

DISPERSAL, WING MORPHOLOGY AND PHYSIOLOGY RELATIONSHIPS IN
EUPHYDRYAS GILLETII AND IMPLICATIONS FOR CONSERVATION

by

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Bachelor of Arts
Illinois Wesleyan University, 2018

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Earth and Environmental Resources Management

College of Arts and Sciences

University of South Carolina

2020

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DEDICATION

To my parents for being the most supportive and open-minded parents ever. Thank you for trusting me and letting me explore. To my grandparents for being my forever cheerleaders. To Sharon, who was the kindest and most positive soul I have ever met. Love to all.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my advisor, Carol Boggs for her indispensable support and guidance at every step of my master's degree. Thank you for the countless hours you spent editing my thesis, for training me to do good field research, for imparting valuable knowledge about the scientific process of research and for all the career and life advice. Her expertise, positivity and encouragement really helped me progress unhindered. I could not have asked for a better advisor.

I would like to thank Boggs's lab members who worked at the Rocky Mountain Biological Lab in the summer of 2019, during my research.

My research would not have been possible without the financial support from the University of South Carolina to Carol Boggs and from the Rocky Mountain Biological Lab Graduate Fellowship. Many thanks to both institutions.

I would also like to thank my committee members Dr. Daniel Speiser and Dr. Thomas Lekan for giving me your valuable time to offer positive feedback and new perspectives

ABSTRACT

Dispersal is important for recolonization, migration and maintenance of local metapopulations and populations, in case of disruption in an animal's habitat. Morphology (wing loading and wing aspect ratio) and physiology (metabolic rates) are features that have been shown to explain dispersal processes in insects. However, there are no studies indicating the direct relationship between morphology and metabolic rates in association with dispersal. I studied *Euphydryas gillettii* butterflies at Rocky Mountain Biological Laboratory, Colorado to assess the link between wing morphology and metabolic rates and their influence on dispersal within different sites at RMBL. Individuals that dispersed to other sites had greater wing wear (an age estimate) and greater wing loading. The total flight distance increased with increasing residual resting metabolic rate (RMR), that is, RMR adjusted for body mass. Residual flight metabolic rate (FMR) and peak metabolic rates (PMR) were related to wing loading. Residual FMR was greater for greater wing loading. Residual PMR was also greater for greater wing loading, but only in females. Overall, greater wing wear, greater wing loading and greater RMR are indicative of better dispersal in *E. gillettii*, but these three morphological and physiological characteristics are not linked to each other. These findings regarding dispersal characteristics of *E. gillettii* butterflies can help inform conservation strategies prior to any reintroduction and translocation efforts. More species-specific dispersal studies underpinning an organism's dispersal mechanisms can minimize difficulties due to unpredicted dispersal reportedly faced by managers during the translocation-reintroduction processes.

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CHAPTER 1

IMPLICATIONS OF DISPERSAL IN TRANSLOCATION AND REINTRODUCTION

Translocation and reintroduction are conservation management approaches that are meant to prevent extinction of species or populations. Reintroduction is defined as the intentional movement, by humans, of an organism to a new site within its historical range from which it has disappeared (IUCN, 2013). Translocation refers to any general "human-mediated movement of living organisms from one area, with release in another" that could be both outside or within the species' historical or "indigenous" range (IUCN, 2013). The definition of translocation varies in the literature and could be a point of confusion. It is sometimes referred to as specific movement of organisms where humans deliberately move animals from one area (capture site) to another area (release site) that is outside their historical range (Mengak, 2018; Schwartz & Martin, 2013). To maintain consistency, I will refer to translocation as the overarching term defined by IUCN. Both translocation and relocation efforts require thorough planning, design, implementation, assessment and monitoring. It is intuitive that everything related to the target species/organisms needs to be thought out in detail to ensure the success of the conservation effort in re-establishing population or preventing declining numbers. In the process, although the majority of the movement is done by humans, it is also important to factor in the organism's dispersal and movement because depending on the goal of the project, wildlife managers and conservation practitioners want to avoid certain types of movement. They either do not

want the animals to disperse long distance and return to the capture site or they want them to disperse only locally to explore resources and establish meta populations in the release site.

Since dispersal is a crucial factor in determining the translocation-reintroduction success, it is imperative to know how and when dispersal is considered in the translocation and reintroduction process. What type of dispersal-related studies are performed during the conservation projects? What needs improvement in terms of dispersal considerations in translocation and reintroductions?

The International Union for Conservation of Nature provides guidelines and international standards for translocation and reintroductions projects. IUCN had formed a task force within their Reintroduction and Invasive Species Specialist Groups and created the guidelines document called the "2013 International Union for the Conservation of Nature's Guidelines for Reintroductions and Other Conservation Translocations" (IUCN/SSC 2013). All conservation translocations follow the standard stages of project design, implementation and management as outlined in the Guidelines. Among the many project stages discussed, the Guidelines clearly denote the importance of assessing the biological feasibility of the proposed translocation operation after the planning and design phase. Biological feasibility assessment includes the process of acquiring basic biological and ecological knowledge about the candidate species including its biotic and abiotic habitat needs and interspecific relationships. Dispersal is certainly mentioned as one of the components of biological and ecological knowledge that the wildlife managers and conservation practitioners need to acquire for a translocation project. (This is in addition to knowledge about reproduction, development, habitat requirements, phenology,

population dynamics, local adaptations, interspecific relationships, etc). It is recommended they refer to the best information available about the candidate species' biology and ecology. It is likely that translocation and reintroduction practitioners acquire that information from either scientific studies published in journals or from case study reports available elsewhere (for example, Global Re-introduction Perspective Series by IUCN). IUCN also recommends consultations with relevant species experts including both professional and amateur naturalists.

Scientific studies have provided relevant information that aid translocation strategies. For example, a study of 34 mammalian species found that the maximum dispersal distance mammals move post-translocation is 40 times the linear dimension of their home range (Bowman et al., 2002). This formula became the recommended minimum translocation distance in order to prevent mammals from returning to their capture site. While such finding gives useful tips for planning translocations, we should bear in mind that such general dispersal information (based on 34 species) could be very different for a particular species, particular populations and particular habitats or matrices. Therefore, it is crucial to not only gather dispersal information that is already available about the target species of concern but to also perform very specific pre-translocation as well as-post translocation dispersal studies. If we study the specific individuals or population's dispersal behaviour pre-translocation then it can increase the possibility of translocation success because we can better predict dispersal tendencies post-translocation and design translocation procedures accordingly. Post-translocation dispersal studies can help confirm predictions made pre-translocation, and also serve as a good way of performing the 'outcome assessment and monitoring' phase as outlined in the IUCN guidelines.

Unfortunately, dispersal studies related to translocation and reintroduction are heavily biased towards post-translocation dispersal only. Dispersal is mostly considered in the post-release management phase, one of the last phases of implementation of a translocation project (Schwartz and Martin, 2013). Some examples are studies on thick-shelled river mussel (*Unio crassus*) (Zajac et al., 2019), hatchery-reared bloater fish (*Coregonus hoyi*), wild cougars (*Puma concolor*) (Ruth, 1998), wolves (*Canis lupus*) (Fritts et al., 1984), Chinese giant salamanders (*Andrias davidianus*) (Fritts et al., 1984), white-tailed deer (Jones et al., 1997). These studies do not mention what specific information about the species' dispersal characteristics and behaviour was used prior to translocating them to the release sites. They only studied dispersal after translocation to the new site, mostly to monitor the translocation success. The lack of attention to dispersal prior to translocating the animals could be a reason why wildlife managers report dispersal and movement as the most common types of difficulties in the translocations of animals (including invertebrates, fishes, amphibians, reptiles, birds and mammal translocations) (Berger-Tal et al., 2020). Such translocation difficulties need to be resolved.

The few pre-translocation dispersal studies are mostly dispersal modelling and simulations rather than direct experimental observations of the target organisms themselves. Recently, researchers estimated lynx dispersal if they were to be reintroduced to Scotland (Philips, 2020). They created a dispersal model based on movement information derived from observation of wild lynx. While such kind of dispersal modelling is increasingly useful and promising for translocation-reintroduction success, it is also important to have specific and concrete observations of the organism's or population's dispersal before actually moving the valuable organism elsewhere for conservation

purposes. People could use mark-release-recapture data to track dispersal and to identify the individuals that are more dispersive or less dispersive based on the conservation goals. They could observe movement behaviours in the field or conduct experiments by selecting few individuals from the candidate populations. Alternatively, one could also collect surrogate measures of dispersal, for example, the physiology (example, metabolic rates in butterflies) and morphology (features such as wings that cause movement) of the organisms that contribute to dispersal. If managers and scientists together can focus on studying dispersal (pre-translocation) of the specific individuals and populations they are targeting to translocate, it can provide a more robust foundation for successful translocations. Thus, there is a need to publish more pre-release/pre-translocation dispersal studies in scientific journals for a proper understanding of pre and post translocation/reintroduction differences and to minimize the difficulties faced by conservation practitioners and managers during the translocation process. I believe that this in turn can help achieve successful translocation and reintroduction outcomes to save species from extirpation and extinctions.

In an attempt to contribute to species-specific dispersal studies, I have conducted research on the dispersal of *Euphydryas gillettii* (Gillette's checkerspot butterflies) in Gothic, Colorado. I report specific details in the following chapter. I did not only look at dispersal and movement but also at flight morphology and physiology to determine any associations with dispersal. Hopefully this kind of study will be meaningful in identifying and selecting individuals to be moved in case this species needs to be translocated and conserved in the future.

CHAPTER 2

DISPERSAL STUDY IN *EUPHYDRYAS GILLETII* : RELATIONSHIPS WITH WING MORPHOLOGY AND PHYSIOLOGY

INTRODUCTION

Dispersal can be an adaptive mechanism employed by organisms during range shifts and environmental disturbances (Boualil et al., 2019; Singh et al., 2012). Studying dispersal of individuals and species is crucial to understanding their ability to relocate and adapt to changes in their habitat or environment. The organisms that disperse can increase their chances of survival and sustain their populations and species. Considering climate change effects and anthropogenic landscape disturbances in wildlife habitat, it is even more crucial to understand the fate of different species and biological organisms. We need to understand how individual organisms move, whether they can disperse on their own and what might contribute to successful dispersal to a location where the species is able to thrive in new habitats. Sometimes when human management by translocation and re-introduction is needed, we also need to ensure they remain in the new site and don't move away. Understanding dispersal before and after any conservation translocation and reintroduction to new sites is crucial as this can determine the success of that conservation project.

To understand dispersal of organisms, we can assess their physiological and morphological features associated with movement. Morphology and physiology of

organisms are condition-dependent factors that facilitate dispersal. The structural features that aid their movement can be categorized under morphology. Bodily functions such as metabolic rates or the energy expenditure capacity for movement account for physiology. In aerial dispersers, we can look at dispersal characteristics that help organisms fly such as wing morphology and the energetic capacity for flight and maintenance. In particular, in ectothermic insects such as Lepidoptera, researchers have used wing morphology and physiology as proxies for movement and dispersal (Niitepõld et al., 2009; Turlure, 2010). Past studies have demonstrated some linkage of each of wing morphology and metabolic rates to dispersal and flight patterns as shown in the interrelationship triangle model made for this study (Figure 2.1).

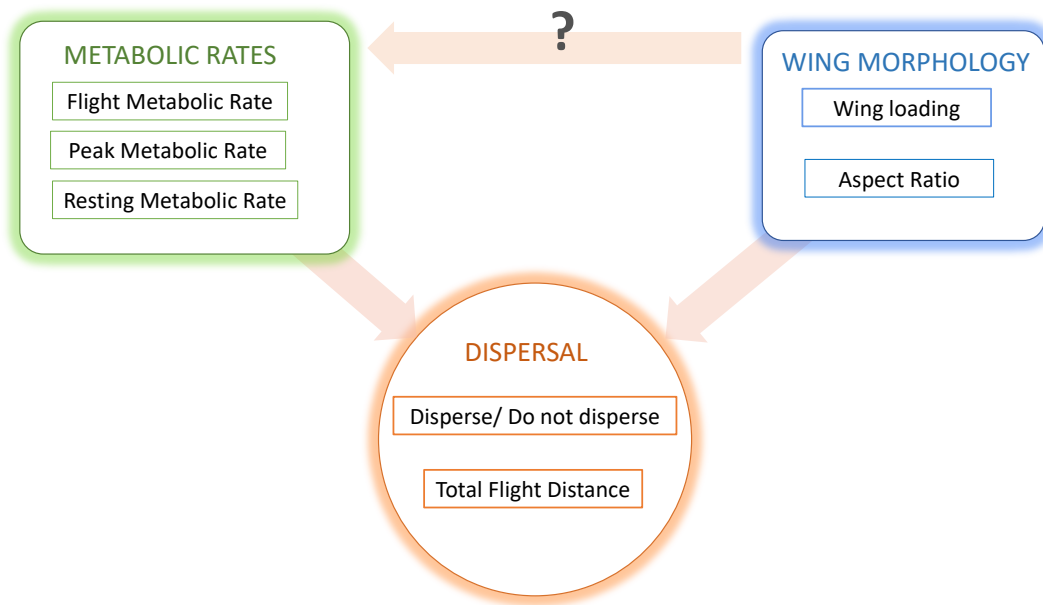


Figure 2.1: The interrelationship triangle. Expected, known and missing relationship links between metabolic rates, wing morphology and dispersal. The direction of the arrows shows known effects of one factor towards the other factor being pointed at. The arrow with the question mark shows unknown but expected direction of relationship effects between morphology and metabolic rates.

In butterflies, we assess the physiology of flight with three factors: resting metabolic rate (RMR), flight metabolic rate (FMR) and peak metabolic rate (PMR) (Niitepold, 2015; Hanski, 2006; Haag et al. 2005). It is important to consider metabolic rates because insects have to spend a significant amount of energy to create a lift during take-off and flight (Marden, 1987), which can affect dispersal (as demonstrated in Figure 1). The metabolic rates measure the energy expenditure during flight and at rest. Specifically, resting metabolic rate accounts for energy expenditure, measured by CO₂ output (Haag et al. 2005), for repair and maintenance. Flight metabolic rate explains the total energy expenditure during flight over a specified time period. It reflects flight capacity, endurance, and behavior (Niitepold, 2014). Peak metabolic rate is the maximum level of CO₂ produced during flight, which indicates maximum flight capacity.

Wing morphology is an important butterfly trait that affects dispersal and suggests whether the organisms are long-distance fliers or not (Altizer and Davis, 2010; Dockx, 2007). Some specific wing morphological features that are most studied for lepidopteran movement are wing area, aspect ratio and wing loading (e.g. Kuussaari et al., 1996; Berwaerts et al., 2002; Turlure et al., 2010b; Davis and Holden, 2015; Shi et. al, 2015; Turlure et. al., 2016). Wing loading specifically indicates the amount of mass per wing area the wings must lift in order to fly. It reflects the amount of effort needed to sustain flight, thus inferring that less energy is required for lower wing loading (Almbro & Kullberg, 2012; Duthie et al., 2015). However, the association between wing loading and dispersal (or flight) is not very clear since some studies show a positive association (Berwaerts, 2002; Turlure, 2010) and others show a negative association (Davis & Holden, 2015; Shi et al., 2015). This may be due to the differences in how wing loading is measured; some factor

in the body mass whereas other used only thorax mass. The lack of clarity may also be due to how dispersal is assessed; for example, studies look at only on aspect such as flight acceleration, flight endurance, glidability or flight distance or a combination of those. Additionally whether these flight characteristics are functionally contributing to short-distance or long- distance dispersal affects the interpretation of wingloading-dispersal relationship. Aspect ratio measures the shape of the forewings by considering how long and wide the wings are. Higher aspect ratio or narrower wings reduce drag at the wing tips and are thought to be more efficient for long distance flights (Lockwood et al 1998).

As mentioned above, the relationship between morphology and dispersal, as well as metabolic rates and dispersal have been studied in insects. However, no studies have explored the direct relationships between wing morphology and metabolic rates (See Figure 1). It is not clear how wing morphology and metabolic rate together can affect dispersal of individual organisms. Therefore, in this study, using the butterfly study system of *Euphydryas gillettii* (Lepidoptera: Nymphalidae), I explore the relationship among dispersal, wing morphology and metabolic rates (physiology). The major research questions of this study were:

- A. What is the relationship between metabolic rates (RMR, FMR and PMR) and wing morphology (aspect ratio and wing loading) of the butterflies?
- B. Is dispersal/movement history of individuals reflective of wing morphology, metabolic rates or an interactive relationship between them?

I expected a contribution of both morphological and physiological features to dispersal and that there will be some association between morphology and physiology as well. I hypothesized that greater flight and peak metabolic rate (FMR and PMR) capacities

are positively associated with dispersal since flight is aided by optimal energy investment, indicated by metabolic rates. Smaller wing loading and greater aspect ratio may favor greater dispersal. Smaller wing loading indicates less effort required to lift the body for flight (Almbro and Kullberg, 2012; Duthie et. al 2015) and higher aspect ratio (longer and narrower wings) are thought to reduce drag at the tips during flight (Le Roy et al., 2019). Greater wing loading and smaller aspect ratio require more energy for flight, so they may be related to greater flight metabolic rates.

METHODOLOGY

A. Study site and organisms

My study system is the Gillette's checkerspot butterfly, *Euphydryas gillettii* (Lepidoptera: Nymphalidae), found at three different sites near the Rocky Mountain Biological Laboratory (RMBL), Crested Butte, Colorado (38°57'N, 106°58'W, 2900 m asl). The species is native to northwestern US states of Wyoming, Idaho, Montana and all the way up to Alberta and British Columbia in Canada. In 1977, the species was experimentally introduced to Colorado. Scientists introduced 10,000 eggs and larvae from Granite Creek, Wyoming to a 2-hectare site at RMBL called Main, approximately 500 km south of Granite Creek (Holdren & Ehrlich, 1981; Boggs et al., 2006). This population at RMBL is separated from the native ranges by the Great Divide Basin, thus creating an isolated population that is highly inbred. Between 1977 and 2000 the population size ranged from 20-200 individuals in the Main site, including two bottlenecks prior to the 2000s. From 2002 until present, the population size has undergone significant fluctuations ranging from 100 to nearly 10,000 adult individuals (Boggs unpublished data). Over the last 40 years, the population from the original introduction site has also spread to other

areas near RMBL forming a patchy population or a metapopulation structure. I studied *E. gillettii* in three sites near RMBL namely Main (38°57'33.5"N, 107°00'06.8"W), Avalanche (38°57'05.7"N, 107°00'05.7"W) and 401 (38°57'56.2"N, 106°59'09.6"W).

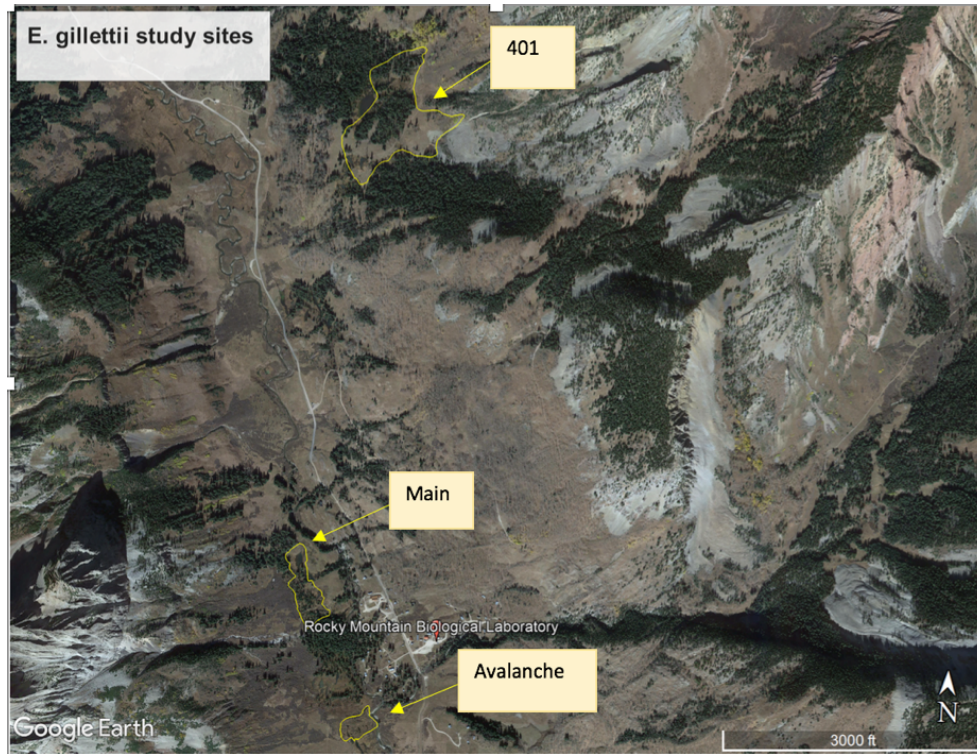


Figure 2.2: Study sites. Aerial view of the location of three sites in and near Rocky Mountain Biological Lab, Gothic, Colorado where butterflies were collected for this study.

Main is the oldest as it is the original site of species introduction. Avalanche site resulted from a population explosion around 2002 and is patchily connected to Main. 401 is the newest site, which originated between 2007-2012. Each study site is divided into 2-8 subsites to monitor dispersal within the sites. Main and Avalanche are about 0.75 km apart (Boggs et al., 2006). Main and 401 are about 1.75 km apart. In previous years, movement of *E. gillettii* between Avalanche and Main has been recorded, indicating that individuals moved away from their original site.

B. Dispersal

Between July 18- August 9, 2019, we conducted mark-release-recapture at the three sites (Main, 401 and Avalanche). We caught butterflies and marked their wings with a unique identifying number using an ultrafine point Sharpie. We recorded the ID, sex, wing wear (an estimate of age) (Boggs, 1987) and the site and subsite that the butterflies were caught in and released them near the center of the subsite. The same process was followed for all recaptures. In total, we recorded data on 590 butterflies, of which 79 butterflies were used in this study.

At the end of the flight season, we used the mark-release recapture data to assess whether the individuals moved from one subsite to another. Most movement data came from Main site. We categorized the individuals that moved as dispersive individuals. We used POPSTRUC program to synthesize movement data (Watt et al. 1977) for the total distance moved between the first and last recapture. The center of each subsite of Main were the reference points, so the distance between the center of one subsite to the other recapture subsite was used in the program. The distance between centers of two subsites in Main and between Main and Avalanche subsites ranged from 35 m to 734 m. The mean of distances between all adjacent subsites within Main was approximately 63m +/- 22m. The shortest distance we could record for dispersing individuals was 35m

C. Respirometry

During the mark-release-recapture in the field, if I found a recaptured butterfly (identified by an ID mark already present), I brought it back to the lab for respirometry. In one single day, only eight to ten individuals were brought into the lab. Each butterfly's ID

number and sex were recorded first. Then the rest of the respirometry data were collected in steps as follows:

i. Age:

A butterfly's wing wear is an estimation of relative age, since damaged wings are not repaired. Age was determined by rating the wing wear on a scale of 1-5 (Boggs, 1987). Each point increment means progressively degraded wings which indicates young to older age. The scores were assigned as follows: 1- a newly eclosed individual with floppy and wet wings; 2- wings that look pristine; from 2-5, I added up to 1.5 in increments of 0.5 for scale erosion and did the same for tears on the wing edges.

ii. Metabolic Rate Measurements:

Data on metabolic rates were acquired by performing flow-through respirometry (see Niitepöld et al. 2009, Niitepold 2011 for details) on 79 individuals. Flow-through respirometry uses 1L cylindrical plastic jars, called the flight/respirometry chamber hereafter, into which the butterflies are introduced for metabolic rate measurements. The flight chamber was situated in a temperature-controlled plywood cabinet with an open front. It was connected to a LI-Cor 7000 differential infrared CO₂ analyzer (Li-Cor Biosciences, Lincoln, NE, USA), UI2 equipment and ExpeData software that recorded butterfly's CO₂ output and the chamber temperature. CO₂-free air flowed into the chamber from the SS4 subsampler air pump (Sable Systems International, Las Vegas, NV, USA) and Mass Flow Controller (Sierra Instruments, Monterey, CA, USA). A fixed NTC thermistor probe (Sable Systems) was used to record the temperature inside the flight chamber. On average, the temperature in the flight chamber was maintained between 28°C to 32°C (mostly 30 C) during resting and flight period.

I introduced one individual at a time to the respirometer's flight chamber to measure the CO₂ output of the butterflies. Specifically, the respirometer measured the CO₂ output during rest (resting metabolic rate (RMR)), the CO₂ output during the optimum flight (peak metabolic rate (PMR)) and the total volume of CO₂ output during the entire flight (flight metabolic rates (FMR)). For the first 90s in the flight chamber, I covered the chamber with a black cloth and allowed the individual to rest. This recorded the RMR of butterflies before flight. After 90s were over, I removed the dark cloth. Then 30s later each individual was stimulated to fly continuously for 7 minutes to record PMR and FMR. At the end of the 7 minutes flight, I waited for one to two minutes for the metabolic rate to come down to baseline RMR levels. Then, I moved on to the next individual. All metabolic rates and associated flight data were recorded by ExpeData software running in a laptop that the respirometry equipment were connected. The data were later translated into calculated metabolic rate values using IGOR software. About eight to nine butterflies were run through the respirometer each day, between 13:00 and 19:00 hrs.

iii. Mass Measurements:

After the flight treatment in the respirometer, each individual was weighed on an OHAUS model SPX123 portable electronic balance to the nearest milligram (mg).

D. Morphology

After collecting respirometry data, I photographed each individual to collect wing morphology data. The photographic apparatus consisted of a platform of a 1x1 cm grid paper topped with a layer of bubble wrap. The butterflies were placed on top of the bubble wrap and held in position underneath a plexiglass. The layer of bubble wrap ensured the butterflies were not harmed by the pressure of the plexiglass above it. Once the butterflies

were oriented in open-wings position, they were photographed with a digital camera placed in a parallel position above the plexiglass platform. The photographs were later analyzed using ImageJ software ((Almbro & Kullberg, 2012; Duthie et al., 2015)) to measure the morphology of the wings, including wing length and area of the right forewing, based on the 1x1 cm square scale reference. The forewing length was measured as the distance from the hinge of the forewing to its tip. The edges of the right forewing were manually outlined, and ImageJ calculated the area (in cm²) of the forewing.

All eight to nine butterflies that were measured each day were fed honey water after metabolic rate and morphology measurements. The next day, they were released at the subsites where they were originally captured.

E. Data analyses

The dependent variables used in this study were resting metabolic rate (RMR), peak metabolic rate (PMR), flight metabolic rate (FMR) and dispersal. The independent variables were sex, wing wear and body mass, total number of days between first and last recapture and, the two features of wing morphology, aspect ratio and wing loading. All dependent variables were checked for normal distribution and all independent variables were checked for cross correlation initially.

The wing morphology data were repurposed into two measures: aspect ratio and wing loading. Aspect ratio represents the shape of the wings and wing loading represents the ease with which the wings can help lift the body mass. I calculated the aspect ratio as $4 \times \text{forewing length}^2 / \text{forewing area}$. I also obtained wing loading by calculating body mass/forewing area. To account for both forewings in wing loading, I multiplied the forewing area by two.

Metabolic rates were obtained by processing the ExpeData record in IGOR software. It calculated the total CO₂ output during the resting period, RMR, before flight. FMR was calculated as the total volume of CO₂ produced during the 7-minute flight period. PMR was calculated as the highest rate of CO₂ produced during the flight. All these metabolic rates were corrected for body mass by taking the residuals of their regression. Therefore, the residual values were used as dependent variables in the statistical analysis. Using the residual metabolic rates (PMR, FMR and RMR) as dependent variables, I conducted general linear model analyses in Systat13 (SYSTAT Software, Inc., San Jose, CA, USA). I used the backward elimination method to eliminate non-significant independent variables at each step after the initial full model.

For dispersal, I did two types of analyses:

1) General Linear Model: I selected only the individuals with recorded movements to subsites other than their original subsite. Total movement distance between the first capture and last recapture was the dependent variable for GLM analysis. The data for total movement distance was log transformed, as the raw data weren't normally distributed. I then performed GLM analyses similar to the GLM analyses for metabolic rates above

2) Binomial regression analysis for two movement categories dependent on whether the butterflies moved outside their original subsite or not.

RESULTS

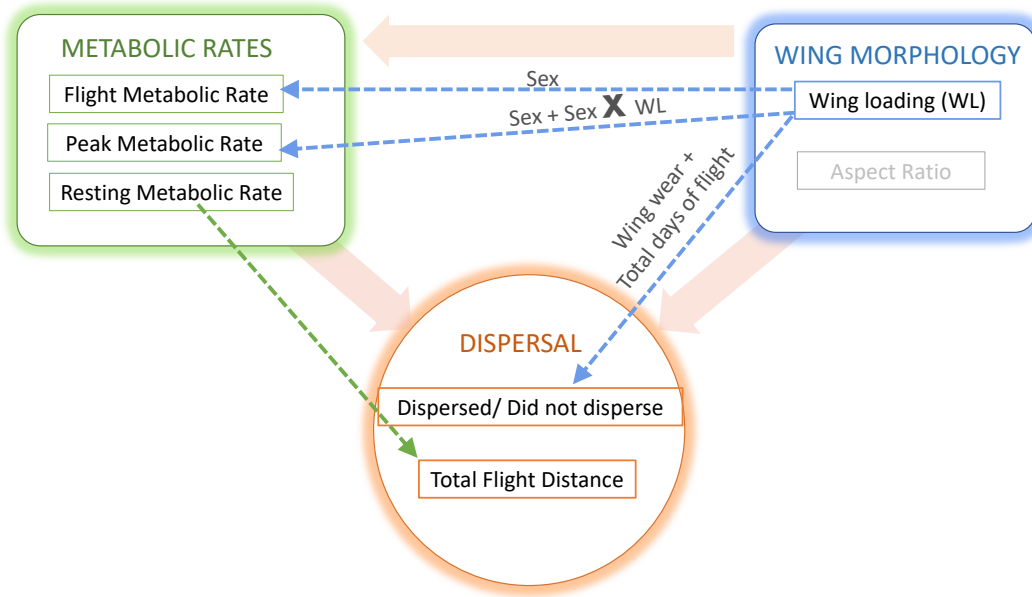


Figure 2.3: Completed interrelationship triangle. The links between metabolic rates (physiology), wing morphology and dispersal in *E. gillettii*. The direction of the arrow indicates the effect of that independent variable 'on' the dependent variable that the arrow points to. The terms indicated on the arrows are other factors that affect the dependent variable. The 'x' mark symbolizes interaction effect, i.e. there is a sex by wing loading effect on peak metabolic rate.

A. Dispersal

According to the binomial regression analysis, *Euphydryas gillettii* butterflies that moved away from their original site had greater wing wear on final or penultimate capture (estimate = 3.301, $z=2.577$, $p=0.010$), and greater wing loading (estimate = 0.211, $z=2.314$, $p=0.021$). However, those that moved had a smaller number of days between the first capture and last recapture (estimate = -0.874, $z=-2.092$, $p=0.036$) (Chi-square = 14.732, $df=4$, $p=.005$). Wing wear and wing loading were measured in the lab mostly on their last recaptures (38 out of 43 individuals).

The General Linear Model (GLM) for total distance moved only included the individuals that showed recorded movements to other subsites outside their original site.

The total distance the individuals moved between the first capture and last capture increased with increasing residual resting metabolic rate, RMR ($F_{1,27} = 7.537$; $p = 0.011$) (Fig. 4). The number of days between the first and last recapture was not significant. 25 individuals out of 29 were measured for metabolic rates on their last re-capture. The rest were measured on their penultimate recapture. Furthermore, there was no sex by RMR interaction effect on the tendency to disperse outside their site of origin.

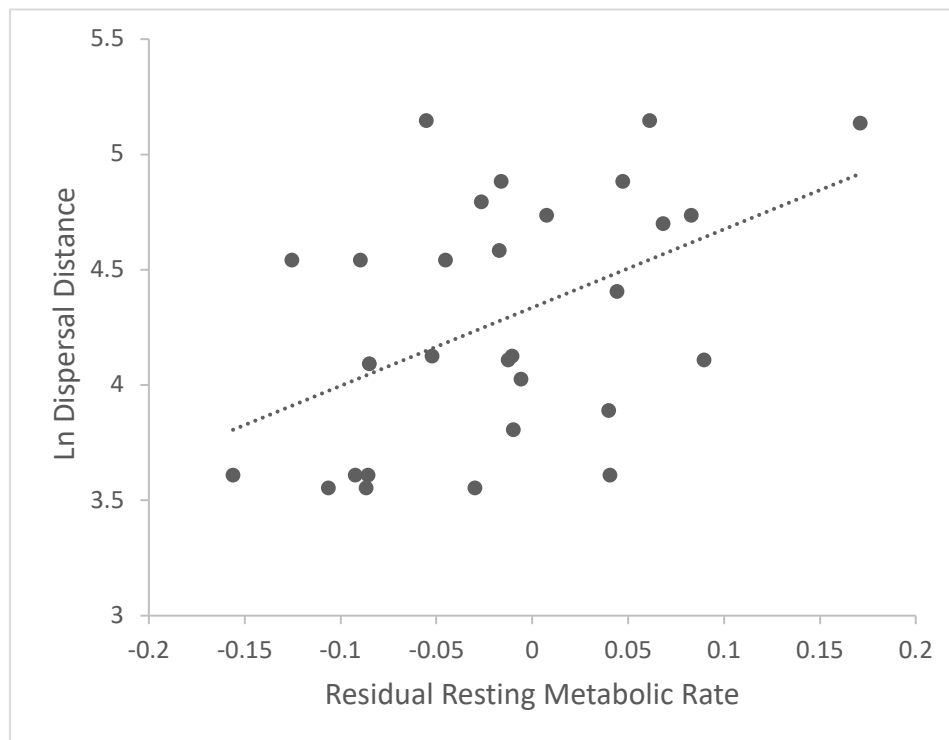


Figure 2.4: Dispersal distance vs. residual resting metabolic rate (RMR). *Euphydryas gillettii* butterflies moved longer distances if they had higher residual resting metabolic rate (RMR corrected for mass).

B. Flight Metabolic Rate (FMR)

Residual FMR, corrected for mass differences, was significantly affected by sex ($F_{1,76} = 5.618$, $p=0.020$) such that males had a higher residual FMR (Least square means= 0.027, SE= 0.013) than females (Least square means= -0.010, SE= 0.007). The butterflies

tended to have higher residual FMR if they had greater wing loading ($F_{1,76}=3.003$, $p=0.087$). There was no significant sex by wing loading interaction.

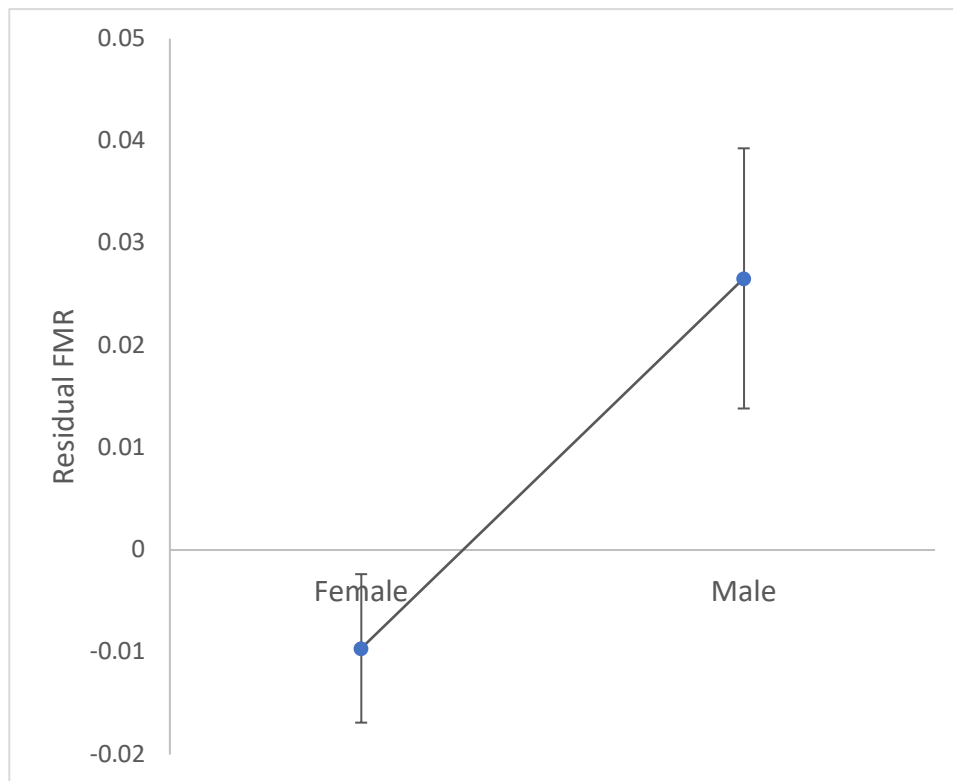


Figure 2.5: Sex differences in residual flight metabolic rate. Male *E.gillettii* had greater residual flight metabolic rate (least square means from a model including wing loading) than females.

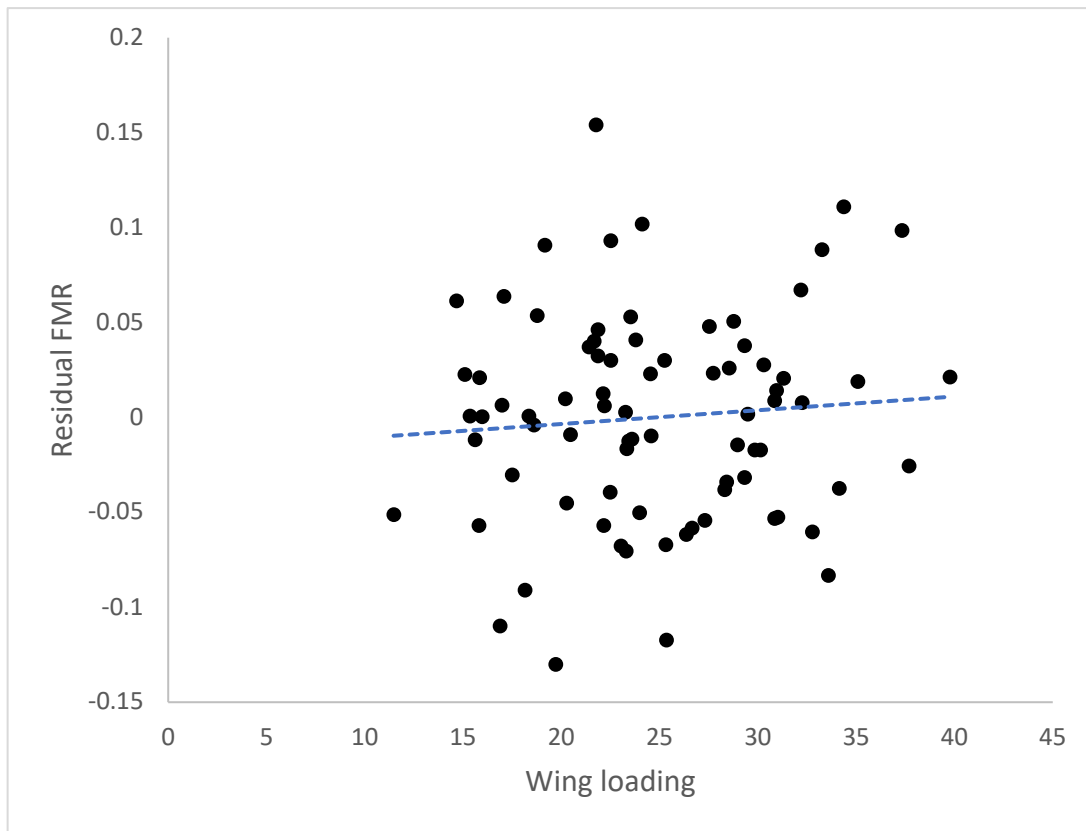


Figure 2.6: Residual flight metabolic rate vs. wing loading. *Euphrydryas gillettii*'s residual flight metabolic rate was greater if they had greater wing loading.

C. Peak Metabolic Rate (PMR)

Residual PMR, corrected for mass differences, depended on sex ($F_{1,76} = 8.879$, $p = 0.004$) where males had greater residual PMR (Least square means = 0.109, SE = 0.13048) than females (Least square means = -0.132, SE = 0.074). Residual PMR also depended on the interaction effect of sex by wing loading ($F_{1,76} = 6.082$, $p = 0.016$). Females had higher residual PMR when they had greater wing loading ($F_{1,56} = 6.496$, $p = 0.014$). The effect of wing loading on residual PMR was not significant for males ($F_{1,19} = 0.148$, $p = 0.705$).

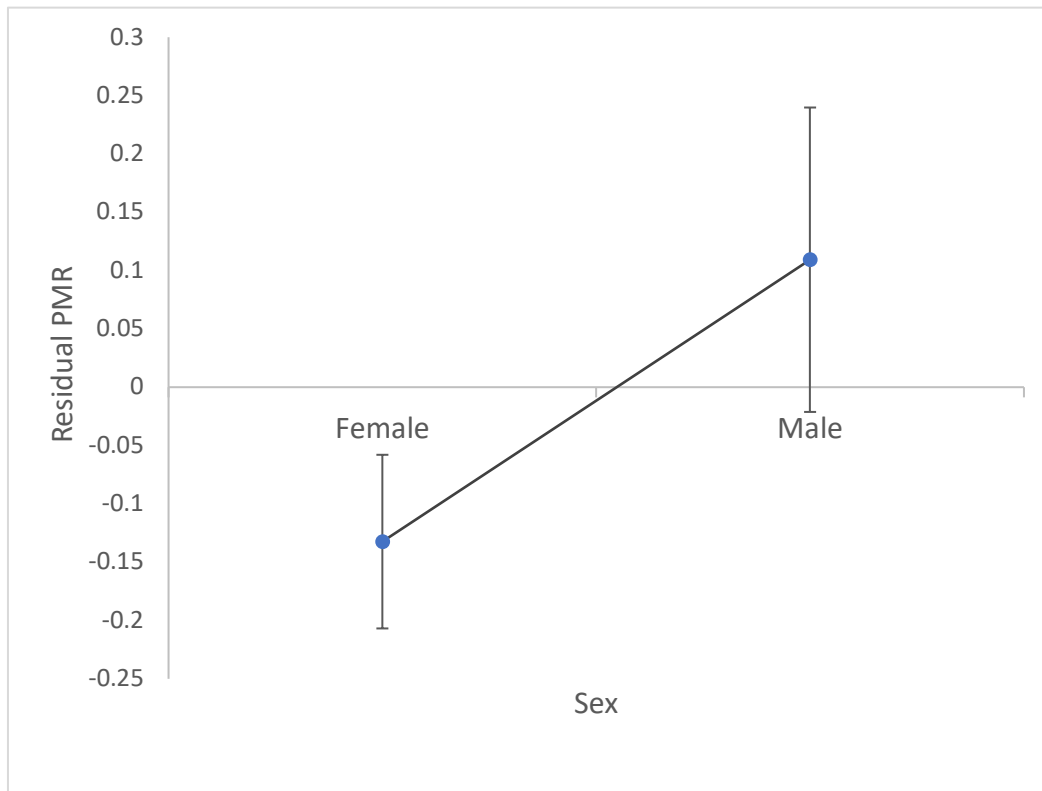


Figure 2.7: Sex differences in residual peak metabolic rate. Male *Euphydryas gillettii* had greater residual peak metabolic rate than females.

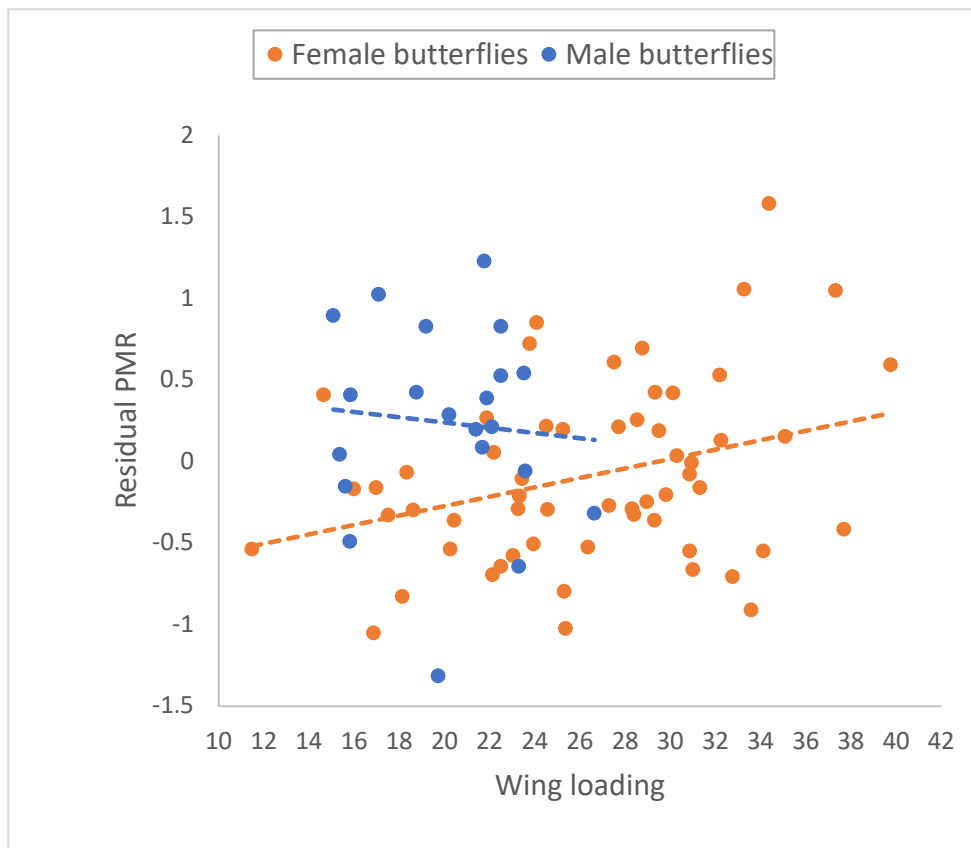


Figure 2.8: Sex by wing loading interaction for residual peak metabolic rate (PMR). Female *E. gillettii*'s residual PMR (orange) was positively associated with wing loading, but male's (blue) were not.

D. Resting Metabolic Rate (RMR)

Residual RMR, corrected for mass differences, did not depend on wing morphology of the butterflies (aspect ratio and wing loading), nor did it depend on sex and wing wear.

E. Morphology

Although wing loading affected dispersal and certain metabolic rates like FMR and PMR (see A, B and C above), aspect ratio did not have any effect on either dispersal or metabolic rates that denote energy expenditure capacity for flight and maintenance.

DISCUSSION

A. Dispersal and morphology

I found that the dispersers among *E. gillettii* (i.e the ones that moved to other sub-sites) had greater wing loading and greater wing wear (an age estimate). Therefore, wing wear and wing loading are indicative of better dispersal in *E. gillettii*. On the other hand, aspect ratio did not have any significant link with dispersal in *E. gillettii* at Gothic, Colorado. The exact implications of these morphological traits on dispersal may however vary depending on how different studies, in the past, have interpreted dispersal and morphological trait associations.

Dispersal is a multifaceted trait, so different studies have measured different aspects of dispersal. Some measure the flight distance; others measure the duration of flight or even flight speed. Some measure dispersal by characterizing individuals from a recently established population considering those individuals as models of higher dispersal, and then link it to specific morphology. Apart from the diversity in dispersal measurements, studies also lack consistency in how wing loading is measured. Some calculate wing loading as body mass divided by wing area (Berwaerts et al., 2002; Betts & Wootton, 1988; Byrne et al., 1952; Davis & Holden, 2015; Reim et al., 2019). Alternatively wing loading is measured as thorax volume by wing area (Turlure et al., 2010, 2016). It is common for dispersal and movement studies to factor in thorax measurements, in addition to wing loading, as a component of movement morphology (Berwaerts et al., 2002; Reim et al., 2019) since butterflies' flight muscles are in the thorax. The differences in what is considered under 'dispersal', what flight parameters are measured and how wing loading is measured could be the reason why there is no clear relationship between wing loading and

dispersal in the literature. I saw similar discrepancies in morphology-dispersal relationship between past studies and ours.

In this study, the dispersers among *E. gillettii* (i.e the ones that moved to other sub-sites) had greater wing loading and greater wing wear (an age estimate). The wing wear-dispersal association can be explained by the fact that the *E.gillettii* that dispersed may have faced degradation of wings due to frequent use of wings for flight, also indicating that they could have lived longer and thus flew more. The wing loading- dispersal association found here was contrary to my hypothesis that greater wing loading would be linked with more dispersive individuals. However, this positive association between wing loading and dispersal was similar to the results for studies by Berwaerts (2002), Betts and Wootton (1988) and Turlure (2010) in other butterfly species. Berwaerts, Betts and Wootton used the same formula for wing loading as I did to explain flight. They have suggested greater wing loading to positively influence different aspects of movement and dispersal like flight acceleration capacity, flight endurance, fast flight and displacement during short distance flights. It is suggested that species that fly fast and use shallow glides have higher wing loading (Betts and Wootton, 1988). Furthermore, high wing loading is known to be compensated with higher wingbeat frequencies (Byrne et al., 1952). So, in our case the greater wing loading could have contributed to fast speed, greater flight acceleration and flight duration or endurance capacity, shallow gliding flights and high wingbeat frequency, and thus, may have aided a dispersive behavior of *E. gillettii* dispersal.

Greater wing loading (calculated using body mass) for more dispersive individuals in this study could have been influenced by either thorax mass or abdomen mass. A greater wing loading could have been a result of heavier abdomen or greater flight muscles which

are in the thorax. If high wing loading is due to abdomen mass, it means greater reproductive potential and energy reserves because a butterfly's abdomen is indicative of reproductive and fat reserves (Stjernholm et al., 2005). If high wing loading is due to heavier or bigger thorax then it would affect flight. Thus, in our case, I would expect thorax mass (i.e. flight muscles) to have contributed to better dispersal than abdomen mass. It is important to note that both thorax and abdomen mass decrease with age in various butterfly species (Stjernholm et al., 2005), so in general, age may also be affecting wing loading indirectly. Older butterflies will potentially have lower wing -loading resulting in lower reproductive and flight ability. For our *E. gillettii* females, increased wing loading was also associated with increased residual PMR per unit body mass. This means that PMR surged to expend greater energy needed for lift and flight due to higher wing loading.

Contrary to my results, other studies have found a negative relationship between smaller wing loading and dispersal or flight. For example, smaller wing loading has been found to be associated with more dispersive individuals when comparing migratory vs non-migratory monarch individuals (Davis & Holden, 2015) and flight capable vs. flightless strains of gypsy moths (Shi et al., 2015). My results might have been opposite to such studies because I measured short distance movements of butterflies rather than long-distance migratory flight. Long and short distance movements are different type of movement behaviors motivated by different reasons; long distance flight is generally for migration away from home with no intention to return whereas short distance flight happens within current habitat patches to search for food, resources and for mate location, for example. Longer flights may require greater wing loading, since higher wing loading is meant to allow for faster flights that is more useful to cover longer distances. Shorter

flights within habitat are more discontinuous but frequent, on the other hand, so they might need a non-energy- expensive easier lift capacity provided by lower wing loading (Almbro, 2012; Duthie et. al, 2015). Wing morphology in different species of insects could also function differently, thus comparison of wing loading across species (butterflies vs. moths) might not yield the same relationship between wing loading and dispersal.

The results show that aspect ratio does not have any relation to dispersal or metabolic rates. My hypothesis for aspect ratio did not stand true since aspect ratio did not affect *E. gillettii* dispersal nor did it affect any metabolic rates. Our study samples may have lacked variation in aspect ratio which caused it to be insignificant in explaining dispersal and metabolic rates. *Euphydryas gillettii* in our study sites in Gothic, Colorado have been isolated from their native range for years and there are no immigrating individuals from native sites. A study done in 2018, found that *E. gillettii* at RMBL have lower aspect ratio compared to most populations in their native range (Bacon unpub). Moreover, populations from different sites within the native range significantly differed in aspect ratio (Bacon unpub). So, variation in wing aspect ratio still exists in their native ranges, where there is a possibility to find a clear relationship between aspect ratio and dispersal. This means that isolation has probably caused a lack of variation in wing shape or aspect ratio at Gothic. The insignificance of aspect ratio in our study may also indicate that aspect ratio or the wing shape may not play an important role in aiding dispersal in *E.gillettii*. Rather, other morphological characters related to flight, like total body mass, thorax mass, forewing area and forewing length may be influencing a butterfly's dispersal related processes, like shown in *Pararge aegeria* (Berwaerts et al., 2002). These other morphological variables were not separately measured and analyzed in my study.

Some studies have shown elongated forewings (higher aspect ratio) to be related to migratory populations of monarch butterflies, *Danaus plexippus*, indicating higher movement and dispersal capacity (Dockx, 2007; Altizer and Davis, 2009). Higher aspect ratio is linked to higher flight acceleration capacity in male butterflies of different species like *Troides radamantus*), *Pararge aegeria* (speckled wood), *Lycaena tityrus* (sooty copper), (Betts & Wootton, 1988; Berwaerts et al., 2002; Reim et. al., 2019). Since I did not find sex by aspect ratio interaction effect on dispersal, I could not verify the above-mentioned relationship. In *Morpho* (Nymphalidae), rounder wings that indicate lower aspect ratio, have been linked to greater maneuverability of several species of butterflies in the genus *Morpho* (DeVries et al. 2010) and efficiency in long-distance flight (Hassall, 2015).

B. Dispersal and metabolic rates

Surprisingly, neither residual flight metabolic rate (FMR) nor residual peak metabolic rate (PMR) was significantly associated with dispersal in our species, *E.gillettii*, contrary to past studies in other species. Flight metabolic rate has been shown to be positively associated with dispersal rate in butterflies (eg. *Melitaea cinxia*) (Niitepõld et al., 2009). Greater FMR has also been associated with the tendency to disperse and establish new populations (Haag et al. 2005). FMR is thought to reflect a combination of flight capacity, endurance, and behavior in *Melitaea cinxia* (Niitepõld et al., 2014). Similar to the argument for dispersal and morphology above, different studies measure different facets of dispersal which makes it difficult to establish a clear and consistent relationship between metabolic rates and dispersal. As for peak metabolic rate, greater values have been positively correlated with longer-distance dispersal in female *Melitaea cinxia* (Niitepõld et

al., 2010). Since the dispersal distance I measured are short-distance movements, I may not have been able to see such relationship between PMR and dispersal in our study.

Only resting metabolic rate (RMR) of *E.gillettii* was found to explain the total distance dispersed by individuals. To my knowledge, there is no literature explaining this direct relationship between resting metabolic rate and dispersal. Higher RMR associated with longer distance dispersal could mean that having a greater energetic capacity for general maintenance of the bodily functions while not active is more important in aiding movement in *E.gillettii* than its FMR and PMR. RMR may also be more important because ultimately an elevated RMR may be induced to help maintain and recover the body from an increased energy investment in flight (Careau et al. 2008).

C. What is considered dispersal?

In this study, I refer to dispersal as the movement of the butterflies outside of their site or subsite of origin. This is mostly short-distance local dispersal between patchy populations of butterflies in our study sites. It could be argued that such short-distance movements may not explain the full picture about dispersal. Scientists have contested whether dispersal could be realised through short-distance routine movements or long-distance migratory type of movement only (Baars, 1979; Ims, 1995; Van Dyck & Baguette, 2005). One could contend that dispersal is a result of only one specific type of movement that causes displacement outside of natal site, and thus, only such type of long-distance movement behaviour can be considered as dispersal. Others may contend that dispersal can also be explained by a sum of short-distance routine movements mostly seen during foraging or mate-searching. So, since short-distance and long-distance dispersal can be two behaviorally different types of movements, it could be debated that we cannot make

inferences about long distance dispersal by looking at short-distance movement patterns. MRR studies like ours is biased towards short-distance dispersal of possibly routine and explorative nature rather than migratory type of dispersal. However, this study is still relevant and reveals important information as both short and long-distance movement behaviors may be contributing to migratory dispersal. It is suggested that behaviors typical of both short-distance as well as long-distance movements may contribute to net displacement (Van Dyck & Baguette 2005). Although our study only looks at short local dispersal, an addition of another study specifically looking at long-distance dispersal behaviors can produce a very clear understanding of dispersal patterns and mechanisms of *E. gillettii*'s dispersal in Gothic.

The *E. gillettii* population that I studied at Rocky Mountain Biological Lab (RMBL), Colorado was a good system to evaluate dispersal because these are non-native butterfly populations that have survived in this site where they were introduced 40 years ago. Due to years of isolation the populations that remain are thought to be less dispersive than their native counterparts, as the dispersers may have already emigrated. However, local dispersal still happens which can help populations to relocate to new places. In fact, movement among the different sites at RMBL have been recorded in previous years (Boggs, unpublished). Studying what affects their dispersal has helped us understand what types of individuals (with certain morphological and physiological features) can disperse and relocate in case of future habitat disturbances. Movement data recorded in past years, prior to my study, at RMBL had not been studied in relation to the individual's wing morphology and flight metabolic rates until now. I explored a novel idea to fill the gap in the understanding of connections among the different dispersal traits of *E. gillettii*.

CONCLUSION

Euphydryas gillettii butterflies that are older (or have higher wing wear due to more frequent flying) and have higher wing loading are the ones that can disperse. Additionally, the ones that have higher RMR can fly greater distances. Referring to the interrelationship triangle (Figure 3) proposed in this study, there is no chain association between morphology, metabolic rate and dispersal, where morphology is linked to metabolic physiology and that in turn is linked to dispersal. For example, wing loading (morphology) was associated with FMR and PMR (physiology), but FMR and PMR was not associated with dispersal at all. Rather morphology and metabolic physiology, specifically wing loading and resting metabolic rate, separately influence the dispersal processes in *E. gillettii*.

This study has identified the dispersive characteristics and features of *E. gillettii* individuals. Wildlife managers and translocation conservation practitioners who require biological and ecological knowledge of a candidate species (according to IUCN guidelines, IUCN/ SSC 2013), can use such information to plan translocation strategies. The results of this study can be used for translocation projects of *E. gillettii* if needed in the future. Dispersal is reportedly the top animal behavior issue that has negatively affected the post-release success of a translocation project (Berger-Tal et al., 2020). So, if such dispersal-related information is available prior to implementing translocation and reintroduction, wildlife managers can identify and choose more dispersive or less dispersive animals to translocate depending on their conservation goals. That could ensure greater translocation conservation success.

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