mass and flight duration. Also, for bats that have been in captivity

over a longer period of time, combining systematic flight trials with a restricted diet or housing large enough to allow voluntary flight improves flight duration. These are important results to inform adequate bat husbandry because captive bat flight is crucial for both animal welfare and scientific research.

References

Barnard, S. M. (2011). Environment and housing: insectivorous bats (pp. 47–70). *Bats in Captivity Volume 3: Diet and Feeding—Environment and Housing* (S. M. Barnard, Ed.). Logos Press. City of Publisher and pp

Barnard, S. M., Griffiths, M. A., & Dierenfeld, E. S. (2011). Diet and feeding: insectivorous bats (pp. 357–402). *Bats in Captivity Volume 3: Diet and Feeding—Environment and Housing* (S. M. Barnard, Ed.). Logos Press, Washington, DC.

Bauer, S. A., Pearl, D. L., Leslie, K. E., Fournier, J., & Turner, P. V. (2012). Causes of obesity in captive cynomolgus macaques: Influence of body condition, social and management factors on behaviour around feeding. *Laboratory Animals*, *46*(3), 193–199. https://doi.org/10.1258/la.2012.011120

Beer, J. R., & Richards, A. G. (1956). Hibernation of the big brown bat. *Journal of Mammalogy*, *37*(1), 31–41. https://doi.org/10.2307/1375523

Brigham, R. M. (1987). The significance of winter activity by the big brown bat (*Eptesicus fuscus*): The influence of energy reserves. *Canadian Journal of Zoology*, *65*(5), 1240–1242. https://doi.org/10.1139/z87-192

Campbell, B. A., & Richardson, R. (1988). Effect of chronic undernutrition on susceptibility to cold stress in young adult and aged rats. *Mechanisms of Ageing and Development*, *44*(2), 193–202. https://doi.org/10.1016/0047-6374(88)90091-7

Cao, T., & Jin, J.-P. (2020). Evolution of flight muscle contractility and energetic efficiency. *Frontiers in Physiology*, *11*, 1038. https://doi.org/10.3389/fphys.2020.01038

Cheney, J. A., Konow, N., Middleton, K. M., Breuer, K. S., Roberts, T. J., Giblin, E. L., & Swartz, S. M. (2014). Membrane muscle function in the compliant wings of

bats. *Bioinspiration & Biomimetics*, *9*(2), 025007. https://doi.org/10.1088/1748-3182/9/2/025007

D'Eath, R. B., Tolkamp, B. J., Kyriazakis, I., & Lawrence, A. B. (2009). 'Freedom from hunger' and preventing obesity: The animal welfare implications of reducing food quantity or quality. *Animal Behaviour*, *77*(2), 275–288. https://doi.org/10.1016/j.anbehav.2008.10.028

Fleming, T. H. (2019). Bat migration. *Encyclopedia of Animal Behavior*, 605–610. https://doi.org/10.1016/B978-0-12-809633-8.20764-4

Foster, C., Hector, L. L., Welsh, R., Schrager, M., Green, M. A., & Snyder, A. C. (1995). Effects of specific versus cross-training on running performance. *European Journal of Applied Physiology and Occupational Physiology*, *70*(4), 367–372. https://doi.org/10.1007/BF00865035

Hermanson, J. W., Cobb, M. A., Schutt, W. A., Muradali, F., & Ryan, J. M. (1993). Histochemical and myosin composition of vampire bat (*Desmodus rotundu*s) pectoralis muscle targets a unique locomotory niche. *Journal of Morphology, 217*, 347356.

Jones, M. F., & Hasiotis, S. T. (2018). Terrestrial behavior and trackway morphology of neotropical bats. *Acta Chiropterologica*, *20*(1), 229–250. https://doi.org/10.3161/15081109ACC2018.20.1.018

Jones, M. F., & Hasiotis, S. T. (2023). Terrestrial locomotor behaviors of the big brown bat (Vespertilionidae: *Eptesicus fuscus*). *Mammal Research*, *68*(2), 253–262. https://doi.org/10.1007/s13364-022-00669-9

Jordan, B. (2005). Science-based assessment of animal welfare: wild and captive animals. *Revue scientifique et technique, 24*(2), 515–528.

Kobayashi, M. (2018). Anatomical attributes of the musculus quadriceps femoris responsible for poor crawling ability in the greater horseshoe bat (*Rhinolophus ferrumequinum*). *International Journal of Morphology*, *36*(1), 69–73. https://doi.org/10.4067/S0717-95022018000100069

Kunz, T. H. (Ed.). (1982). *Ecology of Bats* (pp. 1–55). Springer, Boston, MA. https://doi.org/10.1007/978-1-4613-3421-7

Kurta, A., & Baker, R. H. (1990). *Eptesicus fuscus*. *Mammalian Species*, *356*, 1. https://doi.org/10.2307/3504258

Lawrence, M. J. (1969). Some observations on non-volant locomotion in Vespertilionid bats. *Journal of Zoology*, *157*(3), 309–317. https://doi.org/10.1111/j.1469-7998.1969.tb01705.x

Marques, J. T., Rainho, A., Carapuço, M., Oliveira, P., & Palmeirim, J. M. (2004). Foraging behaviour and habitat use by the European free-tailed bat *Tadarida teniotis*. *Acta Chiropterologica*, *6*(1), 99–110. https://doi.org/10.3161/001.006.0108

Martins, V. J. B., Toledo Florêncio, T. M. M., Grillo, L. P., Do Carmo P. Franco, M., Martins, P. A., Clemente, A. P. G., Santos, C. D. L., Vieira, M. D. F. A., & Sawaya, A. L. (2011). Long-lasting effects of undernutrition. *International Journal of Environmental Research and Public Health*, *8*(6), 1817–1846. https://doi.org/10.3390/ijerph8061817

McMillan, F. D. (2013). Stress-induced and emotional eating in animals: A review of the experimental evidence and implications for companion animal obesity. *Journal of Veterinary Behavior: Clinical Applications and Research*, *8*(5), 376–385. https://doi.org/10.1016/j.jveb.2012.11.001

Mellor, E. L., Cuthill, I. C., Schwitzer, C., Mason, G. J., & Mendl, M. (2020). Large lemurs: ecological, demographic and environmental risk factors for weight gain in captivity. *Animals (Basel)*, *10*(8), NA-NA. https://doi.org/10.3390/ani10081443

Oftedal, O. T. (1985). Pregnancy and lactation. In *Bioenergetics of Wild Herbivores*. CRC Press.

Orr, R. T. (1958). Keeping bats in captivity. *Journal of Mammalogy*, *39*(3), 339–344. https://doi.org/10.2307/1376142

Pearce, R. D., O'Shea, T. J., & Wunder, B. A. (2008). Evaluation of morphological indices and total body electrical conductivity to assess body composition in big brown bats. *Acta Chiropterologica*, *10*(1), 153–159. https://doi.org/10.3161/150811008X331171

Popa-Lisseanu, A. G., Bontadina, F., & Ibáñez, C. (2009). Giant noctule bats face conflicting constraints between roosting and foraging in a fragmented and heterogeneous landscape. *Journal of Zoology*, *278*(2), 126–133. https://doi.org/10.1111/j.1469-7998.2009.00556.x

Riskin, D. K., Bertram, J. E. A., & Hermanson, J. W. (2005). Testing the hindlimb-strength hypothesis: Non-aerial locomotion by Chiroptera is not constrained by the dimensions of the femur or tibia. *Journal of Experimental Biology*, *208*(7), 1309–1319. https://doi.org/10.1242/jeb.01522

Riskin, D. K., & Hermanson, J. W. (2005). Independent evolution of running in vampire bats. *Nature*, *434*(7031), 292–292. https://doi.org/10.1038/434292a

Roeder, L. M., & Chow, B. F. (1972). Maternal undernutrition and its long-term effects on the offspring. *The American Journal of Clinical Nutrition*, *25*(8), 812–821. https://doi.org/10.1093/ajcn/25.8.812

Rossi, G. S., & Welch, K. C. (2024). Vampire bats rapidly fuel running with essential or non-essential amino acids from a blood meal. *Biology Letters*, *20*(11), 20240453. https://doi.org/10.1098/rsbl.2024.0453

Skrinyer, A. J., Faure, P. A., Dannemiller, S., Ball, H. C., Delaney, K. H., Orman, R., Stewart, M., & Cooper, L.N. (2017). Care and husbandry of the world's only flying mammals. *Laboratory Animal Science Professional, 6*, 24–27.

Strickler, T. L. (1978). Functional osteology and myology of the shoulder in the Chiroptera. In M.K. Hecht & Szalay (Eds.), *Contributions to Verterbrate Evolution* (Vol. 4, pp. 198). S. Karger.

Tanaka, H. (1994). Effects of cross-training. Transfer of training effects on VO^2max between cycling, running and swimming. *Sports Medicine (Auckland, N.Z.)*, *18*(5), 330–339. https://doi.org/10.2165/00007256-199418050-00005

Tanaka, H., & Swensen, T. (1998). Impact of resistance training on endurance performance. *Sports Medicine*, *25*(3), 191–200. https://doi.org/10.2165/00007256-199825030-00005

Thomas, D. W. (1983). The annual migrations of three species of West African fruit bats (Chiroptera: Pteropodidae). *Canadian Journal of Zoology*, *61*(10), 2266–2272. https://doi.org/10.1139/z83-299

Von Busse, R., Hedenström, A., Winter, Y., & Johansson, L. C. (2012). Kinematics and wing shape across flight speed in the bat, *Leptonycteris yerbabuenae*. *Biology Open*, *1*(12), 1226–1238. https://doi.org/10.1242/bio.20122964

Wormell, D., Ramsay, S., Masefield, W., Houston, D., & Price, E. (2018). Assessing muscle condition in captive Livingstone's fruit bats. *Research Report*, *27*.

Yacoe, M. E. (1983). Maintenance of the pectoralis muscle during hibernation in the big brown bat, *Eptesicus fuscus*. *Journal of Comparative Physiology B, 152*(1), 97–104. https://doi.org/10.1007/BF00689733

Appendix

Animals

Appendix Table 1. *E. fuscus* used in all studies.

Experiment 1: Quantifying decline in captive bat flight

Bat ID	Sex	Pregnancy status at start	Capture date
Orange 4	Male	n/a	May 24, 2024
Orange 12	Male	n/a	May 25, 2024
Orange 13	Male	n/a	May 25, 2024
Orange 16	Male	n/a	May 25, 2024
Orange 19	Female	Pregnant	May 25, 2024
Orange 24	Female	Pregnant	May 25, 2024
Orange 25	Female	Pregnant	May 25, 2024
Orange 27	Female	Pregnant	May 25, 2024
Orange 30	Female	Pregnant	May 25, 2024
Orange 31	Female	Non-pregnant	May 25, 2024

Pilot study 1

Bat ID	Sex	Capture date
Sky 101	Female	May 18,2023
Sky 111	Female	May 18,2023
Sky 151	Female	May 18,2023
Sky 154	Female	May 18,2023
Sky 155	Female	May 18,2023
Sky 156	Female	May 18,2023

Pilot study 2

Bat ID	Sex	Capture date
Violet 113	Female	May 2, 2023*
Violet 125	Female	May 2, 2023*
Violet 127	Female	May 2, 2023*

Experiment 2: Methods to improve flight in captive bats

Bat ID	Sex	Experimental group	Capture date
Grey 82	Female	Non-exercise/unrestricted-diet	May 20, 2022
Grey 100	Female	Exercise/restricted-diet	May 23, 2022
Sky 101	Female	Non-exercise/unrestricted-diet	May 18, 2023
Sky 110	Female	Colony	May 18, 2023
Sky 111	Female	Exercise/restricted-diet	May 18, 2023
Sky 114	Female	Colony	May 18, 2023
Sky 140	Female	Non-exercise/unrestricted-diet	May 18, 2023
Sky 141	Female	Colony	May 18, 2023
Sky 144	Female	Non-treadmill/restricted-diet	May 18, 2023
Sky 146	Female	Non-treadmill/restricted-diet	May 18, 2023
Sky 147	Female	Colony	May 18, 2023
Sky 150	Female	Non-treadmill/restricted-diet	May 18, 2023
Sky 151	Female	Colony	May 18, 2023
Sky 154	Female	Non-treadmill/restricted-diet	May 18, 2023
Sky 155	Female	Non-treadmill/restricted-diet	May 18, 2023
Sky 156	Female	Exercise/restricted-diet	May 18, 2023
Sky 157	Female	Exercise/restricted-diet	May 18, 2023
White 166	Female	Exercise/restricted-diet	May 24, 2019
White 167	Female	Non-treadmill/restricted-diet	May 24, 2019
Violet 111	Female	Colony	May 2, 2023*
Violet 170	Female	Non-exercise/unrestricted-diet	August 22, 2023*
Yellow 84	Female	Non-exercise/unrestricted-diet	May 24, 2018
Yellow 151	Female	Exercise/restricted-diet	May 24, 2019
Yellow 152	Female	Non-exercise/unrestricted-diet	May 26, 2021

*refers to date that bat was acquired from Hobbitstee Wildlife Refuge; information on when bat entered the Refuge not available

Appendix Table 2. Flight groups for Experiment 2.

Group 1	Group 2
Grey 82	Grey 100
Sky 101	Sky 111
Sky 110	Sky 114
Sky 140	Sky 144
Sky 141	Sky 146
Sky 147	Sky 151
Sky 150	Sky 156
Sky 154	Sky 157
Sky 155	Violet 170
Violet 111	White 166
Yellow 84	White 167
Yellow 152	Yellow 151

Experiment 1: Quantifying decline in flight performance

Appendix Table 3. Experiment 1. *E. fuscus* (*n* = 10) mass (ln(g)) LMM results.

Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Non-reproductive individuals,						
first phase (intercept)	2.79	0.04	8	78.50	<.0001	[2.70, 2.87]
Second phase	0.50	0.05	8	5.96	<.001	[0.31, 0.70]
Pregnant-at-start females	0.46	0.05	8	9.11	<.0001	[0.34, 0.57]
Second phase:						
Pregnant-at-start females	-0.51	0.06	8	-4.32	<.01	[-0.79, -0.24]
Intercept SD	0.07					[0.04, 0.11]
Intercept & slope correlation	-0.53					[-0.86, 0.15]
Slope SD	0.18					[0.11, 0.28]
Residual SD	0.05					[0.05, 0.07]

Appendix Table 4. Experiment 1. *E. fuscus* (*n* = 10) mass (ln(g)) study phase EMM contrasts. A negative EMM contrast signifies an increase in group mass EMM from the first to the second phase, and vice versa.

Term	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
Non-reproductive individuals	-0.50	0.08	8	-5.96	<.001	[-0.70, -0.31]
Pregnant-at-start females	0.01	0.08	8	0.15	0.88	[-0.18, 0.21]

Appendix Table 5. Experiment 1. *E. fuscus* (*n* = 10) mass (ln(g)) group EMM contrasts. A negative EMM contrast signifies the pregnant-at-start females group had greater mass EMM, and vice versa.

Term	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
First phase	-0.46	0.05	8	-9.11	<.0001	[-0.57, -0.34]
Second phase	0.06	0.05	8	0.55	0.60	[-0.18, 0.29]

Appendix Table 6. Experiment 1. *E. fuscus* (*n* = 10) flight duration (ln(s)) LMM results.

Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Non-reproductive individuals,						
first phase (intercept)	1.97	0.34	22.4	9.35	<.0001	[1.28, 2.67]
Second phase	-0.55	0.36	174	-2.80	0.12	[-1.26, 0.15]
Mass	-0.16	0.03	165	-4.85	<.0001	[-0.21, -0.11]
Pregnant-at-start females	0.01	0.46	20.1	-1.11	0.98	[-0.95, 0.97]
Second phase:						
Pregnant-at-start females	0.17	0.41	175	1.43	0.67	[-0.63, 0.98]
Intercept SD	0.50					[0.24, 0.79]
Residual SD	0.96					[0.86, 1.06]

Appendix Table 7. Experiment 1. *E. fuscus* (*n* = 10) flight duration (ln(s)) study phase EMM contrasts. A negative EMM contrast signifies an increase in group flight duration EMM from the first to the second phase, and vice versa.

Term	EMM	SE	df	t	*p*	95% CI
Non-reproductive individuals	0.55	0.36	174	2.80	0.13	[-0.16, 1.27]
Pregnant-at-start females	0.38	0.20	167	1.71	0.06	[-0.02, 0.78]

Appendix Table 8. Experiment 1. *E. fuscus* (*n* = 10) flight duration (ln(s)) group EMM contrasts. A negative EMM contrast signifies the pregnant-at-start females group had greater flight duration EMM, and vice versa.

Term	EMM	SE	df	t	*p*	95% CI
First phase	-0.01	046	20.5	-0.02	0.98	[-0.97, 0.95]
Second phase	-0.18	0.38	10.8	-0.48	0.64	[-1.02, 0.65]

Appendix Table 9. Experiment 1. *E. fuscus* (*n* = 10) take-off latency (ln(s)) LMM results.

Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Non-reproductive individuals,						
first phase (intercept)	0.68	0.24	39.4	2.79	<.01	[0.19, 1.17]
Second phase	-0.61	0.33	122	-1.82	0.07	[-1.26, 0.05]
Mass	-0.01	0.02	72.1	-0.34	0.74	[-0.06, 0.04]
Pregnant-at-start females	0.42	0.33	37	1.29	0.20	[-0.24, 1.008
Second phase:						
Pregnant-at-start females	-0.30	0.39	148	-0.78	0.44	[-1.07, 0.47]
Intercept SD	0.16					[0.00, 0.34]
Residual SD	0.97					[0.87, 1.07]

Appendix Table 10. Experiment 1. *E. fuscus* (*n* = 10) take-off latency (ln(s)) study phase EMM contrasts. A negative EMM contrast signifies an increase in group take-off latency EMM from the first to the second phase, and vice versa.

Term	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
Non-reproductive individuals	0.61	0.34	120	1.76	0.08	[-0.08, 1.29]
Pregnant-at-start females	0.91	0.20	167	4.45	<.0001	[0.51, 1.31]

Appendix Table 11. Experiment 1. *E. fuscus* (*n* = 10) take-off latency (ln(s)) group EMM contrasts. A negative EMM contrast signifies the pregnant-at-start females group had greater take-off latency EMM, and vice versa.

Term	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
First phase	-0.42	0.34	35.5	-1.29	0.22	[-1.10, 0.26]
Second phase	-0.12	0.23	20.7	-0.52	0.61	[-0.60, 0.36]

Experiment 2: Methods to improve flight in captive bats

Appendix Table 12. Experiment 2. Mass (g) LMM results.

Including data from all bats (*n* = 24)						
Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Colony (intercept)	19.7	0.88	20.1	22.30	<.0001	[17.9, 21.6]
Trial day	0.004	0.02	20.0	0.21	0.83	[-0.03, 0.04]
No tread./unrest. diet	-0.49	1.25	20.1	-0.39	0.70	[-3.09, 2.12]
No tread./rest. diet	-4.29	1.25	20.1	-3.44	<.01	[-6.89, -1.69]
Tread./rest. diet	-4.63	1.25	20.1	-3.71	<.01	[-7.23, -2.03]
Trial day:No tread./unrest. diet	0.03	0.03	20.0	1.10	0.28	[-0.03, 0.08]
Trial day:No tread./rest. diet	-0.07	0.03	20.0	-2.59	<.05	[-0.12, -0.01]
Trial day:Tread./rest. diet	-0.04	0.03	20.0	-1.35	0.19	[-0.09, 0.02]
Intercept SD	2.16					[1.52, 2.70]
Intercept & slope correlation	0.36					[-0.03, 0.66]
Slope SD	0.05					[0.03, 0.06]
Residual SD	0.59					[0.56, 0.61]
Excluding data from mass outlier (see text; *n* = 23)						
Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Colony (intercept)	18.6	0.75	19.0	24.7	<.0001	[17.0, 20.1]
Trial day	-0.02	0.01	19.0	-1.86	0.08	[-0.05, 0.003]
No tread./unrest. diet	0.67	1.02	19.0	0.66	0.52	[-1.46, 2.80]
No tread./rest. diet	-3.14	1.02	19.0	-3.08	<.01	[-5.27, -1.01]
Tread./rest. diet	-3.48	1.02	19.0	-3.42	<.01	[-5.61, -1.35]
Trial day:No tread./unrest. diet	0.06	0.02	19.0	3.20	<.01	[0.02, 0.10]

Trial day:No tread./rest. diet	-0.04	0.02	19.0	-2.20	<.05	[-0.08, -0.002]
Trial day:Tread./rest. diet	-0.01	0.02	19.0	-0.38	0.71	[-0.05, 0.03]
Intercept SD	1.68					[1.17, 2.10]
Intercept & slope correlation	-0.31					[-0.63, 0.10]
Slope SD	0.03					[0.02, 0.04]
Residual SD	0.59					[0.57, 0.61]

Appendix Table 13. Experiment 2. Mass (g) first and final trial day EMM contrasts.

A negative EMM contrast signifies an increase in group mass EMM from first to final trial day, and vice versa.

Including data from all bats (*n* = 24)

Group	EMM	SE	df	t	*p*	95% CI
Colony	-0.23	1.09	20	-0.21	0.83	[-2.52, 2.05]
No tread./unrest. diet	-1.94	1.09	20	-1.77	0.09	[-4.22, 0.35]
No tread./rest. diet	3.78	1.09	20	3.46	<.01	[1.50, 6.07]
Tread./rest. diet	1.86	1.09	20	1.70	0.10	[-0.42, 4.14]

Excluding data from mass outlier (see text; *n* = 23)

Group	EMM	SE	df	t	*p*	95% CI
Colony	1.46	0.78	19	1.86	0.08	[-0.18, 3.09]
No tread./unrest. diet	-1.94	0.71	19	-2.71	<.05	[-3.43, -0.44]
No tread./rest. diet	3.78	0.71	19	5.30	<.0001	[2.29, 5.28]
Tread./rest. diet	1.86	0.71	19	2.61	<.05	[0.37, 3.36]

Appendix Table 14. Experiment 2. Mass (g) group EMM contrasts. A negative EMM contrast signifies the subtracted group had a greater mass EMM, and vice versa.

Including mass outlier (*n* = 24)						
First trial day						
Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	1.30	1.21	20	1.08	0.71	[-2.09, 4.69]
No tread./unrest. diet - Tread./rest. diet	2.25	1.21	20	1.86	0.28	[-1.14, 5.64]
No tread./unrest diet – No tread./rest diet	0.95	1.21	20	0.78	0.86	[-2.45, 4.33]
Colony - Tread./rest. diet	3.59	1.21	20	2.96	<.05	[0.20, 6.97]
Colony - No tread./rest. diet	2.28	1.21	20	1.88	0.26	[-1.11, 5.67]
Colony - No tread./unrest. diet	1.34	1.21	20	1.10	0.69	[-2.05, 4.73]
Final trial day						
Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	-0.62	1.69	20	-0.37	0.98	[-5.34, 4.10]
No tread./unrest. diet - Tread./rest. diet	6.05	1.69	20	3.59	<.01	[1.33, 10.77]
No tread./unrest diet – No tread./rest diet	6.67	1.69	20	3.95	<.01	[1.94, 11.39]
Colony - Tread./rest. diet	5.68	1.69	20	3.37	<.05	[0.96, 10.40]
Colony - No tread./rest. diet	6.30	1.69	20	3.73	<.01	[1.58, 11.02]
Colony - No tread./unrest. diet	-0.37	1.69	20	-0.22	1.00	[-5.09, 4.35]
Excluding data from mass outlier (see text; *n* = 23)						
First trial day						
Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	1.30	1.22	19	1.07	0.71	[-2.14, 4.74]
No tread./unrest. diet - Tread./rest. diet	2.25	1.22	19	1.84	0.29	[-1.19, 5.69]

No tread./unrest diet – No tread./rest diet	0.95	1.22	19	0.77	0.87	[-2.49, 4.38]
Colony - Tread./rest. diet	3.28	1.28	19	2.55	0.08	[-0.33, 6.88]
Colony - No tread./rest. diet	1.97	1.28	19	1.54	0.44	[-1.63, 5.58]
Colony - No tread./unrest. diet	1.03	1.28	19	0.80	0.85	[-2.58, 4.64]

Final trial day

Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	-0.62	0.95	19	-0.65	0.91	[-3.29, 2.05]
No tread./unrest. diet - Tread./rest. diet	6.05	0.95	19	6.38	<.0001	[3.38, 8.71]
No tread./unrest diet – No tread./rest diet	6.67	0.95	19	7.03	<.0001	[4.00, 9.33]
Colony - Tread./rest. diet	3.68	0.99	19	3.70	<0.01	[0.89, 6.48]
Colony - No tread./rest. diet	4.30	0.99	19	4.32	<0.01	[1.50, 7.10]
Colony - No tread./unrest. diet	-2.37	0.99	19	-2.38	0.12	[-5.16, 0.43]

Appendix Table 15. Experiment 2. Flight duration (ln(s)) LMM results.

Including data from all bats (*n* = 24)

Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Colony (intercept)	5.12	0.43	20.6	11.90	<.0001	[4.22, 6.02]
Trial day	0.16	0.03	19.6	5.55	<.0001	[0.10, 0.22]
Mass	-0.30	0.06	58.9	-4.90	<.0001	[-0.42, -0.17]
Days in captivity	-0.001	0.0003	18.3	-1.96	0.07	[-0.001, 0.0001]
No tread./unrest. diet	-0.42	0.61	20.6	-0.69	0.50	[-1.69, 0.86]
No tread./rest. diet	-0.80	0.59	20.0	-1.36	0.19	[-2.02, 0.43]
Tread./rest. diet	-1.29	0.63	21.4	-2.06	0.05	[-2.59, 0.01]
Trial day:No tread./unrest. diet	-0.16	0.04	21.7	-3.94	<.001	[-0.25, -0.08]
Trial day:No tread./rest. diet	-0.01	0.04	23.8	-0.14	0.89	[-0.09, 0.08]
Trial day:Tread./rest. diet	-0.11	0.04	21.5	-2.67	<.05	[-0.20, -0.02]
Mass:No tread./unrest. diet	0.06	0.10	153	0.57	0.57	[-0.14, 0.26]
Mass:No tread./rest. diet	0.22	0.08	148	2.70	<.01	[0.06, 0.37]
Mass: Tread./rest. diet	-0.06	0.10	243	-0.56	0.58	[-0.26, 0.15]
Intercept SD	0.97					[0.64, 1.17]
Intercept & slope correlation	-0.22					[-0.61, 0.22]
Slope SD	0.06					[0.04, 0.08]
Residual SD	0.44					[0.40, 0.47]

Excluding data from mass outlier (see text; *n* = 23)

Term	β	SE	df	t	*p*	95% CI
Colony (intercept)	5.24	0.47	19.1	11.20	<.0001	[4.26, 6.22]
Trial day	0.17	0.03	19.9	5.43	<.0001	[0.11, 0.24]
Mass	-0.20	0.09	309	-2.27	<.05	[-0.38, -0.03]
Days in captivity	-0.001	0.0004	17.6	-1.89	0.08	[-0.001, 0.0001]
No tread./unrest. diet	-0.46	0.65	20.1	-0.70	0.49	[-1.81, 0.90]
No tread./rest. diet	-0.88	0.62	18.6	-1.43	0.17	[-2.18, 0.41]
Tread./rest. diet	-1.29	0.65	19.5	-1.99	0.06	[-2.65, 0.07]
Trial day:No tread./unrest. diet	-0.18	0.04	21.1	-4.06	<.001	[-0.27, -0.09]
Trial day:No tread./rest. diet	-0.02	0.04	23.1	-0.44	0.67	[-0.11, 0.07]
Trial day:Tread./rest. diet	-0.12	0.04	21.0	-2.86	<.01	[-0.22, -0.03]
Mass:No tread./unrest. diet	-0.03	0.12	270	-0.28	0.78	[-0.28, 0.21]
Mass:No tread./rest. diet	0.13	0.11	325	1.21	0.23	[-0.08, 0.33]
Mass:Tread./rest. diet	-0.15	0.12	326	-1.20	0.23	[-0.39, 0.09]
Intercept SD	0.98					[0.65, 1.19]
Intercept & slope correlation	-0.30					[-0.67, 0.16]
Slope SD	0.06					[0.04, 0.08]
Residual SD	0.45					[0.41, 0.48]

Excluding data from visualized outliers (see text; *n* = 22)

Term	β	SE	df	t	*p*	95% CI
Colony (intercept)	4.80	0.46	20.4	10.4	<.0001	[3.84, 5.76]
Trial day	0.18	0.03	17.4	6.05	<.0001	[0.11, 0.24]
Mass	-0.28	0.06	55.1	-4.25	<.0001	[-0.41, -0.15]

Days in captivity	-0.001	0.0004	17.0	-1.75	0.10	[-0.001, 0.0001]
No tread./unrest. diet	-0.47	0.67	21.3	-0.70	0.49	[-1.85, 0.92]
No tread./rest. diet	-0.51	0.59	19.4	-0.85	0.40	[-1.74, 0.73]
Tread./rest. diet	-1.00	0.63	20.6	-1.59	0.13	[-2.31, 0.31]
Trial day:No tread./unrest. diet	-0.16	0.04	20.8	-3.84	<.001	[-0.25, -0.07]
Trial day:No tread./rest. diet	-0.02	0.04	21.2	-0.58	0.57	[-0.11, 0.06]
Trial day:Tread./rest. diet	-0.13	0.04	19.1	-3.11	<.01	[-0.21, -0.04]
Mass:No tread./unrest. diet	0.07	0.11	206	0.65	0.51	[-0.15, 0.30]
Mass:No tread./rest. diet	0.19	0.08	126	2.30	<.05	[0.03, 0.36]
Mass:Tread./rest. diet	-0.05	0.11	199	-0.51	0.61	[-0.26, 0.15]
Intercept SD	0.91					[0.59, 1.10]
Intercept & slope correlation	0.04					[-0.43, 0.50]
Slope SD	0.06					[0.03, 0.07]
Residual SD	0.44					[0.41, 0.48]

Appendix Table 16. Experiment 2. Flight duration (ln(s)) first and final trial day EMM contrasts. A negative EMM contrast signifies an increase in flight duration EMM from first to final trial day, and vice versa.

Including data from all bats (*n* = 24)

Group	EMM	SE	df	t	*p*	95% CI
Colony	-2.21	0.40	19.5	-5.55	<.0001	[-3.05, -1.38]
No tread./unrest. diet	0.07	0.42	23.8	0.16	0.88	[-0.80, 0.94]
No tread./rest. diet	-2.13	0.44	28.1	-4.85	<.0001	[-3.03, -1.23]
Tread./rest. diet	-0.67	0.42	23.3	-1.60	0.12	[-1.54, 0.20]

Excluding data from mass outlier (see text; *n* = 23)

Group	EMM	SE	df	t	*p*	95% CI
Colony	-2.41	0.44	20.0	-5.43	<.0001	[-3.34, -1.48]
No tread./unrest. diet	0.07	0.42	23.1	0.16	0.87	[-0.81, 0.94]
No tread./rest. diet	-2.14	0.44	7.5	-4.85	<.0001	[-3.04, -1.23]
Tread./rest. diet	-0.67	0.42	22.7	-1.59	0.13	[-1.54, 0.20]

Excluding data from visualized outliers (see text; *n* = 22)

Group	EMM	SE	df	t	*p*	95% CI
Colony	-2.45	0.41	17.4	-6.05	<.0001	[-3.30, -1.59]
No tread./unrest. diet	-0.15	0.44	24.7	-0.35	0.73	[-1.07, 0.76]
No tread./rest. diet	-2.11	0.41	26.3	-5.13	<.0001	[-2.96, -1.27]
Tread./rest. diet	-0.70	0.39	21.2	-1.80	0.09	[-1.51, 0.12]

Appendix Table 17. Experiment 2. Flight duration (ln(s)) group EMM contrasts. A negative EMM contrast signifies the subtracted group had a greater flight duration EMM, and vice versa.

Including data from all bats (*n* = 24)

First trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	-0.24	0.69	19.4	-0.34	0.99	[-2.18, 1.71]
No tread./unrest. diet - Tread./rest. diet	1.24	0.69	19.4	1.81	0.30	[-0.69, 3.17]
No tread./unrest diet – No tread./rest diet	1.48	0.68	18.9	2.16	0.17	[-0.45, 3.40]
Colony - Tread./rest. diet	0.52	0.72	20.8	0.72	0.89	[-1.49, 2.53]
Colony - No tread./rest. diet	0.76	0.69	19.7	1.10	0.70	[-1.18, 2.70]
Colony - No tread./unrest. diet	-0.72	0.71	20.4	-1.01	0.74	[-2.71, 1.27]

Final trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	1.23	0.65	27.0	1.87	0.26	[-0.57, 3.01]
No tread./unrest. diet - Tread./rest. diet	0.50	0.65	27.0	0.78	0.87	[-1.28, 2.29]
No tread./unrest diet – No tread./rest diet	-0.72	0.64	24.5	-1.13	0.68	[-2.48, 1.04]
Colony - Tread./rest. diet	2.06	0.67	23.9	3.10	<.05	[0.23, 3.90]
Colony - No tread./rest. diet	0.84	0.63	22.3	1.34	0.55	[-0.90, 2.58]
Colony - No tread./unrest. diet	1.56	0.65	22.1	2.40	0.11	[-0.25, 3.37]

Excluding data from mass outlier (see text; *n* = 23)

First trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	-0.33	0.72	18.2	-0.46	0.97	[-2.35, 1.69]
No tread./unrest. diet - Tread./rest. diet	1.20	0.71	18.3	1.69	0.36	[-0.80, 3.21]
No tread./unrest diet – No tread./rest diet	1.53	0.71	18.2	2.14	0.18	[-0.48, 3.54]
Colony - Tread./rest. diet	0.42	0.78	20.0	0.54	0.95	[-1.77, 2.61]
Colony - No tread./rest. diet	0.75	0.76	19.3	0.98	0.76	[-1.39, 2.88]
Colony - No tread./unrest. diet	-0.78	0.78	20.1	-1.01	0.75	[-2.96, 1.40]

Final trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	1.14	0.63	24.7	1.82	0.29	[-0.59, 2.88]
No tread./unrest. diet - Tread./rest. diet	0.47	0.64	26.4	0.73	0.88	[-1.29, 2.22]
No tread./unrest diet – No tread./rest diet	-0.68	0.63	24.5	-1.07	0.71	[-2.42, 1.07]
Colony - Tread./rest. diet	2.16	0.66	21.2	3.29	<.05	[0.33, 3.99]
Colony - No tread./rest. diet	1.02	0.62	20.3	1.64	0.38	[-0.72, 2.75]
Colony - No tread./unrest. diet	1.70	0.66	21.4	2.57	0.08	[-0.14, 3.53]

Excluding data from visualized outliers (see text; *n* = 22)

First trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	-0.21	0.60	17.3	-0.35	0.98	[-1.92, 1.50]
No tread./unrest. diet - Tread./rest. diet	0.81	0.63	17.9	1.28	0.58	[-0.97, 2.58]
No tread./unrest diet – No tread./rest diet	1.02	0.63	17.3	1.61	0.40	[-0.78, 2.81]
Colony - Tread./rest. diet	0.13	0.67	19.1	0.19	1.00	[-1.75, 2.01]
Colony - No tread./rest. diet	0.34	0.64	18.4	0.53	0.95	[-1.46, 2.13]

Colony - No tread./unrest. diet	-0.68	0.70	19.2	-0.97	0.77	[-2.64, 1.29]

Final trial day

Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	1.20	0.68	24.5	1.77	0.31	[-0.66, 3.06]
No tread./unrest. diet - Tread./rest. diet	0.26	0.73	27.4	0.35	0.98	[-1.73, 2.24]
No tread./unrest diet – No tread./rest diet	-0.94	0.72	25.6	-1.30	0.57	[-2.93, 1.04]
Colony - Tread./rest. diet	1.87	0.72	22.2	2.59	0.07	[-0.14, 3.88]
Colony - No tread./rest. diet	0.67	0.69	20.6	0.98	0.76	[-1.25, 2.59]
Colony - No tread./unrest. diet	1.62	0.78	23.9	2.08	0.19	[-0.52, 3.75]

Appendix Table 18. Experiment 2. Take-off latency (ln(s)) LMM results.

Including data from all bats (*n* = 24)

Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Colony (intercept)	2.06	0.54	21.6	3.83	<.001	[0.95, 3.18]
Trial day	-0.01	0.03	19.5	-0.21	0.84	[-0.07, 0.06]
Mass	0.15	0.06	128	2.55	<.05	[0.03, 0.27]
No tread./unrest. diet	-0.82	0.74	19.9	-1.11	0.28	[-2.37, 0.72]
No tread./rest. diet	0.65	0.77	22.5	0.85	0.41	[-0.95, 2.25]
Tread./rest. diet	0.36	0.78	23.2	0.46	0.65	[-1.25, 1.97]
Trial day: No tread./unrest. diet	-0.002	0.04	19.7	-0.04	0.97	[-0.10, 0.10]
Trial day: No tread./rest. diet	0.05	0.05	23.4	1.06	0.30	[-0.05, 0.15]
Trial day: Tread./rest. diet	0.23	0.05	20.4	5.00	<.0001	[0.13, 0.32]
Intercept SD	1.26					[0.86, 1.57]
Intercept & slope correlation	0.52					[-0.48, 1.00]
Slope SD	0.05					[0.0003, 0.08]
Residual SD	0.99					[0.92, 1.08]

Excluding data from mass outlier (see text; *n* = 23)

Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Colony (intercept)	2.07	0.60	19.3	3.49	<.01	[0.83, 3.32]
Trial day	-0.01	0.04	19.3	-0.38	0.71	[-0.09, 0.06]
Mass	0.15	0.07	166	2.06	<.05	[0.01, 0.28]
No tread./unrest. diet	-0.87	0.80	18.8	-1.10	0.29	[-2.55, 0.80]
No tread./rest. diet	0.58	0.81	20.1	0.71	0.49	[-1.12, 2.27]
Tread./rest. diet	0.28	0.82	20.7	0.34	0.74	[-1.43, 1.99]

Trial day: No tread./unrest. diet	0.01	0.05	21.1	0.12	0.90	[-0.10, 0.11]
Trial day: No tread./rest. diet	0.06	0.05	21.0	1.13	0.27	[-0.05, 0.16]
Trial day: Tread./rest. diet	0.23	0.05	18.8	4.88	<.001	[0.13, 0.33]
Intercept SD	1.29					[0.87, 1.62]
Intercept & slope correlation	0.53					[-0.50, 1.00]
Slope SD	0.05					[0.0003, 0.08]
Residual SD	1.01					[0.94, 1.10]

Excluding data from visualized outlier (see text; *n* = 23)

Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Colony (intercept)	2.07	0.47	21.3	4.40	<.001	[1.09, 3.04]
Trial day	-0.01	0.03	18.5	-0.19	0.85	[-0.07, 0.06]
Mass	0.14	0.06	110	2.39	<.05	[0.02, 0.26]
No tread./unrest. diet	-1.41	0.67	19.1	-2.10	<.05	[-2.81, -0.01]
No tread./rest. diet	0.61	0.67	22.0	0.91	0.37	[-0.78, 2.01]
Tread./rest. diet	0.32	0.68	22.9	0.46	0.65	[-1.09, 1.72]
Trial day: No tread./unrest. diet	-0.02	0.05	18.6	-0.36	0.73	[-0.12, 0.08]
Trial day: No tread./rest. diet	0.05	0.05	22.2	1.00	0.33	[-0.05, 0.15]
Trial day: Tread./rest. diet	0.23	0.05	19.4	4.97	<.0001	[0.13, 0.32]
Intercept SD	1.07					[0.72, 1.34]
Intercept & slope correlation	0.39					[-1.00, 1.00]
Slope SD	0.05					[0.00, 0.07]
Residual SD	1.00					[0.93, 1.09]

Appendix Table 19. Experiment 2. Take-off latency (ln(s)) first and final trial day EMM contrasts. A negative EMM contrast signifies an increase in group take-off latency EMM from first to final trial day, and vice versa.

Including data from all bats (*n* = 24)

Group	EMM	SE	df	t	*p*	95% CI
Colony	0.09	0.44	19.6	0.21	0.84	[-0.83, 1.02]
No tread./unrest. diet	0.12	0.45	20.8	0.26	0.80	[-0.83, 1.06]
No tread./rest. diet	-0.62	0.50	25.6	-1.25	0.22	[-1.64 0.40]
Tread./rest. diet	-3.09	0.45	20.7	-6.83	<.0001	[-4.03, -2.15]

Excluding data from mass outlier (see text; *n* = 23)

Group	EMM	SE	df	t	*p*	95% CI
Colony	0.19	0.50	19.3	0.38	0.71	[-0.85, 1.23]
No tread./unrest. diet	0.10	0.47	20.9	0.22	0.83	[-0.87, 1.07]
No tread./rest. diet	-0.59	0.52	29.0	-1.14	0.27	[-1.65, 0.47]
Tread./rest. diet	-3.08	0.47	20.7	-6.62	<.0001	[-4.04, -2.11]

Excluding data from visualized outlier (see text; *n* = 23)

Group	EMM	SE	df	t	*p*	95% CI
Colony	0.09	0.44	18.6	0.19	0.85	[-0.84, 1.01]
No tread./unrest. diet	0.32	0.49	19.3	0.65	0.52	[-0.71, 1.35]
No tread./rest. diet	-0.58	0.50	24.3	-1.18	0.25	[-1.60, 0.44]
Tread./rest. diet	-3.07	0.45	19.7	-6.80	<.0001	[-4.02, -2.13]

Appendix Table 20. Experiment 2. Take-off latency (ln(s)) group EMM contrasts. A negative EMM contrast signifies the subtracted group had a greater take-off latency EMM, and vice versa.

Including data from all bats (*n* = 24)

First trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	1.53	0.71	20.1	2.16	0.17	[-0.46, 3.51]
No tread./unrest. diet - Tread./rest. diet	0.42	0.71	20.4	0.59	0.93	[-1.57, 2.41]
No tread./unrest diet – No tread./rest diet	-1.11	0.70	19.7	-1.57	0.42	[-3.08, 0.87]
Colony - Tread./rest. diet	1.23	0.73	21.8	1.69	0.35	[-0.80, 3.26]
Colony - No tread./rest. diet	-0.30	0.71	20.2	-0.42	0.97	[-2.28, 1.69]
Colony - No tread./unrest. diet	0.81	0.71	20.0	1.15	0.67	[-1.17, 2.79]

Final trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	-0.94	0.89	19.6	-1.06	0.72	[-3.45, 1.56]
No tread./unrest. diet - Tread./rest. diet	-2.79	0.95	23.0	-2.94	<.05	[-5.42, -0.16]
No tread./unrest diet – No tread./rest diet	-1.85	0.96	23.7	-1.92	0.25	[-4.50, 0.81]
Colony - Tread./rest. diet	-1.95	0.95	23.1	-2.05	0.20	[-4.58, 0.68]
Colony - No tread./rest. diet	-1.01	0.96	23.9	-1.05	0.72	[-3.67, 1.65]
Colony - No tread./unrest. diet	0.84	0.89	19.6	0.94	0.79	[-1.67, 3.34]

Excluding data from mass outlier (see text; *n* = 23)

First trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	1.54	0.73	19.1	2.12	0.18	[-0.51, 3.59]
No tread./unrest. diet - Tread./rest. diet	0.44	0.73	19.5	0.60	0.93	[-1.62, 2.49]
No tread./unrest diet – No tread./rest diet	-1.10	0.72	18.6	-1.53	0.44	[-3.13, 0.93]
Colony - Tread./rest. diet	1.35	0.78	20.2	1.74	0.33	[-0.82, 3.53]
Colony - No tread./rest. diet	-0.19	0.76	18.9	-0.25	0.99	[-2.33, 1.95]
Colony - No tread./unrest. diet	0.92	0.76	18.7	1.21	0.63	[-1.21, 3.05]

Final trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	-0.95	0.92	18.9	-1.03	0.73	[-3.53, 1.63]
No tread./unrest. diet - Tread./rest. diet	-2.74	0.99	24.1	-2.78	<.05	[-5.47, -0.02]
No tread./unrest diet – No tread./rest diet	-1.80	1.00	25.3	-1.79	0.30	[-4.55, 0.96]
Colony - Tread./rest. diet	-1.91	0.99	20.7	-1.94	0.24	[-4.67, 0.84]
Colony - No tread./rest. diet	-0.97	1.00	21.3	-0.97	0.77	[-3.74, 1.81]
Colony - No tread./unrest. diet	0.83	0.97	19.5	0.86	0.83	[-1.89, 3.55]

Excluding data from visualized outlier (see text; *n* = 23)

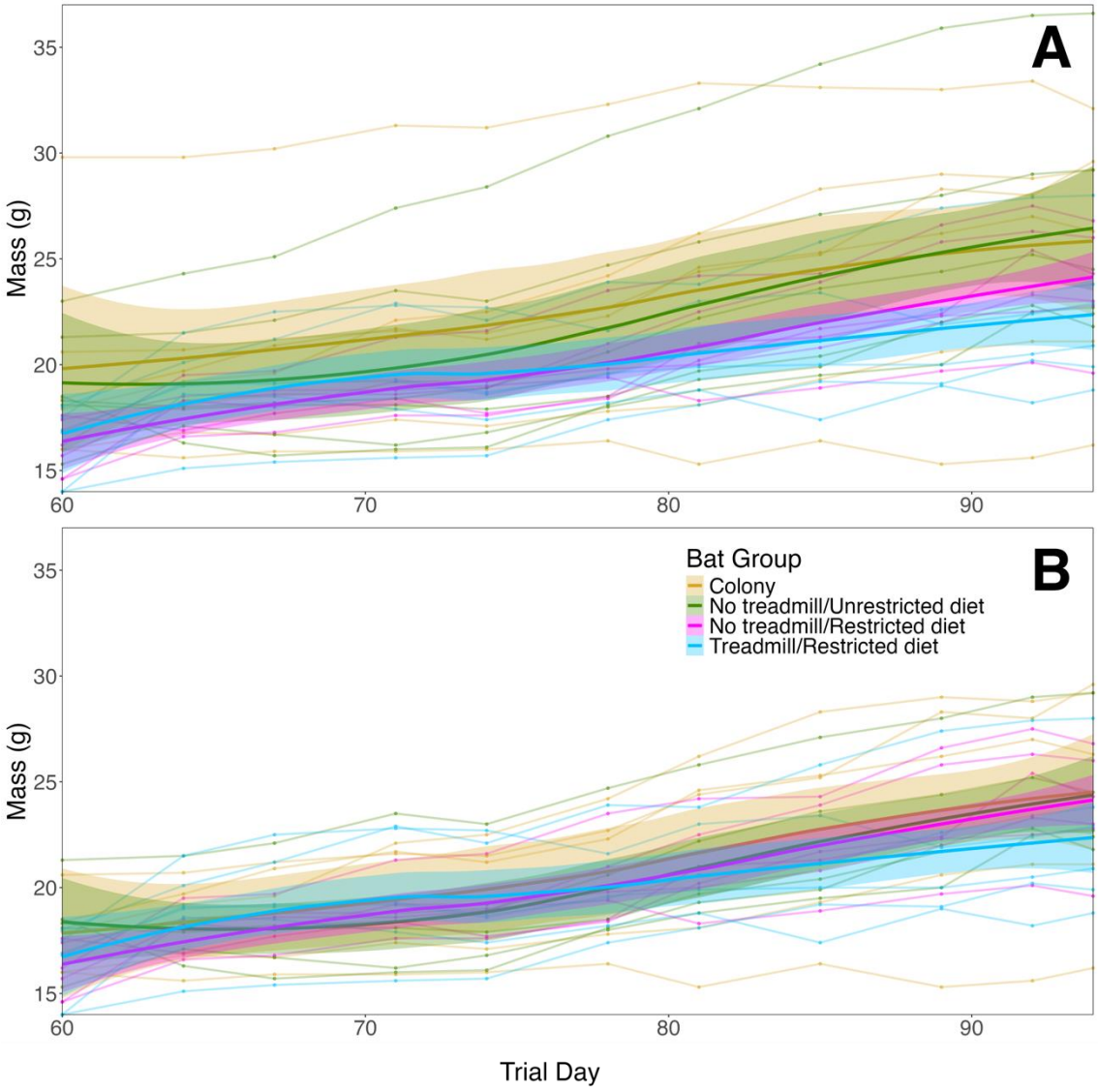
First trial day

Group	EMM	SE	df	t	*p*	95% CI
No tread./rest. diet - Tread./rest. diet	1.54	0.65	19.1	2.39	0.11	[-0.27, 3.36]
No tread./unrest. diet - Tread./rest. diet	-0.03	0.68	19.2	-0.04	1.00	[-1.93, 1.88]
No tread./unrest diet – No tread./rest diet	-1.57	0.67	18.6	-2.34	0.12	[-3.46, 0.32]
Colony - Tread./rest. diet	1.26	0.67	20.9	1.90	0.26	[-0.60, 3.12]
Colony - No tread./rest. diet	-0.28	0.65	19.2	-0.43	0.97	[-2.10, 1.54]

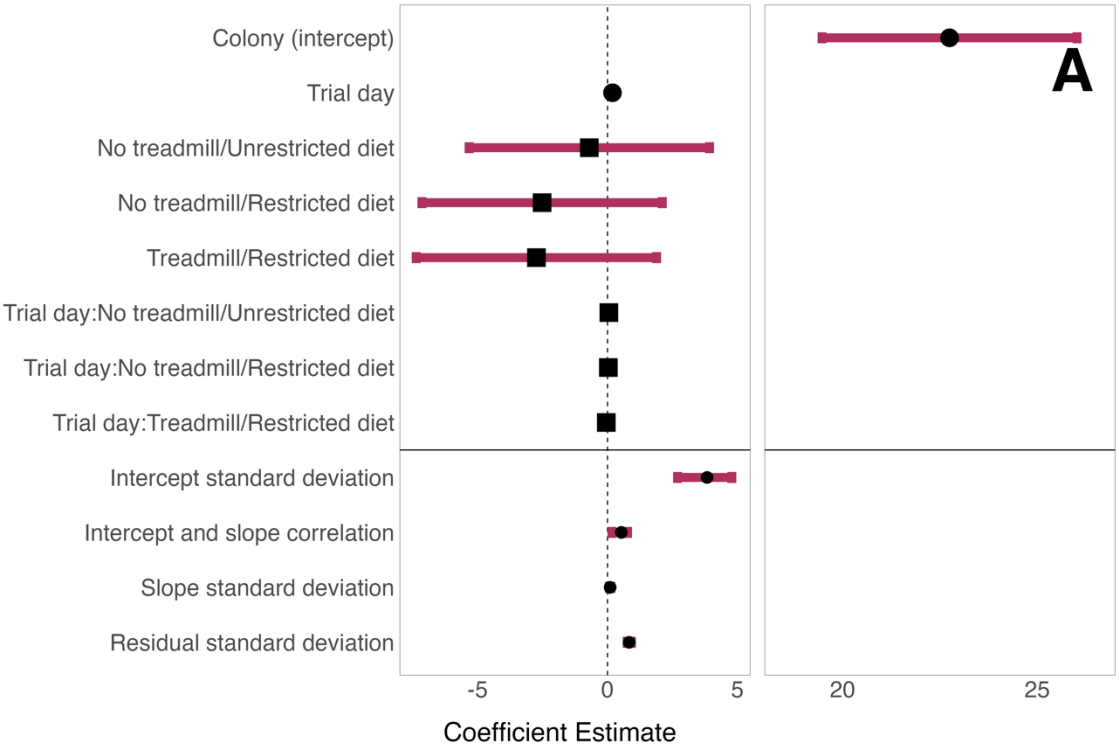
Colony - No tread./unrest. diet	1.29	0.68	19.1	1.91	0.26	[-0.61, 3.19]

Final trial day

Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	-0.95	0.77	18.5	-1.23	0.62	[-3.13, 1.23]
No tread./unrest. diet - Tread./rest. diet	-3.42	0.86	21.4	-3.970	<.01	[-5.82, -1.02]
No tread./unrest diet – No tread./rest diet	-2.47	0.87	22.1	-2.83	<.05	[-4.89, -0.05]
Colony - Tread./rest. diet	-1.90	0.84	22.4	-2.26	0.14	[-4.22, 0.43]
Colony - No tread./rest. diet	-0.95	0.85	23.3	-1.11	0.69	[-3.30, 1.41]
Colony - No tread./unrest. diet	1.52	0.81	18.5	1.88	0.27	[-0.76, 3.81]



Appendix Fig. 1. Post-Experiment 2. Change in mass (g) for *E. fuscus* after returning all bats to colony including and excluding outliers. (**A**) Data from all bats (*n* = 24). (**B**) Same data but with values of visualized outliers (Violet 111 and Grey 82) excluded (see text; *n* = 22). Points and connecting lines represent individual bats; thicker lines represent group averages fitted with local regression smoothing curves and shaded 95% CIs.

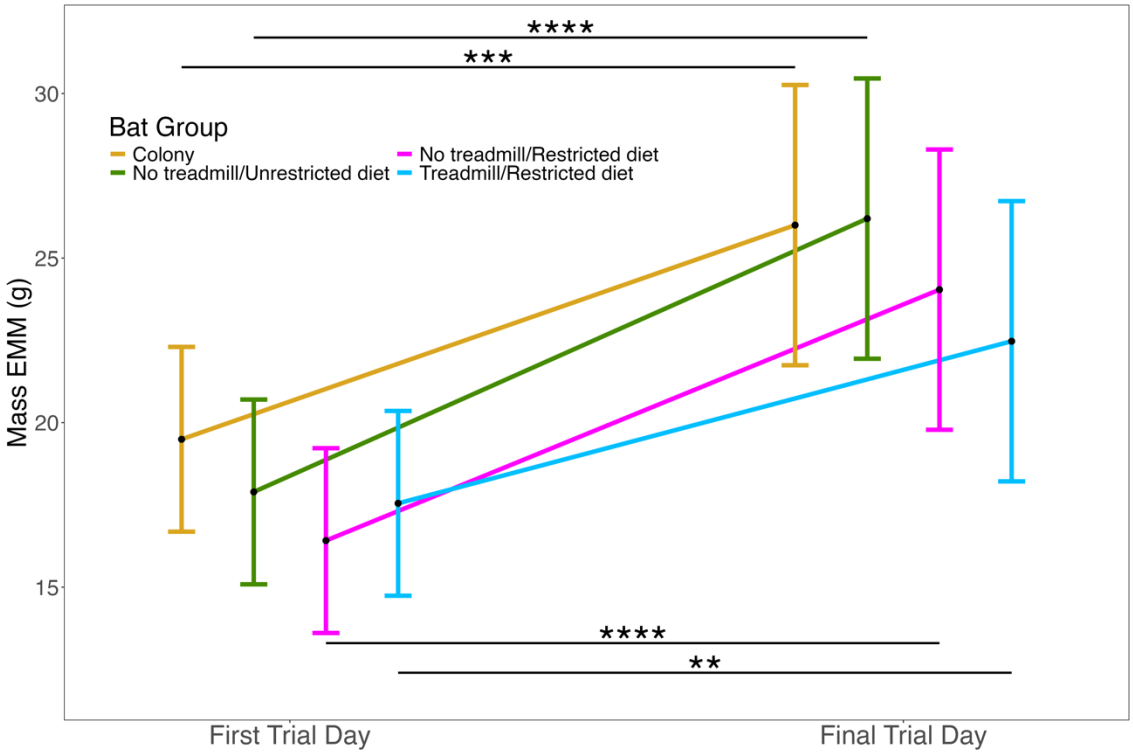


Appendix Fig. 2. Post-Experiment 2. *E. fuscus* (*n* = 24) mass (g) LMM results. Points represent β; maroon lines represent 95% CIs. Fixed effects (above horizontal line) include: colony group as intercept, centered trial day, three cage group deviations, and centered trial day and three cage group interactions; random effects (below horizontal line) include: intercept SD, intercept and slope correlation, slope SD, and residual SD.

Appendix Table 21. Post- Experiment 2. Mass (g) LMM results.

Including data from all bats (*n* = 24)						
Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Colony (intercept)	22.7	1.57	20	14.5	<.0001	[19.5, 26.0]
Trial day	0.19	0.04	20	4.49	<.001	[0.10, 0.28]
No tread./unrest. diet	-0.70	2.22	20	-0.76	0.76	[-5.33, 3.93]
No tread./rest. diet	-2.52	2.22	20	-1.14	0.27	[-7.15, 2.11]
Tread./rest. diet	-2.74	2.22	20	-1.23	0.23	[-7.37, 1.89]
Trial day:No tread./unrest. diet	0.05	0.06	20	0.88	0.39	[-0.07, 0.18]
Trial day:No tread./rest. diet	-0.03	0.06	20	0.55	0.59	[-0.09, 0.16]
Trial day:Tread./rest. diet	-0.05	0.06	20	-0.77	0.45	[-0.17, 0.08]
Intercept SD	3.84					[2.70, 4.79]
Intercept & slope correlation	0.53					[0.17, 0.77]
Slope SD	0.10					[0.07, 0.13]
Residual SD	0.83					[0.76, 0.92]
Excluding data from two visualized outliers (see text; *n* = 22)						
Term	**β**	**SE**	**df**	**t**	***p***	**95% CI**
Colony (intercept)	21.0	1.16	18	18.0	<.0001	[18.5, 23.4]
Trial day	0.21	0.04	18	4.86	<.001	[0.12, 0.30]
No tread./unrest. diet	-0.52	1.65	18	-0.31	0.76	[-3.98, 2.94]
No tread./rest. diet	-0.73	1.58	18	-0.46	0.65	[-4.04, 2.58]
Tread./rest. diet	-0.95	1.58	18	-0.60	0.56	[-4.26, 2.37]
Trial day:No tread./unrest. diet	-0.003	0.06	18	-0.04	0.97	[-0.13, 0.13]
Trial day:No tread./rest. diet	0.02	0.06	18	0.27	0.79	[-0.11, 0.14]

Trial day:Tread./rest. diet	-0.06	0.06	18	-1.10	0.29	[-0.19, 0.06]
Intercept SD	2.59					[1.78, 3.26]
Intercept & slope correlation	0.75					[0.47, 0.90]
Slope SD	0.09					[0.06, 0.12]
Residual SD	0.85					[0.77, 0.94]



Appendix Fig. 3. Post-Experiment 2. *E. fuscus* (*n* = 24) mass (g) group EMMs during first and final trial days. Significant contrasts are labeled; horizontal labels represent within-group contrasts from first to final trial day.

Appendix Table 22. Post-Experiment 2. Mass (g) first and final trial day EMM contrasts. A negative EMM contrast signifies an increase in group mass EMM from first to final trial day, and vice versa.

Including data from all bats (*n* = 24)

Group	EMM	SE	df	t	*p*	95% CI
Colony	-6.51	1.45	20	-4.49	<.001	[-9.53, -3.48]
No tread./unrest. diet	-8.31	1.45	20	-5.73	<.0001	[-11.33, -5.28]
No tread./rest. diet	-7.63	1.45	20	-5.26	<.0001	[-10.65, -4.60]
Tread./rest. diet	-4.93	1.45	20	-3.40	<.01	[-7.95, -1.90]

Excluding data from two visualized outliers (see text; *n* = 22)

Group	EMM	SE	df	t	*p*	95% CI
Colony	-7.09	1.46	18	-4.86	<.001	[-10.15, -4.03]
No tread./unrest. diet	-7.00	1.46	18	-4.80	<.001	[-10.06, -3.94]
No tread./rest. diet	-7.63	1.33	18	-5.73	<.0001	[-10.42, -4.83]
Tread./rest. diet	-4.93	1.33	18	-3.70	<.01	[-7.72, -2.13]

Appendix Table 23. Post-Experiment 2. Mass (g) group EMM contrasts. A negative EMM contrast signifies the subtracted group had a greater mass EMM, and vice versa.

Including data from all bats (*n* = 24)						
First trial day						
Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	-1.13	1.90	20	-0.60	0.93	[-6.46, 4.19]
No tread./unrest. diet - Tread./rest. diet	0.35	1.90	20	0.18	1.00	[-4.98, 5.67]
No tread./unrest diet – No tread./rest diet	1.48	1.90	20	0.78	0.86	[-3.85, 6.81]
Colony - Tread./rest. diet	1.95	1.90	20	1.02	0.74	[-3.38, 7.27]
Colony - No tread./rest. diet	3.08	1.90	20	1.62	0.39	[-2.25, 8.40]
Colony - No tread./unrest. diet	1.60	1.90	20	0.84	0.83	[-3.73, 6.93]
Final trial day						
Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	1.57	2.89	20	0.54	0.95	[-6.51, 9.65]
No tread./unrest. diet - Tread./rest. diet	3.73	2.89	20	1.29	0.58	[-4.35, 11.81]
No tread./unrest diet – No tread./rest diet	2.16	2.89	20	0.75	0.88	[-5.92, 10.24]
Colony - Tread./rest. diet	3.53	2.89	20	1.22	0.62	[-4.55, 11.61]
Colony - No tread./rest. diet	1.96	2.89	20	0.68	0.90	[-6.12, 10.04]
Colony - No tread./unrest. diet	-0.20	2.89	20	-0.07	1.00	[-8.28, 7.88]
Excluding data from two visualized outliers (see text; *n* = 22)						
First trial day						
Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	-1.13	1.06	18	-1.07	0.71	[-4.12, 1.85]
No tread./unrest. diet - Tread./rest. diet	-0.61	1.11	18	-0.55	0.95	[-3.74, 2.52]

No tread./unrest diet – No tread./rest diet	0.53	1.11	18	0.48	0.96	[-2.60, 3.65]
Colony - Tread./rest. diet	-0.13	1.11	18	-0.12	1.00	[-3.26, 2.99]
Colony - No tread./rest. diet	1.00	1.11	18	0.90	0.80	[-2.13, 4.13]
Colony - No tread./unrest. diet	0.47	1.16	18	0.41	0.98	[-2.80, 3.74]

Final trial day

Group	**EMM**	**SE**	**df**	**t**	***p***	**95% CI**
No tread./rest. diet - Tread./rest. diet	1.57	2.28	18	0.69	0.90	[-4.86, 8.00]
No tread./unrest. diet - Tread./rest. diet	1.47	2.39	18	0.62	0.93	[-5.28, 8.22]
No tread./unrest diet – No tread./rest diet	-0.10	2.39	18	-0.04	1.00	[-6.85, 6.65]
Colony - Tread./rest. diet	2.03	2.39	18	0.85	0.83	[-4.72, 8.78]
Colony - No tread./rest. diet	0.46	2.39	18	0.19	1.00	[-6.28, 7.21]
Colony - No tread./unrest. diet	0.56	2.49	18	0.23	1.00	[-6.49, 7.61]