Effect of flight and winter conditions on adult life history traits of the Mountain Pine Beetle (*Dendroctonus ponderosae* Hopkins)

by

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Abstract

The mountain pine beetle, *Dendroctonus ponderosae*, represents a significant ecological and economic threat to North American forests. This study investigates multiple facets of the biology of *D. ponderosae*, focusing on metabolic rate, flight capacity, and host entry behaviour under varying winter conditions. I first test the impact of flight on the subsequent metabolic rate of adult *D. ponderosae* using respirometry. Results reveal that flight activity positively impacts the metabolic rate of *D. ponderosae*, showing increased CO_2 production rate with flight distance and distinct respiratory patterns post-flight. My study is the first to identify distinct continuous and discontinuous gas exchange patterns in adult *D. ponderosae*, significantly influencing metabolic activity. Beetles with discontinuous respiration exhibit overall higher metabolic rates. I then test how exposing beetles to different winter conditions demonstrates that the duration and severity of overwintering during the larval stage influence the metabolic rate and respiratory behaviors of the adult *D. ponderosae*. Prolonged exposure to cold temperatures negatively affects metabolic rates in adult *D. ponderosae*, resulting in metabolic suppression and reduced CO_2 production rates. My study highlights significant interactions between physiological state, environmental stressors, and metabolic regulation in adult *D. ponderosae*. Furthermore, my research shows that winter conditions and pre-flight physiological states significantly influence subsequent flight capacity in *D. ponderosae*. Heavier beetles show higher flight propensity and distance, with winter conditions having an indirect or secondary impact. Overwinter conditions do not affect the subsequent host entry of female *D. ponderosae*; instead, it is the weight of the females that influences their rates of host entry. This study is one of the first to explore how flight and winter conditions impact adult mountain pine beetle, providing insights that may help predict its spread as the climate changes.

Preface

This thesis consists of a series of studies conducted as part of my Master's research at the University of Alberta and within the scope of the TRIA-FoR Project. My research focuses on the mountain pine beetle, *Dendroctonus ponderosae*; Coleoptera: Curculionidae, aiming to clarify and interpret findings from experimental work. I was fortunate to collaborate with respected faculty and researchers who significantly contributed to the planning, execution, and record of these studies.

Chapter 2 experiment was developed closely with Dr. Maya Evenden, Taylor Volappi, and Dr. Antonia Musso. I was responsible for creating methods, doing experiments, collecting, and analyzing data, interpreting results, and writing the manuscript. Dr. Evenden and Taylor Volappi came up with the original experimental design, and I helped finalize it. Drs. Evenden, Heath MacMillan, and Lien Luong gave feedback on the concept and design. Dr. Musso was crucial in analyzing the data and interpreting the statistical results. Dr. Evenden reviewed and provided feedback on my thesis drafts. Chapter 3 was a team effort with Dr. Evenden and Dr. Musso, where I did similar tasks as in Chapter 2. Both Drs. Evenden and Musso helped shape the study. Dr. Musso also developed the methods for analyzing overwintering conditions, which was essential for our findings. Dr. Evenden provided feedback on this chapter and the further drafts. Drs. Evenden, Heath MacMillan, Lien Luong, and Keith Tierney provided valuable feedback on the final manuscript's editing.

This research was funded by a Teaching Assistantship from the University of Alberta and the TRIA-FoR Project, Activity 4, which focuses on D. ponderosae fitness, dynamics, and local adaptation. This support was essential for the work presented in this thesis. All research adhered to ethical standards set by provincial and federal regulations, the TRIA-FoR Project, and the University of Alberta. I completed all necessary training and permits before data collection. Chapter 2 is formatted for submission to *Physiological Entomology*, and Chapter 3 for *Journal of Experimental Biology*.

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I owe a profound thank you to my parents, whose love and guidance have shaped me into the person I am today. I extend a special thank you to my father, who has not only cared for my mother during the most challenging times but has also been a pillar of strength and resilience. His dedication and lessons on being strong and independent, no matter the circumstances, have been invaluable. His example is a guiding light in my life, especially when I am far from homeland. I also want to thank my mother, who, although she can no longer hear my voice or respond, I know continues to bless me in her own way. Her spirit and love continue to guide and inspire me every day. I know it was her wish for me to focus more on academia, but life had other plans, and I had to redirect my path. I want to say sorry, Mom, for not following the path you hoped for me. However, your influence and strength have always been with me, helping me navigate every challenge. I must express my deepest gratitude to my boyfriend, Hems Jay, for his constant love and support. His unwavering presence and encouragement have been my anchor during tough times, lifting my spirits and bringing happiness into my life when I needed it most. I thank my cats, Tuna, and Samosa, for their presence, which brought joy and relaxation to my days. I am also grateful for my part-time job at Fantuan Delivery, which has been instrumental in supporting my career. A special thank you to my managers and co-workers for their flexibility, understanding, and support, making it possible for me to balance work and my academic pursuits.

I am grateful to all my family, relatives, the Evenden Lab and friends at the University of Alberta and the TRIA-FoR Project for creating a supportive research environment. Thank you all for your contributions, big and small, which have guided me through this challenging and fulfilling journey.

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Chapter 1: General Introduction

1.1 Introduction

The mountain pine beetle, *Dendroctonus ponderosae* (Coleoptera: Curculionidae: Scolytinae) has an extensive native range in western North America where its primary host is mature lodgepole pine, *Pinus contorta* Dougl. ex Loud var. *latifolia* Engelm (Reid 1962; Carroll et al. 2004). The range of *D. ponderosae* has expanded in latitude and altitude since the late 1990s and 2000s by entering formerly unsuitable habitat due to climate change (Safranyik et al. 1975; Carroll et al. 2004; Safranyik et al 2010). The *D. ponderosae* outbreak in British Columbia resulted in the death of over 18 million hectares of pine forests. This massive infestation caused significant ecological and economic impacts, including the loss of timber resources and increased wildfire risks (Corbett et al. 2016). As part of this range expansion, *D. ponderosae* moved eastward across the Rocky Mountains and encountered a novel host, jack pine, *Pinus banksiana* (Cullingham et al. 2011).

As shown in Figure 1.1, the mountain pine beetle follows a univoltine lifecycle, where dispersal and reproduction occur in summer, followed by larval development in winter. All stages of beetle development occur under the bark except for adults that disperse in July or August to find new host trees for brood development (Carroll & Safranyik 2004). Adult beetles exhibit flight polymorphisms (Jones et al. 2020) and some beetles can fly long distances, as measured by flight mill bioassays (Evenden et al. 2014). Flight polymorphisms in *D. ponderosae* involve distinct flight capacities that may be a bet hedging strategy in the host colonization process (Jones et al. 2020). Understanding flight activity is critical for predicting the eastward expansion of *D. ponderosae* and its potential spread throughout the boreal forest (Safranyik et al. 2010). As *D. ponderosae* establishes populations in Alberta, it significantly expands its range into new habitats, posing a threat to the boreal forest through increased tree mortality and ecosystem disruption (Cullingham et al. 2011; Cook & Carroll, 2017; Shegelski et al. 2021).This expansion underscores the adaptability of *D. ponderosae* and the pressing need for comprehensive management strategies.

Female *D. ponderosae* are the pioneers that coordinate a mass attack on host trees by releasing an aggregation pheromone that attracts mostly male and some female beetles (Libbey et al. 1985; Borden et al. 1987; Progar et al. 2014). Males joining the aggregation release a different aggregation pheromone that attracts mainly females (Vité & Pitman 1968). Aggregation is slowed to avoid intraspecific competition by the subsequent release of anti-aggregation pheromones from both sexes (Raffa & Berryman 1983; Byers et al. 1984; Hunt and Borden 1990; Borden et al. 1990; Chiu & Bohlmann 2022). Mated female beetles lay eggs along the sides of vertical tunnels excavated in the phloem. *Dendroctonus ponderosae* overwinters as late instar larvae and develop fully the next spring before pupating in June or July (Reid 1962).

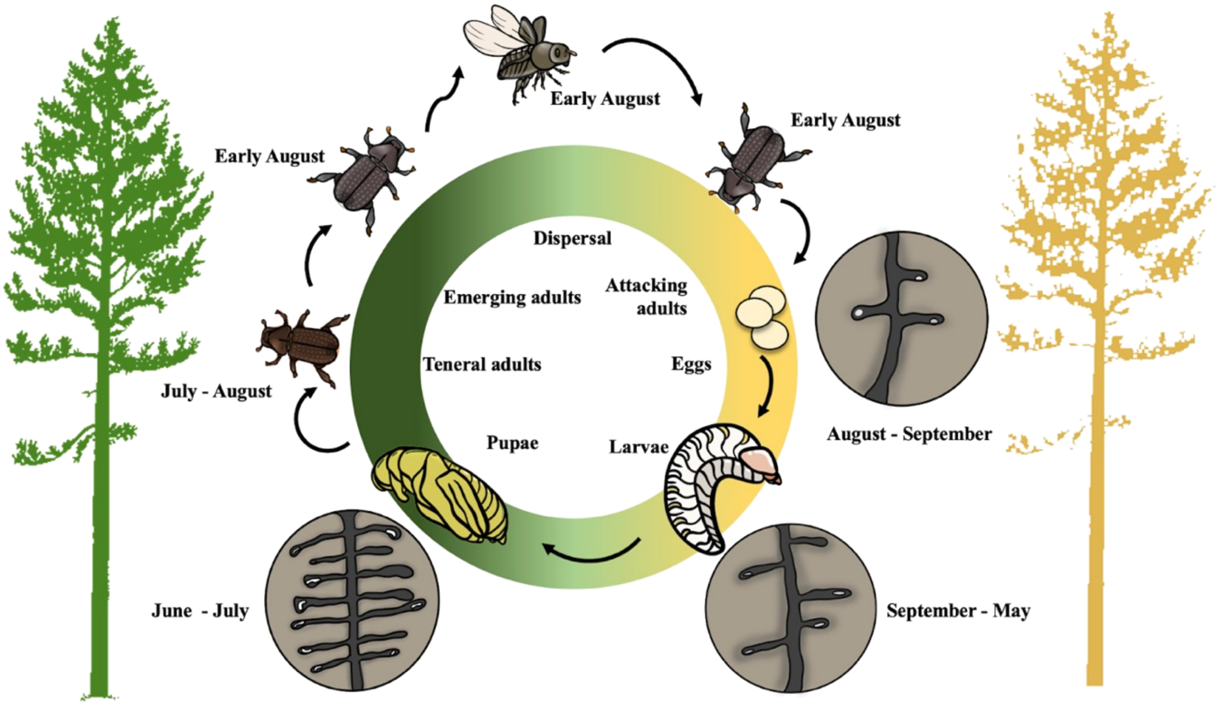

Figure 1.1 Mountain pine beetle univoltine life cycle. During the summer, the beetles disperse and reproduce, with females laying eggs in galleries they excavate beneath the bark. Mountain pine beetles overwinter as larvae and resume development in the spring, eventually emerging as adults later in the summer.

A trade-off exists between flight and host colonization in *D. ponderosae*, as the energy expended during flight limits the beetle's ability to colonize well-defended hosts (Jones et al. 2020). The energy expended during flight also affects subsequent reproductive success (Wijerathna et al. 2019). If beetles have enough energy following flight to enter hosts, those with the lowest energy stores enter hosts first, indicating they are less capable of further dispersal and less selective in their choice of hosts (Latty & Reid 2010; Jones et al. 2020). What is not known in this system is how different winter conditions affect adult emergence, flight capacity, and host colonization after they survive the winter (Sambaraju et al. 2012; Thompson et al. 2020; Sambaraju 2021; Sambaraju & Goodsman 2021).

Dendroctonus ponderosae is a freeze avoiding species that relies on the production of cryoprotectants such as glycerol (Fraser et al. 2017) or modifications in enzymes involved in RNA metabolism for survival during the cold periods (Bonnett et al. 2012). For example, RNA-binding proteins and modifications in RNA processing ensure that the beetles’ cells continue to function during overwintering, preventing damage from cold temperatures (Storey & Storey, 2012). The overwintering behavior of *D. ponderosae* plays a critical role in its lifecycle and population dynamics (Bentz & Mullins 1999, Robert et al. 2016 Rosenberger et al. 2017, Bleiker et al. 2017; Bleiker & Smith, 2019). During the winter months, *D. ponderosae* larvae enter a state of dormancy characterized by a suppressed metabolic rate, which reduces energy expenditure and enhances their ability to survive freezing temperatures until warmer conditions return in the spring. This metabolic suppression allows them to survive prolonged periods of low temperatures with limited energy reserves. For example, metabolic rates of *D. ponderosae* decline sharply from October to November and remain low during the colder months before gradually rising again in the spring. This metabolic reduction is minimizes energy use and beetles rely on their stored metabolic reserves (Lester & Irwin, 2012). Additionally, after overwintering, beetles must rapidly restore their metabolic functions, which can influence their subsequent flight capacity, energy use, and overall fitness as they prepare for dispersal and host colonization. This is crucial because stored energy, primarily lipids, fuels their flight during the obligatory dispersal phase, enabling them to locate new hosts for colonization. After overwintering, beetles rapidly mobilize their previously suppressed energy reserves to support survival and reproduction during the next life cycle stages (Evenden et al. 2014; Wijerathna et al. 2019). This metabolic shift is influenced by several factors, including overwintering temperature, dormancy duration, and the beetle's pre-winter energy reserves. During the winter months, *D. ponderosae* enter a state of dormancy to conserve energy and withstand freezing temperatures. The metabolic rate of overwintering *D. ponderosae* larvae is suppressed to conserve energy until warmer temperatures return in the spring (Hahn & Denlinger, 2011). This period of dormancy is crucial for survival, as it allows them to endure the cold temperatures and harsh environmental conditions. Factors such as temperature, and moisture levels can influence beetle survival rates and subsequent infestation in pine forests (Lester & Irwin 2012).

1. 2 Objectives

I address the following objectives in this thesis:

Chapter 2. Objective 1: Effect of Flight on Metabolic Rate in Adult *D. ponderosae*

- **Hypothesis:** Flight increases subsequent metabolic rate of mountain pine beetle.
- **Prediction:** *Dendroctonus ponderosae* that fly will exhibit higher post-flight metabolic rates compared to non-flying beetles. The increased metabolic activity is necessary to replenish depleted energy reserves and repair muscle tissues that were heavily utilized during flight (Scholander et al. 1953). These muscles are also essential for subsequent behaviors (Chari et al. 2021), such as host entry, where further exertion is required for successful colonization. Additionally, flight triggers the activation of specific metabolic pathways, leading to the upregulation of enzymes involved in tolerating monoterpene, energy metabolism and recovery (see review paper in Chiu et al. 2022).
- **Rationale:** Previous studies show that flight in bark beetles, including *D. ponderosae*, leads to increased energy consumption, indicating a physiological cost of dispersal (Evenden et al. 2014). Flight is an energetically demanding activity that requires the engagement of muscles and the activation of metabolic pathways responsible for energy production and recovery.

Chapter 3. Objective 1. Effect of Winter and cold Duration on Metabolic Rate and Respiratory Patterns

- **Hypothesis:** Prolonged severe winter conditions reduce subsequent metabolic rates of *D. ponderosae*. They lower their metabolic rate to conserve energy during overwintering, limiting the use of stored reserves. This reduction is crucial for survival in cold environments, optimizing energy use and potentially facilitating synchronized mass attack behavior post-winter.
- **Prediction:** Beetles exposed to the severe winter conditions and longer cold periods will exhibit lower subsequent metabolic rates than those exposed to the cooler, and shorter cold durations.
- **Rationale:** Studies have shown that prolonged cold exposure leads to suppressed metabolic activity in *D. ponderosae* (Lester & Irwin, 2012), which aligns with energy conservation strategies common in overwintering insects (Bale & Hayward, 2010; Chown et al. 2006). Additionally, metabolic suppression may contribute to the synchronization of emergence post-winter, which is critical for mass attack behavior in bark beetles (Bentz et al. 2014). However, no studies have yet examined how winter conditions affect subsequent adult metabolic rates and whether this plays a role in synchronized emergence and collective host colonization efforts.

Chapter 3. Objective 2: Effect of Winter Conditions on Flight Capacity

- **Hypothesis**: Winter conditions reduce flight capacity by depleting energy reserves through prolonged cold exposure, reducing lipid stores essential for flight. Harsh conditions can cause weight loss, limiting flight ability while facilitating synchronized emergence (Bentz et al. 2014) .
- **Predictions**: (1) beetles with higher pre-flight weight will have a greater propensity to fly and will fly farther than lighter beetles, and (2) harsh winter conditions will negatively affect subsequent flight capacities.
- **Rationale:** Studies on *D. ponderosae* indicate that energy reserves and body mass, influenced by winter conditions, play a key role in flight capacity. McCambridge (1971) found that beetles exposed to colder conditions exhibited reduced flight ability, while Jones et al. (2019) demonstrated the importance of body mass in determining flight distance and propensity in bark beetles. Harsh winters may drain energy reserves, thus limiting the beetles’ flight capacity, but also create strong temperature cues that synchronize beetle emergence, facilitating coordinated dispersal and host colonization despite reduced individual flight performance.

Chapter 3. Objective 3: Effect of Winter Conditions on Host Colonization Behavior

- **Hypothesis:** Winter conditions affect host entry rates, as prolonged cold exposure reduces energy reserves and physiological fitness, making it more challenging for beetles to perform demanding tasks like boring into trees. Synchronization of emergence, influenced by winter conditions, also affects collective host colonization behavior.
- **Predictions:** (1) Beetles with higher body weights will show higher rates of host entry, and (2) longer overwintering durations will negatively affect host entry success, but synchronized emergence may partially reduce the effects of depleted energy reserves.
- **Rationale:** Beetles with higher body weights are more successful at colonizing hosts (Bentz & Powell, 2014). Ullah et al. (2024) shows that cold stress can significantly alter energy metabolism and reduce physiological performance in insects. Harsh winter conditions may lead to reduced individual performance due to lower energy reserves, impairing the beetles' ability to bore into trees.

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Chapter 2: Effect of flight activity on subsequent metabolism of adult *Dendroctonus ponderosae*

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2.1 Abstract

The mountain pine beetle (*Dendroctonus ponderosae*) is a huge threat to pine forests in North America, particularly as it has expanded into Alberta, encountering novel environmental conditions and pine hosts. This study investigates how flight activity affects the post-flight metabolic rate of adult *D. ponderosae* using respirometry. Beetles were subjected to different energy manipulation treatments (flown, room, fridge) and post-flight VCO_2 levels were measured. Results showed that flight activity did not significantly alter VCO_2 levels, but body weight positively correlated with metabolic rate. Beetles using discontinuous gas exchange had significantly higher VCO_2 levels than those using continuous respiration during the period of measurement. Increased metabolic cost of transport (MCOT) resulted in lower post-flight metabolic rates, indicating an energy trade-off. We found that beetles employing cyclic or discontinuous respiration exhibited higher post-flight metabolic rates compared to those with continuous respiration. These findings highlight the critical role of body weight, respiratory strategies, and energy expenditure in shaping the dispersal potential and ecological success of *D. ponderosae* under current environmental conditions.

Key words: *Dendroctonus ponderosae*, post-flight metabolic rates, range expansion, energy expenditure, flow-through respirometry, respiratory pattern.

2.2 Introduction

The relationship between flight and metabolism in insects is a well-studied area, revealing intricate physiological adaptations that support the energetic demands of flight. Initiating a flight is one of the most energetically demanding behaviors, requiring substantial energy expenditure derived from mobilization of stored carbohydrates and lipids which are oxidized to fuel muscle activity during flight (Jutsum & Goldsworthy, 1976). Flight exercise significantly influences the metabolic rate in various insect species. In the fruit fly (*Drosophila melanogaster*), flight activity increases metabolic rate substantially, with elevated levels of oxygen consumption and carbon dioxide production during and immediately following flight (Privalova et al. 2021). This heightened metabolic state is necessary to meet the energy demands of sustained flight, which can be up to 50-100 times higher than resting metabolic rates. In addition to the immediate metabolic demands of flight, flight exercise also has longer-term effects on subsequent metabolism in insects. In honeybees (*Apis mellifera*), repeated flight activity induces changes in the expression of genes associated with energy metabolism, enhancing the efficiency of energy production pathways (Hunt 2007; Margotta et al. 2012). Similarly, in locusts (*Locusta migratoria*), prolonged flight can lead to an increase in mitochondrial density and activity in flight muscles, improving overall metabolic capacity (Suarez & Moyes, 1992).

The mountain pine beetle, *Dendroctonus ponderosae* (Hopkins), is a major forest disturbance agent that not only affects timber production but also plays a significant ecological role in pine forests in western North America (Romme et al. 1986; Fettig & Hilszczański, 2015). This tree-killing bark beetle causes extensive damage during the outbreak population phase when healthy trees are colonized by mass-attack and subsequent phloem feeding by the larval brood contributes to the death of mature trees (Safranyik et al. 2010). The successful spread and persistence of epidemic populations of *D. ponderosae* depends on its capacity to execute well-timed flights and effectively colonize hosts through mass attack (Raffa & Berryman, 1982; Raffa & Berrymanm, 1983; Carroll & Safranyik, 2003; Roth et al. 2018; Wijerathna & Evenden, 2019). The north-south distribution of *D. ponderosae* is influenced by a combination of climatic conditions, host tree availability, and the beetle's physiological adaptations (Carroll & Safranyik, 2003). In the northern range, colder temperatures historically limited *D. ponderosae* survival and reproduction; however, with climate change, warmer winters and longer growing seasons have enabled their expansion into previously inhospitable northern areas. In the southern range, warmer temperatures allow for faster development and potentially multiple generations per year, increasing population densities (Carroll & Safranyik, 2003). The availability of suitable host trees, such as lodgepole pines in the north and other pine species in the south, also plays a critical role in determining the extent and success of *D. ponderosae* infestations across their distribution. These factors together shape the *D. ponderosae*'s capacity to expand and establish in different regions.

In the early 2000s, *D. ponderosae* expanded its range across the Rocky Mountains eastward into Alberta. It now faces a new set of challenges of different climatic conditions and novel pine hosts (Esch et al. 2016). Range expansion into pine forests east of the Rocky Mountains has revealed the importance of beetle body condition for dispersal and survival in the new range (Latty & Reid, 2010; Chubaty et al. 2014; Jones et al. 2020). Energy used by *D. ponderosae* during flight is crucial for their dispersal and ecological impact. Wijerathna & Evenden (2019), found that dispersal flights demand substantial energy, primarily derived from lipid reserves. This balance between flight energy expenditure and resource conservation is vital for their survival and reproduction. Jones et al. (2020) explored flight polyphenisms in *D. ponderosae*, showing that beetles with varying flight capabilities exhibit distinct mate finding and host colonization strategies based on energetic state. This adaptability enhances their ability to exploit various habitats and contributes to population spread on the landscape. Environmental factors significantly impact flight performance (Wijerathna et al. 2020). Optimal temperatures enable flight, while extreme conditions impair flight capacity (Shepherd 1966; McCambridge 1971; Safranyik 1978). Morphological variation of *D. ponderosae* also affects flight capacity, as beetles with larger flight muscles have superior flight performance. For example, research shows that beetles with proportionally larger flight muscles relative to their body size exhibit improved flight performance, including longer flight durations and better maneuverability. This is because the increased muscle mass allows for more powerful wing strokes and better energy utilization during flight (Chari et al. 2021). These morphological and physiological traits determine the flight capacity of *D. ponderosae* and their ability to invade new territories and colonize new hosts.

Dendroctonus ponderosae show a trade-off between flight and colonization of well-defended tree hosts. Energy expended during flight limits beetle potential to overcome host defenses and establish in pine trees (Jones et al. 2020). When beetles have sufficient energy left after flying to enter hosts, those with the lowest energy reserves are the first to do so, indicating their urgency and inability to disperse further (Latty & Reid, 2010; Jones et al. 2020). Physiological condition, which includes factors like energy reserves and hydration, plays a key role in the beetles' ability to overcome host monoterpene defenses, with beetles in better condition being more likely to survive monoterpene exposure (Reid & Purcell, 2011). Larger beetles lose proportionally less mass during this exposure period (Reid et al. 2017). Flight-related energy expenditure also has repercussions on subsequent reproduction (Wijerathna et al. 2019). Beetle physiological condition influences offspring egg size, which decreases with the body condition of the parent beetle (Elkin & Reid, 2005).

The primary objective of this experiment is to determine how energy expenditure through flight activity influences the subsequent metabolic rate of adult *D. ponderosae*. This involves measuring both the overall metabolic rate during the respirometry test and identifying specific respiratory patterns, such as continuous and discontinuous gas exchange. I test the hypothesis that flight activity significantly increases the metabolic rate of *D. ponderosae* post-flight. This is based on the understanding that flight is an energy-intensive activity, requiring substantial energy expenditure, which should be reflected in elevated metabolic rates afterward. I predict that beetles that engage in flight will exhibit higher metabolic rates compared to those that do not fly, as their energy demands during flight should lead to increased post-flight metabolic activity.

Despite the extensive research on the physiological adaptations of *D. ponderosae*, the specific effects of flight on post-flight metabolism remain poorly understood. Previous studies have focused on flight capacity and immediate energy use (Evenden et al. 2014; Jones et al. 2020), but little is known about how flight affects long-term metabolic recovery and respiratory strategies. This study assesses this gap by investigating the effects of flight on post-flight metabolic rates and gas exchange patterns, contributing to a better understanding of the trade-offs in energy expenditure that may influence dispersal and range expansion.

2.3 Methods & Materials

Specimens and Study system

In September 2021, a total of three lodgepole pine trees infested by *D. ponderosae* were felled at two sites in Alberta, Canada: one tree in Hinton (53.5096000°N, -117.2661760°E) and two trees in Cynthia (53°16’58”N, 115°25’22”W). Twelve 1-m bolts from the Hinton site and eleven 1-m bolts from the Cynthia site, harvested 1 m above the soil surface from each tree, were cut into two 50-cm sections. These bolts were transported to the University of Alberta, where the cut ends were sealed with paraffin wax (Parowax®) to prevent desiccation. Bolts were stored at 5°C for ~ 30 days until use in October 2021 (Figure 2.1).

At the initiation of trials, bolts were removed from cold storage and placed in 121 L emergence bins equipped with a glass jar to collect emerging beetles. The emergence bins were kept at a constant temperature of 21°C with a light cycle of 16 h of light followed by 8 h of darkness. Bolts were monitored daily for beetle emergence and adult beetles were collected starting on 18 October 2021.

Energetic manipulation treatments

The energetic manipulation treatments were based on the methods outlined in Evenden et al. (2014). Emerging beetles were collected daily, sorted by sex, labeled, and placed in 1.5 mL microcentrifuge tubes containing a small strip of paper (Evenden et al. 2014). They were stored at 4°C with controlled humidity to prevent desiccation until used in the bioassay, 3-5 days after emerging from the bolt. Beetles were separated by sex (McCambridge 1962; Michael & Rudinsky, 1972; Fleming et al.

2013) and weighed to the nearest 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, Ohio). Beetles were randomly assigned to one of three energetic manipulation treatments by placing the entire group into a Petri dish, from which beetles were arbitrarily selected without consideration of factors such as sex, color, or body size, ensuring an unbiased sample for the experiment.

The energetic manipulation treatments include:

(1) **Flown**: those that were flown on the flight mills for 23 h.

(2) **Room**: control beetles that were held individually in 1.5 mL microcentrifuge tubes in the flight mill room for 23 h, but not given the opportunity to fly.

(3) **Fridge**: beetles held individually in 1.5 mL microcentrifuge tubes in a refrigerator maintained at 4°C to minimize activity and energy expended.

Two to 22 beetles were flown per day. Flown beetles were tethered with 0.3 mm aluminum wire tethers with a small loop at the end, and a 2 cm straight wire segment oriented perpendicular to the loop using Press-Tite Contact Cement (LePage, Mississauga, Ontario) so that elytra movement was not restricted. Tethered beetles were carefully transferred to a dedicated flight mill room, specifically designed to control environmental conditions conducive to flight trials. In this room, the ambient temperature was consistently maintained at 24°C to simulate optimal conditions for beetle activity. Additionally, the room followed a light-dark cycle of 16 h of light and 8 h of darkness (16:8 L:D. These controlled conditions ensured that the beetles' flight behavior was observed under standardized and ecologically relevant settings. Beetles were individually secured to flight mills by inserting the tether into a small piece of wire insulation at the distal end of each flight mill arm, positioned at ~ 100° from the axis of the flight mill arm. Flight treatments started at 4 h into the photoperiod and lasted 23 h. Control beetles were kept with a piece of paper in perforated 1.5 mL microcentrifuge tubes in the flight mill room during the treatment period. Light in the flight mill room was provided by high flicker frequency fluorescent lights during the photophase (550 lux) and no light (0 lux) during the scotophase (Evenden et al. 2014). A small magnetic transmitter, located on the flight mill arm, detected the arm's rotation driven by the flight of the beetles. This transmitter sent the signal to the connected computer system. LabView software (National Instruments Corporation, Austin, TX, USA) measured each revolution of the flight mill arm, which had a circumference of 94.4 cm. The recorded data included the duration of flight and the number of revolutions completed during each flight burst initiated by the beetles. Key flight parameters such as total flight distance, flight duration, flight velocity, and the number of flight bursts were computed from this recorded information.

Following the 23 h treatment, tethers were removed from flown beetles. All beetles, including those from the flown, room, and fridge treatments, were weighed to the nearest 0.01 mg to determine post-treatment weight loss. Beetles that became detached from tethers during the flight treatment were excluded from subsequent bioassays and statistical analyses.

Respirometry Setup

Fifteen minutes after the energy manipulation treatment, beetles that were still alive as was determined by movement of tarsi following stimulation, were placed in respirometry chambers to measure their metabolic rate. Why 15 mintues? The 15-minute delay was chosen to allow for a brief recovery period after treatment to minimize stress without impacting the metabolic measurements. Beetle metabolism (VCO_2 in µL/min) was measured using the Multiple Animal Versatile Energetics Flow Through (MAVEn-FT) system (Sable Systems International, Las Vegas, NV) following the method described by Horn et al. (2018), and Brophy & Luong (2021). The respirometry trials were conducted between 11:00 AM and 12:30 PM each experimental day to ensure consistent timing, accounting for natural daily variations. In each respirometry trial, beetles were randomly selected by placing the entire group into a Petri dish, and then 3 to 10 beetles were arbitrarily chosen without considering factors like sex, color, or body size, ensuring an unbiased sample for the experiment. They were placed individually in separate chambers (46 × 7 mm diameter) and were allowed to freely move about the activity chamber. In our respirometry setup, a lab-grade compressed air cylinder equipped with a series of filters provided a constant flow of clean, dry air to the chambers where beetles were placed. The air was filtered to remove moisture and CO_2, ensuring accurate measurement of the beetle’s metabolic rate (Lighton 2008). After passing through the respirometry chamber, the air was analyzed for changes in CO_2 concentration, which reflects the beetle's metabolic activity. The airflow rate was controlled to ensure a stable environment inside the chamber, preventing any external fluctuations from affecting the measurements. Respiration data was automatically recorded by the system for 2 min from the baseline chamber, 7 min from the experimental chamber, followed by another 2 min of baseline; throughout the flow rate was 30 mL/sec (Lighton 2008). Zeroing in respirometry was done by running the clean, filtered air through the system without any beetles in the chamber and recording the baseline CO_2 and O_2 levels. This ensures that any detected changes in gas concentrations after introducing the beetle are solely due to the beetle's metabolic activity. The average behavioral activity of each beetle within the chamber was simultaneously monitored alongside its metabolic rate using the MAVEn-FT system. This system allows for precise tracking of both physiological and behavioral responses during the experiment. The MAVEn-FT system’s activity monitor is equipped with a proximity detector that operates by emitting a wide cone of infrared light, which is reflected off the beetle's body. The system then detects the intensity and frequency of the reflected light to determine the beetle's movements. All respirometry data transformation and calculations were performed in the Expedata-P Software (V1.9.27, Sable Systems International, Las Vegas, NV). The respirometry measurements in this study focus on Standard Metabolic Rate (SMR) rather than Basal Metabolic Rate (BMR) (Chown & Nicolson, 2004; Lighton 2008).

Post-flight Metabolic Rates

The rate of CO_2 production (µL/min) was calculated using the formula:

$$VCO_2 = FR_i \ (F'_eCO_2 - F_iCO_2)$$

Here, VCO_2 stands for the rate of carbon dioxide production (Lighton 2008), FR_i is the flow rate of incoming air, and F'_eCO_2 and F_iCO_2 are excurrent fractional concentration and incurrent fractional concentration of CO_2, respectively. The excurrent fractional concentration of CO_2 is equal to the experimental chamber measurement minus the baseline measurement. Water vapor was dropped from calculations as inflow and outflow water vapor was scrubbed, and hence equals zero. Since the incoming air was scrubbed of CO_2 ($F_iCO_2 = 0$) (Lighton 2008), the formula was further converted for convenience:

$$VCO_2 = FR_i \times F'_eCO_2$$

Measuring post-flight metabolic rate (MR) is important because it offers insights into the physiological costs of flight in insects (Jenni-Eiermann, 2017; Darveau, 2024). While it may seem equivalent to a recovery period in my study, post-flight MR provides valuable data on the extent of energy depletion and how long it takes for the beetle to return to baseline metabolic rates. Studies like those by Giladi & Pinshow (1999, on pigeons) and Guglielmo et al. (2017, on yellow-rumped warblers) show that energy demands from flight extend into recovery periods, highlighting the need to measure post-flight MR to fully assess the impact of flight. This helps find out the energetic investment in flight, assesses the beetles' ability to replenish energy reserves, and understand how flight impacts subsequent behaviors like respiration. Post-flight MR can also be indicative of how well insects can recover, which is especially relevant in *D. ponderosae* where flight is essential prior to mass attack and reproduction.

Given the importance of body weight on the beetles' subsequent metabolic rate (Figure 2.4), we included mass-specific VCO_2 (µL/min/mg) as a parameter to analyze the results. The formula is:

$$\text{Mass-specific } VCO_2 = \frac{V\,CO2}{pre-respirometry\;body\;weight}$$

The Gas Exchange Patterns

Based on the measurement of VCO_2, beetles were divided into groups based on their respiratory pattern (Lighton, 1996). Beetles that exhibited continuous gas exchange were characterized by a steady release of CO_2 throughout their activity cycles. In contrast, beetles conducting discontinuous gas exchange showed intermittent bursts of CO_2 release, where periods of zero gas exchange could be observed within a single breathing cycle.

The gas exchange patterns were categorized based on the shape and timing of CO_2 peaks (refer to Figures 2.8A & 8B) using ExpeData software (V1.9.27, Sable Systems International, Las Vegas, NV). To get the rates of each gas exchange pattern, we used Expedata-P Data Analysis Software (V1.9.27, Sable Systems International, Las Vegas, NV. Website: https://www.sablesys.com), which processes the CO_2 data collected from respirometry trials from the MAVEn-FT chambers. We identified and quantified the distinct phases of gas exchange—closed, flutter, and open for discontinuous respiration, and continuous CO_2 output for the continuous pattern—using Expedata-P. A detailed method for differentiating these gas exchange patterns visually is based on the approach described by Lighton (1996). By analyzing the timing and amplitude of CO_2 peaks, Expedata-P provides direct measurements of the rate at which CO_2 is released during each trial, giving metabolic rate estimates for both gas exchange patterns.

The Metabolic Cost of Transport (MCOT)

Investigating the relationship between metabolic rate (VCO_2) and Cost of Transport (MCOT) in *D. ponderosae* is crucial for understanding how efficiently these beetles use energy during movement, which influences their dispersal capacity and overall fitness in varying environmental conditions. To investigate the relationship between the VCO_2 and the MCOT in *D. ponderosae*, I calculated the MCOT for each beetle using the formula:

$$\text{MCOT} = \frac{Percentage\ weight\ loss\,(\%)}{Distance\,(km)}$$

This calculation resulted in a COT value representing the percentage of body weight lost per kilometer flown.

Data analyses

All statistical analyses were conducted in R (R Core Team 2023) using RStudio (RStudio Team 2023). Data processing and sorting were carried out using packages from the *tidyverse* collection (Wickham & Grolemund, 2016; Wickham et al. 2019). To evaluate the statistical model fit, I used the *DHARMa* package (*simulateResiduals*; Hartig 2022) to create scaled residuals and q-q plots. Additionally, I conducted the Kolmogorov-Smirnov test for overdispersion, the outlier test, and the Shapiro-Wilk test to assess the normality of residuals and determine if model assumptions were met. Final models were compared to a null model using likelihood ratio tests, and only those models that showed significant differences from the null model were interpreted. Model summaries included *Chi*-squared test and adjusted R^2 values for the overall models, and ANOVA tables were generated using the *car* package (Fox & Weisberg 2019). Figures displaying raw data and fitted values of linear models were created using the *visreg* (Breheny & Burchett 2017) and *ggplot2* (Wickham 2016) packages. To examine the relationship between beetle weight and VCO_2, I generated predicted values using the *ggpredict* function (Lüdecke 2018) from the *ggeffects* package and visualized the results with *ggplot2* (Wickham 2016), displaying the predicted values alongside the actual data points with jitter and custom theme settings.

To analyze the effect of flight through the energetic manipulation treatments on mass-adjusted VCO_2 levels, I used linear mixed-effects models (LMMs) implemented with the *lme4* package (version 1.1-27.1) (Table 2.2). Panel A of Table 2.2 presents general data on VCO_2 levels in relation to treatment, weight, and activity, while Panel B provides a comparative analysis of mass-specific VCO_2, flight distance, weight loss, and the metabolic cost of transport (MCOT), highlighting significant findings and interaction effects. Random effects for bolt nested within tree were included to account for the hierarchical structure of the data. I examined the effect of energy manipulation treatments (control, room, fridge) and body weight on VCO_2 by fitting a linear mixed-effects model. The effect of continuous vs. discontinuous respiratory patterns on VCO_2 was measured using a linear mixed-effects model with random effects for tree/bolt and chamber. Tree/bolt and flight mill were included as random effects to account for variability in factors like differences between trees (e.g., nutrient content) or flight mills (e.g., calibration), which could affect beetle performance unpredictably but are not central to the study. ANOVA analyses were conducted to evaluate the overall significance of the model terms if the model showed a good fit according to the residual diagnostics. To test the effect of flight distance, duration, and average speed on VCO_2 levels, I fit linear mixed-effects models where the VCO_2 was the dependent variable and flight distance, duration and average speed, weight, were independent variables in separate models that included random effects for tree/bolt and flight mill (Table 2.2). The residual diagnostics confirmed a satisfactory fit for only the flight duration. An ANOVA analysis was conducted to assess the overall significance of the model terms. All predicted values of the linear mixed models and raw data were plotted using *ggpredict* (Lüdecke 2018) and *ggplot2* . All plots were generated using *ggplot2* (version 3.3.5) for clear visualization of the results. Statistical significance was determined at a p-value threshold of 0.05.

For the metabolic cost of transport MCOT after beetle’s flight, Initially, I observed that some beetles had extremely high MCOT values, including several infinite values due to a division by zero where the total distance flown was zero. To address this, I performed the following steps: (1) removal of infinite values: I filtered out rows with infinite MCOT values to ensure that all observations had finite, meaningful MCOT values for subsequent analysis. This step was essential to avoid skewing the results with extreme or non-informative values; (2) exploration of MCOT distribution: I plotted a histogram of the MCOT values to explore their distribution. The histogram revealed that MCOT values were highly skewed, with most data clustered near the lower end of the scale; (3) log transformation: to normalize the distribution and reduce skewness, I applied a log transformation to the MCOT values. A small constant (0.001) was added to avoid taking the logarithm of zero. The transformed data (log_mcot) was then plotted, showing a more normally distributed set of values.

To assess the relationship between VCO_2 and MCOT, I fitted a linear mixed-effects model using the *lmer* function in R. The model included log_mcot as the fixed effect and random intercepts for both mill and the nested effect of tree/bolt, accounting for the hierarchical structure of the data. I assessed the model fit by simulating residuals and using diagnostic plots, which indicated that the model fit the data well. An analysis of variance (*ANOVA*) was performed on the model to evaluate the significance of the fixed effect. To visualize the relationship between VCO_2 and MCOT, I generated predicted values using the *ggpredict* function, focusing on the log_mcot variable. These predictions, along with the 95% confidence intervals, were plotted against the observed data. The resulting plot displayed the predicted VCO_2 values as a function of the log transformed MCOT, with a shaded ribbon representing the 95% confidence interval. The observed data points were overlaid on the plot, showing a clear negative relationship between MCOT and VCO_2. To maintain consistency, all significant figures are reported to three decimal places throughout the thesis.

2.4 Results

All results are summarized in Figure 2.7, which presents a proposed cause-and-effect map illustrating the relationships between the variables.

The energy manipulation treatment (flown, room, or fridge) did not significantly affect VCO_2 levels ($X^2 = 2.981$, df = 2, $p = 0.225$). In the flown group, post-flight VCO_2 levels ranged from a minimum of 0.311 µL/min to a maximum of 19.6 µL/min. The fridge group exhibited a broader variation, with VCO_2 levels between 0.272 µL/min and 34.6 µL/min. In contrast, the room group recorded the lowest overall values, ranging from 0.130 µL/min to 17.8 µL/min.

The test examining the effect of flight treatment on the subsequent mass-specific VCO_2 was not statistically significant ($X^2 = 4.228$, df = 2, $p = 0.121$; Table 3.2). The lack of significant difference in VCO_2 levels across flight treatments suggests that metabolic recovery may occur rapidly after flight or that energy use is more efficiently managed than previously thought.

Body weight had a highly significant impact on VCO_2 levels ($X^2 = 18.708$, $p < 0.001$), indicating that larger beetles require more energy to sustain metabolic functions. VCO_2 levels significantly increased with body weight (Fig. 2.4). However, the interaction between energy manipulation treatment and weight was not significant ($X^2 = 0.101$, df = 2, $p = 0.951$).

Independent of flight impact, we found that the beetle's respiratory pattern significantly influenced VCO_2 levels ($X^2 = 5.831$, df = 1, $p = 0.016$). Beetles with the cyclic/discontinuous respiration pattern showed higher VCO_2 compared to those with the continuous pattern (Fig. 2.5). We also found a statistically significant effect of respiratory pattern on mass-specific VCO_2 ($X^2 = 4.00$, df = 1, $p = 0.045$). However, the interaction effect between energy manipulation treatment, respiratory pattern, and beetle weight on subsequent metabolic rates was not statistically significant ($X^2 = 0.160$, df = 1, $p = 0.691$).

We revealed a significant negative relationship between the metabolic cost of transport (MCOT) and VCO_2 levels in *D. ponderosae* ($\chi^2 = 9.536$, $p = 0.002$). As MCOT increased, beetle VCO_2 levels decreased (Estimate = -1.066), indicating that higher energy demands for transport lead to reduced metabolic rates (Figure 2.6).

The flight duration does not significantly impact VCO_2 levels in beetles ($X^2 = 1.555$, $p = 0.212$). The interaction effect between total duration and weight was not significant ($X^2 = 0.057$, $p = 0.811$).

2.5 Discussion

My study investigated the impact of flight treatment on the metabolic rate of *D . ponderosae* by measuring VCO_2 levels after flight to determine how energy demands during flight influence metabolic performance afterwards. The results showed that flight treatment (whether the beetles had flown, were kept at room temperature, or were stored in a fridge) did not significantly affect subsequent VCO_2 levels. Regardless of whether the beetles experienced active flight or were kept in different environmental conditions without flying, their subsequent metabolic rates, as measured by CO_2 production, remained similar. This result contrasts with prior studies that found significant increases in metabolic rates during flight, likely due to the substantial energy demands required for sustained flight (e.g., Lehmann et al. 2000). Other studies on the Colorado potato beetle (*Leptinotarsa decemlineata*) (Lebenzon et al. 2022), bumblebees (*Bombus terrestris*) (Combes et al. 2020), and fruit flies (*Drosophila melanogaster*) (Lehmann et al. 2000) all demonstrated a marked rise in metabolic rates associated with active flight. The absence of a significant post-flight increase in metabolic rates in our study suggests that beetle recovery may occur rapidly or that energy use is more efficiently managed after flight.

Overall, there is a positive effect of beetle body weight on VCO_2 levels, which supports previous studies suggesting a general relationship between body size and metabolic rate (Glazier 2005; White et al. 2006; Glazier 2008; McNab 2008; Speakman & Król, 2010). The significant effect of body weight on metabolic rate is consistent with findings from other insect species, such as locusts and hornworms, where larger individuals have higher metabolic demands (Du et al. 2022; Messerman 2009). This suggests that in *D. ponderosae*, body size plays a critical role in determining energy use during flight and recovery. This also aligns with the principles of metabolic scaling theory, which posits that metabolic rates scale positively with body size across a wide range of taxa (West et al. 1997; Brown et al. 2004; White et al. 2009). Larger insects generally have higher absolute metabolic rates due to their greater muscle mass and energy demands associated with activity.

My study is the first to document two distinct gas exchange patterns in adult *D. ponderosae* following flight. As the environmental conditions were consistent throughout the respirometry trials, the presence of these two patterns could indicate flexible respiratory strategies in response to internal physiological factors, such as metabolic demand, hydration levels, or energy reserves. This aligns with findings across various insect taxa, where adaptive respiratory mechanisms are observed even under stable external conditions (Slama 1988; Lighton 1996; Marais et al. 2005; Chown et al. 2006). The cyclic, discontinuous gas exchange (DGE) pattern observed in my study optimizes gas exchange efficiency while minimizing water loss, particularly under conditions akin to those beetles experience when boring under bark. This pattern reflects adaptations for conserving water and sustaining metabolism at low levels during unfavorable conditions. DGE is characterized by three phases: the closed (C) phase, the flutter (F) phase, and the open (O) phase (Lighton 1996). During the closed phase, spiracles remain shut, preventing water loss. The flutter phase involves rapid opening and closing of the spiracles, allowing minimal gas exchange with reduced water loss. Finally, the open phase permits the expulsion of accumulated carbon dioxide. This respiratory strategy is particularly beneficial for insects living in arid environments or those that experience fluctuating humidity levels, as it helps in balancing the need for oxygen with the risk of desiccation (Hadley 1994). Studies on other beetles, such as tenebrionid beetles (Hadley 1994) and weevils (Marais et al. 2005), have demonstrated similar gas exchange patterns that enhance survival in dry and hypoxic habitats. These beetles exhibit reduced metabolic rates and increased efficiency in water conservation through cyclic respiration. Furthermore, the DGE pattern occurs in other insects, suggesting its widespread evolutionary significance. For example, cockroaches (Abbas et al. 2020) and fire ants (Ko et al. 2022) use DGE to manage water loss while maintaining efficient gas exchange. In cockroaches, DGE is thought to be an adaptation to their often dry and variable environments, allowing them to thrive in habitats with limited water availability. Similarly, fire ants utilize DGE to balance high metabolic demands with the need to conserve water, particularly in arid environments. For *D. ponderosae,* living under the bark of trees comes with challenges like low oxygen levels and the need to conserve water. Although the lab conditions in my study are not the same as the beetles' natural environment, the discontinuous gas exchange (DGE) pattern I observed likely reflects an adaptation to low-oxygen conditions. This means that even in more controlled lab settings, the beetles still show flexible breathing strategies. This supports the idea that they have developed physiological adaptations to survive in their natural habitat, where oxygen is limited, and breathing strategies must be carefully managed.

The higher CO_2 rates observed in *D. ponderosae* during DGE can be explained by the accumulation of CO_2 during the closed phase, followed by its rapid expulsion during the open phase (Lighton 1996). This intermittent release results in higher peaks of CO_2 output compared to continuous gas exchange. Water loss might not be a major problem for *D. ponderosae*, but the observed respiratory pattern could still be useful. By reducing water loss during the closed and flutter phases, the beetles conserve moisture in dry conditions while still efficiently expelling CO_2 during the open phase. This dual function not only helps them adapt to environments like the subcortical spaces under tree bark, where maintaining moisture balance is crucial, but also ensures CO_2 does not reach toxic levels within their body, which is vital for beetles living in desiccating conditions (Chown et al. 2006). In contrast to DGE, continuous gas exchange involves a more steady and ongoing exchange of gasses through the spiracles (Lighton & Schilman, 2005). Beetles switch to continuous gas exchange when the environmental conditions are stable and conducive to regular gas exchange. For example, when beetles are not actively engaged in activities that require conservation of water or management of oxygen availability, they can sustain continuous gas exchange to support regular physiological functions and metabolic processes. However, my findings contradict much of the existing literature (Levy & Schneiderman, 1966; Lighton 1996; Chown et al. 2000; Hetz & Bradley, 2005), possibly due to the relatively short testing (7 minutes) or baseline period (2 minutes) used for each beetle individual in my study, which may not have been long enough for the beetles to fully reflect their natural respiratory patterns. Longer exposure times allow beetles to fully express their adaptive gas exchange strategies, such as switching from continuous gas exchange to DGE.

Research on the evolution of respiratory patterns in insects suggests that DGE might have evolved multiple times in response to ecological pressures (Chown et al. 2006). This indicates that such respiratory adaptations are not only widespread but also crucial for the survival of various insect species under challenging environmental conditions. For example, *Aphodius fossor* (Scarabaeidae) balances efficient respiration with the need to conserve water through the application of DGE, especially during periods of low metabolic activity, such as diapause, where water conservation is critical for survival (Chown & Holter, 2000; Bentz & Hansen, 2018). However, in my study, beetles were not subjected to such challenging environmental conditions. Instead, we tested their subsequent respiratory patterns following flight. Despite this, the presence of DGE in the post-flight period suggests that these beetles retain flexible respiratory strategies, possibly reflecting inherent adaptations that persist even under more controlled conditions.

A significant negative relationship was observed between the metabolic cost of transport (MCOT) after flight and subsequent VCO_2 levels in *D. ponderosae*, suggesting that beetles experiencing higher transport costs may reduce energy expenditure during recovery to conserve resources for future dispersal or host colonization. One key method of energy recovery in insects involves replenishing glycogen and lipid reserves (Ziegler and Van, 2006; Jones et al. 2019; Bretscher and O'Connor, 2020), which are used during flight to fuel muscle activity and sustain metabolism. After intense flight activity, beetles engage in a recovery process that includes replenishing these reserves, which may contribute to elevated metabolic rates. Additionally, muscle repair and recovery (Schilder and Marden, 2006) may play a crucial role in preparing beetles for post-flight activities such as host entry. Following intense flight, insects often engage in protein synthesis to repair muscle tissue damaged during exertion. This process is vital for maintaining muscle function and ensuring the beetles are physically prepared for subsequent tasks. The repair mechanisms help restore muscle strength and functionality, critical for the beetle's survival and overall fitness, especially when it comes to tasks like boring into host trees. Another important factor is hormonal regulation, particularly through the activation of specific enzymes (Chiu et al. 2022). In *D. ponderosae*, the Cytochrome P450 enzyme enables tolerance to monoterpenes, volatile organic compounds found in pine trees. These compounds can both attract and repel beetles, affecting colonization and survival (see review paper in Chiu et al. 2022). The activation of this enzyme allows beetles to tolerate monoterpenes and convert them into female aggregation pheromones after dispersal, facilitating successful host colonization. Together, these processes—energy recovery, muscle repair, and hormonal regulation

—likely influence the beetle's post-winter metabolism. In beetles with higher MCOT, potentially due to body weight loss during flight, these mechanisms may become particularly important for ensuring survival. However, the trade-off between energy conservation and recovery could impact the beetles' ability to invade new habitats, with those experiencing higher transport costs possibly facing reduced fitness for reproduction or further dispersal. In the context of *D. ponderosae* dispersal ecology, these findings suggest that beetles with higher MCOT may have less energy available for critical tasks like host entry, influencing population dynamics and range expansion. Further research is needed to explore how these energetic constraints vary with environmental conditions and how they affect long-term beetle survival and reproduction.

2.6 Conclusion

Overall, my study provides new insights into the relationship between flight activity and subsequent metabolic performance in adult *D. ponderosae*. Endergy expenditure treatment did not significantly affect post-flight VCO_2 levels, but body weight had a significant influence on metabolic rates, with larger beetles exhibiting higher subsequent VCO_2 levels. The observation of distinct respiratory patterns suggests that *D. ponderosae* employs flexible gas exchange strategies, which may be adaptations to conserve water or manage energy efficiently. Additionally, the negative relationship between the metabolic cost of transport (MCOT) and post-flight metabolic rates highlights an energy trade-off that may influence the beetles' dispersal capacity and success in colonizing new hosts. These findings have significant implications for understanding the dispersal potential of *D. ponderosae* in novel environments, particularly in the context of climate change-driven range expansion. The energy trade-offs observed in this study suggest that beetles with higher metabolic costs after flight may have reduced capacity for further dispersal or host colonization, which could impact the dynamics of population spread and forest infestation. Future research should explore how these metabolic trade-offs influence long-term survival and reproduction in *D. ponderosae*, particularly under different environmental conditions such as temperature extremes or variable food availability.

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Tables

Table 2.1. Sample size (n) distribution by treatment, sex, and respiration pattern in the beetle study.

Treat	Sex	Respiration Pattern	n
flown	Female	Continuous	26
		Cyclic/discontinuous	23
flown	Male	Continuous	31
		Cyclic/discontinuous	10
fridge	Female	Continuous	29
		Cyclic/discontinuous	27
fridge	Male	Continuous	17
		Cyclic/discontinuous	14
room	Female	Continuous	28
		Cyclic/discontinuous	14
room	Male	Continuous	26
		Cyclic/discontinuous	6

Table 2.2. Comparative analysis of factors influencing VCO_2 levels in beetles. Panel A summarizes general VCO_2 data based on treatment, weight, and activity, while Panel B focuses on mass-specific VCO_2, flight distance, weight loss, and metabolic cost of transport (MCOT). Significant findings and interaction effects are highlighted.

Panel A: General VCO_2: Treatment, Weight, and Activity

Question	Response variable	Fixed effects	Random	Diagnostics pass	Chi2, df, p-value
Does weight and flight treatment affect VCO_2 in beetles? Data = all beetles Treatment = room, fridge, flown	VCO_2	- Treatment - Weight (Fig.4) - Interaction	Bolt nested in tree, respirometry chamber	Yes	$X^2 = 2.981$, df = 2, p = 0.225 $X^2 = 18.708$, df = 1, p < 0.001* $X^2 = 0.101$, df = 2, p = 0.951
Does beetle flight treatment, weight, and activity in the respirometry chamber impact VCO_2? Data = all beetles Treatment = room, fridge, flown	VCO_2	- Treatment - Weight - Average Activity in chamber - Interactions	Bolt nested in tree, respirometry chamber	No	
Does beetle flight treatment, weight and respiratory pattern impact VCO_2 in beetles? Data = all beetles Treatment = room, fridge, flown	VCO_2	- Weight - Respiratory pattern (Fig. 5) - Interaction	Bolt nested in tree, respirometry chamber	Yes	$X^2 = 11.624$, df = 1, p = 0.0007 * $X^2 = 5.831$, df = 1, p = 0.0157 * $X^2 = 0.003$, df = 1, p = 0.958

Panel B: Mass-Specific VCO_2: Flight Distance, Weight Loss, MCOT

Question	Response variable	Fixed effects	Random	Diagnostics pass	Chi2, df, p-value
Does flight treatment affect VCO_2 in beetles? Data = all beetles Treatment = room, fridge, flown	Mass -specific VCO_2	- Flight treatment	Bolt nested in tree, respirometry chamber	Yes	$X^2 = 4.228$, df = 2, p = 0.121
Does beetle flight treatment, and activity in the respirometry chamber impact VCO_2? Data = all beetles Treatment = room, fridge, flown	Mass -specific VCO_2	- Average Activity in chamber	Bolt nested in tree, respirometry chamber	No Model highly violates the assumptions	
Does beetle respiratory pattern impact VCO_2 in beetles? Data = all beetles Treatment = room, fridge, flown	Mass -specific VCO_2	- Respiratory pattern	Bolt nested in tree, respirometry chamber	Yes	$X^2 = 4.00$, df = 1, p = 0.045*
Does the flight distance impact VCO_2 in beetles? Data = flew	Mass -specific VCO_2	- Flight distance	Bolt nested in tree, flight mill	Yes	$X^2 = 0.191$, df = 1, p = 0.662

Does the weight and flight distance impact VCO_2 in beetles? Data = flew	VCO_2	- Flight distance - Weight - Interaction	Bolt nested in tree, flight mill	No	
Does flight distance, and proportional weight loss impact VCO_2 in beetles? Data = flew	VCO_2	- Flight distance (Fig. 6) - Proportional Weight Loss (Fig. 6) - Interaction	Bolt nested in tree, flight mill	Yes	X^2 = 3.865, df = 1, p = 0.049* X^2 = 9.357, df = 1, p = 0.00421* X^2 = 13.551, df = 1, p = 0.0002*
Does flight distance and chamber activity impact VCO_2 in beetles? Data = flew	VCO_2	- Flight distance - Average activity in chamber - Weight - Interactions	Bolt nested in tree, flight mill	No	
Does flight distance and respiratory pattern impact VCO_2 in beetles? Data = flew	VCO_2	- Flight distance - Respiratory pattern - Weight - Interactions	Bolt nested in tree, flight mill	Yes	X^2 = 0.969, df = 1, p = 0.325 X^2 = 0.037, df = 1, p = 0.848 X^2 = 1.480, df = 1, p = 0.224 Interactions all non-sig
Does flight duration impact VCO_2 in beetles? Data = flew	VCO_2	- Flight duration - Weight - Interaction	Bolt nested in tree, flight mill	Yes	X^2 = 1.5553, df = 1, p = 0.2123 X^2 = 1.1636, df = 1, p = 0.2807 X^2 = 0.0571, df = 1, p = 0.8112
Does flown beetle flight velocity impact VCO_2 in beetles? Data = flew	VCO_2	- Flight average speed - Weight - Interaction	Bolt nested in tree, flight mill	No	
Does the metabolic cost of transportation (MCOT) impact VCO_2 in beetles? Data = flew	Log(VCO_2)	- MCOT	Bolt nested in tree, flight mill	Yes	X^2 = 11.813, df = 1, p = 0.0006 *
Does flight distance and chamber activity impact VCO_2 in beetles? Data = flew	Mass -specific VCO_2	- Flight distance - Average activity in chamber	Bolt nested in tree, flight mill	No	
Does flight distance and respiratory pattern impact VCO_2 in beetles? Data = flew	Mass -specific VCO_2	- Flight distance - Respiratory pattern - Interaction	Bolt nested in tree, flight mill	Yes	X^2 = 0.248, df = 1, p = 0.619 X^2 = 0.115, df = 1, p = 0.735 X^2 = 0.021, df = 1, p = 0.884
Does flight duration impact VCO_2 in beetles? Data = flew	Mass -specific VCO_2	- Flight duration	Bolt nested in tree, flight mill	No	
Does flown beetle flight velocity impact VCO_2 in beetles? Data = flew	Mass -specific VCO_2	- Flight average speed	Bolt nested in tree, flight mill	Yes	X^2 = 1.917, df = 1, p = 0.166

Table 2.3. Mixed-effects model results testing the effect of weight on VCO_2 in beetles, using both untransformed and log-transformed VCO_2. Weight was measured pre-respirometry, and random effects included bolt nested in tree and respirometry chamber. Model 1 (untransformed) shows a significant positive relationship, while models 2 and 3 (log-transformed) show no significant effects. Model 3 has the lowest AIC but fails diagnostic tests.

Question:	**Response variable**	**Fixed effects**	**Random**	**Diagnostics pass**	**Chi^2, df, p-value**	**AIC**
Does weight affect VCO_2 in beetles? Data = all beetles Treatment = room, fridge, flown Weight = pre-respirometry weight	VCO_2	Weight	Bolt nested in tree, respirometry chamber	Yes	$X^2 = 17.115$, df = 1, $p < 0.001$*	m1: 370.562
Does weight affect VCO_2 in beetles? Data = all beetles Treatment = room, fridge, flown Weight = pre-respirometry weight	Log (VCO_2)	Weight	Bolt nested in tree, respirometry chamber	No		m2: 179.543
Does weight affect VCO_2 in beetles? Data = all beetles Treatment = room, fridge, flown Weight = pre-respirometry weight	Log (VCO_2)	Log (Weight)	Bolt nested in tree, respirometry chamber	No		m3: 173.152

Figures

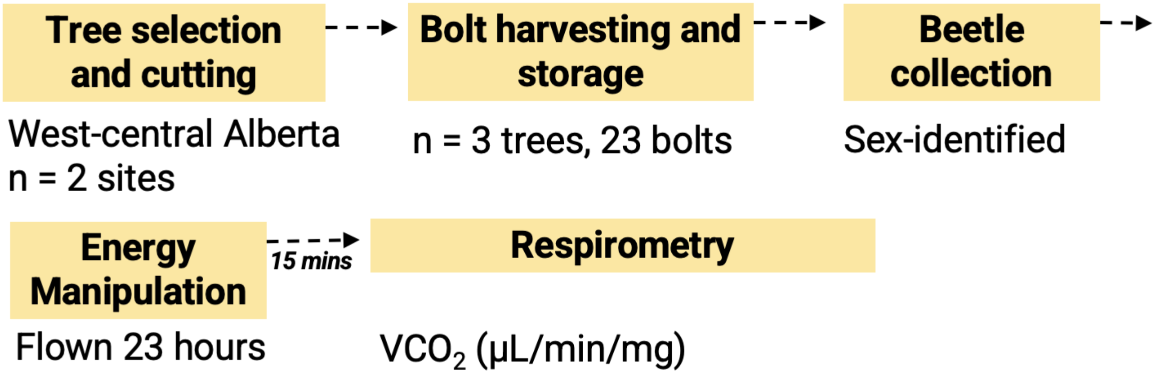

Figure 2.1. Flowchart outlining the sequential experimental procedures conducted in this study. Three *D. ponderosae*-infested lodgepole pine trees were selected from two sites in west-central Alberta and cut into 50 cm bolts. A total of 23 bolts were harvested and stored in a cold room at 5°C for a minimum of 30 days. Following storage, bolts were incubated at 21–22°C to allow beetle emergence. Emerging beetles were sex-identified and subjected to energy manipulation treatments, including flown trials lasting up to 23 hours. Beetle metabolic rates (VCO_2 in µL/min/mg) and respiratory patterns were then measured using MAVEn™, a Small Insect Flow-Through Respirometry system, to assess their physiological responses under varying energy conditions.

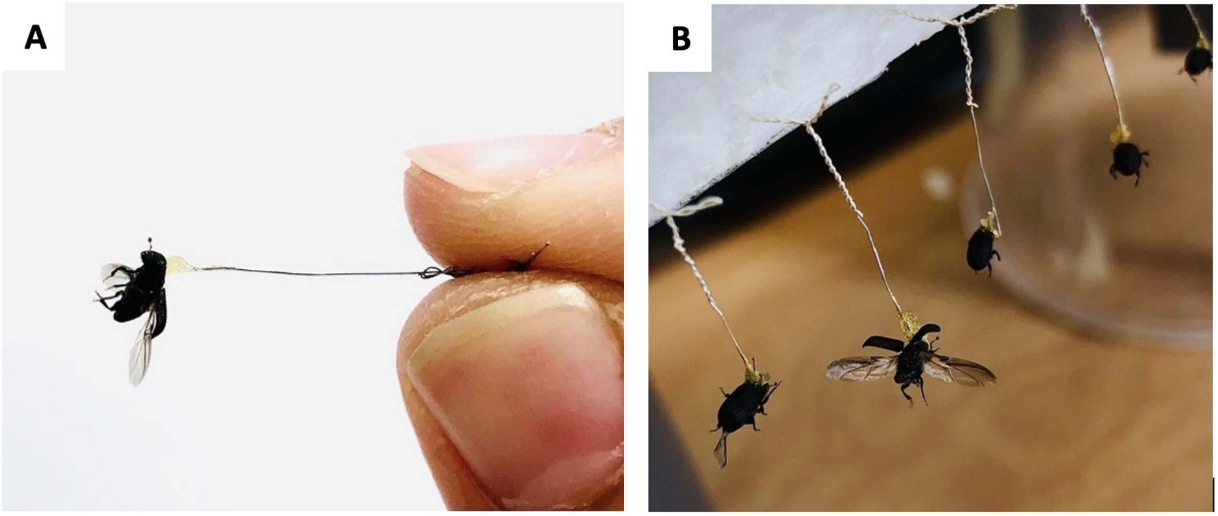

Figure 2.2. Mountain pine beetles with 0.3 mm aluminum wire tethers attached to the pronotum.

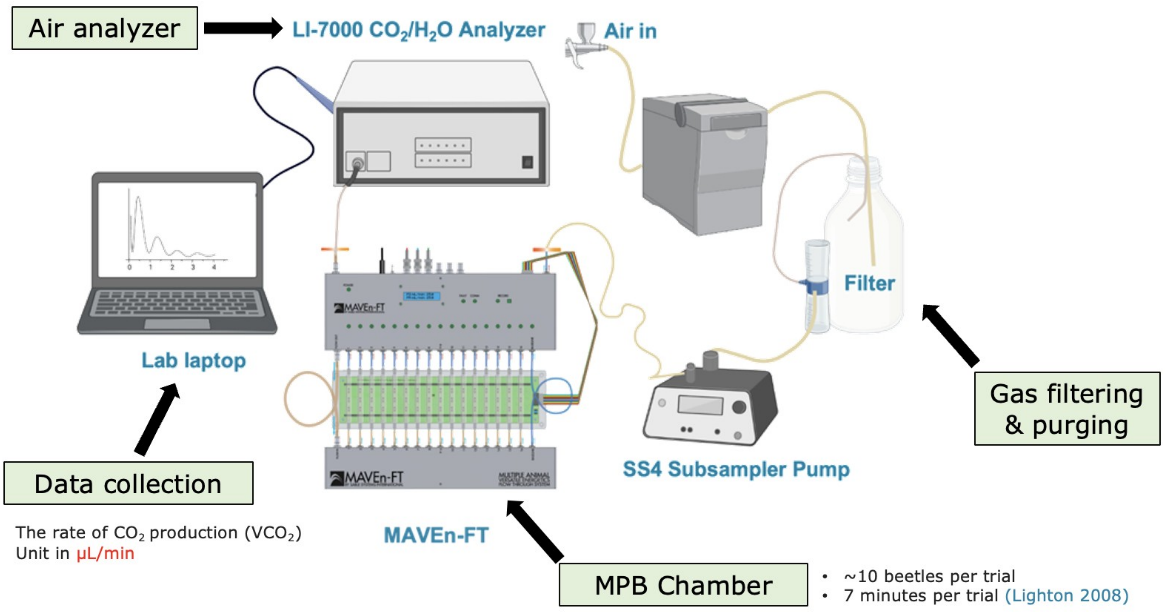

Figure 2.3. A flow-through respirometry setup designed to directly measure the metabolic rate and respiratory characteristics of tested beetles. The system includes a MAVEn-FT (Multiple Animal Versatile Energetics Flow-Through) system, which regulates airflow and houses the beetles during trials. Air is drawn into the system from a lab-grade compressed air cylinder, which supplies clean, dry air after passing through a gas filtration and moisture removal process. The air then flows through the respirometry chambers, where beetles are placed for measurement. An SS4 Subsampler Pump controls the airflow, ensuring consistent flow rates for accurate data collection. CO_2 concentrations in the outflow air are measured by an LI-7000 CO_2/H_2O Analyzer, which quantifies the beetles' metabolic rate (VCO_2) in µL/min. A filter is used to remove water vapor before the gas enters the analyzer. Data collected from the analyzer is processed and recorded on a laboratory laptop for further analysis. Each trial includes approximately 10 beetles, with a measurement duration of around 7 minutes per beetle (Lighton 2008).

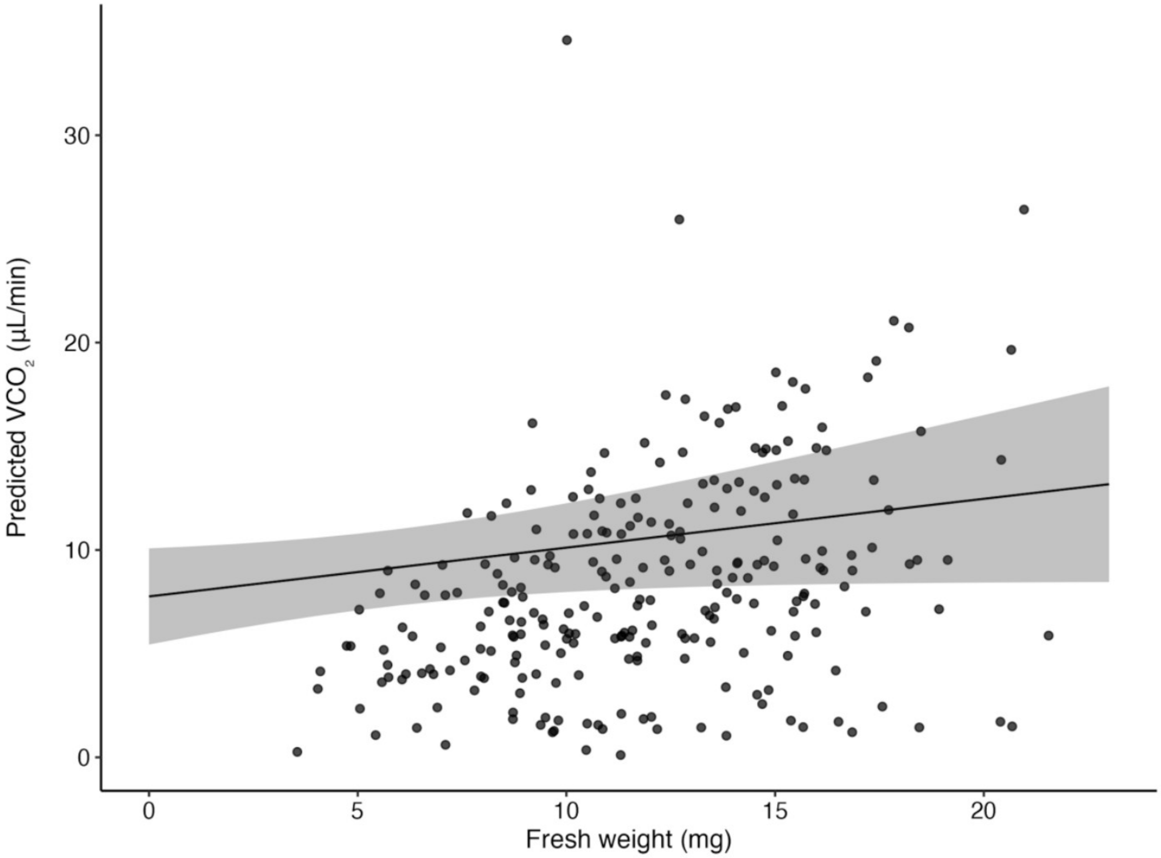

Figure 2.4. The relationship between beetle fresh weight (mg) and predicted metabolic rate (VCO_2, μL/min). The solid black line represents predicted values from the linear mixed-effects model, with actual data points jittered for clarity. The shaded area shows the 95% confidence interval. The analysis reveals a statistically significant positive relationship between beetle weight and metabolic rate ($X^2 = 18.708$, $p < 0.001$), indicating that heavier beetles exhibit higher VCO_2 levels. Model selection was based on diagnostic tests and AIC values, with model 1 (listed in Table 3) chosen as the best-fitting model.

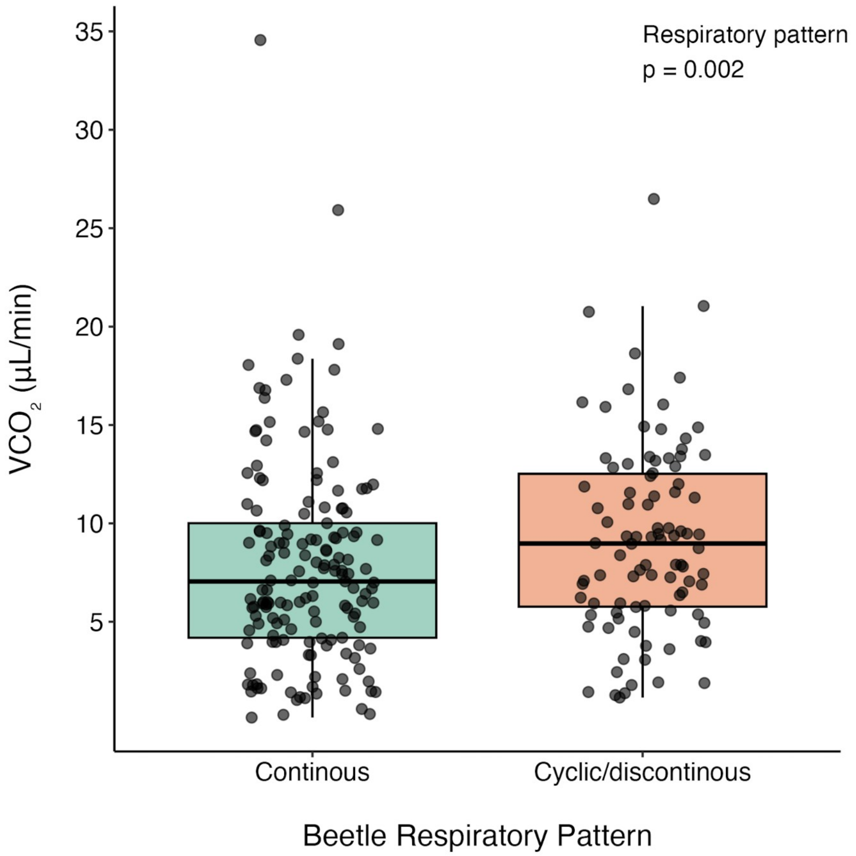

Figure 2.5. Boxplot showing the variation in VCO_2 (µL/min) across different respiratory patterns in beetles. The boxes represent the interquartile range (IQR) with the median indicated by the midline, and whiskers extending to 1.5 times the IQR or the range of the data. Individual data points are jittered for clarity. Beetles exhibiting cyclic/discontinuous gas exchange patterns had significantly higher VCO_2 levels compared to those with continuous gas exchange patterns (ANOVA, p = 0.002). This analysis was based on a linear mixed-effects model.

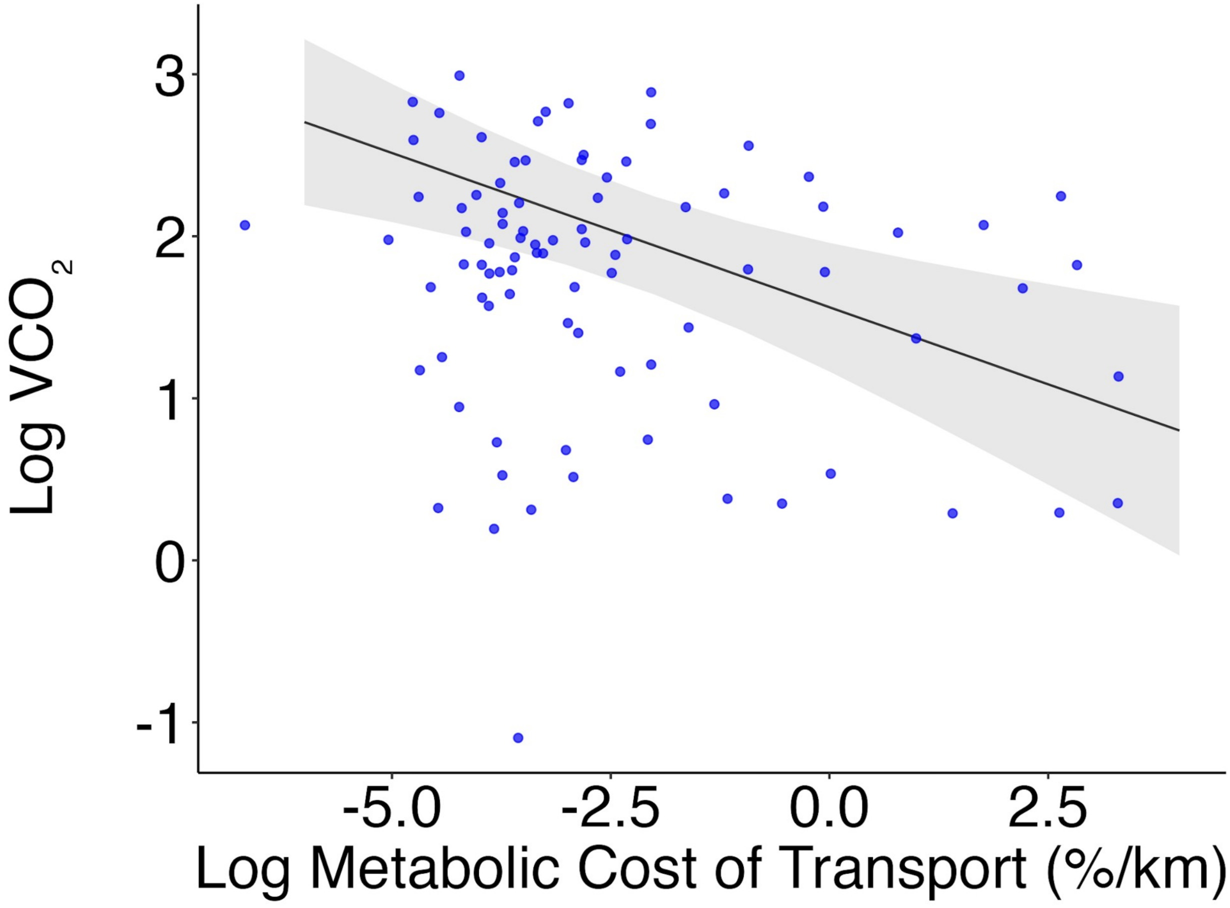

Figure 2.6. Relationship between log-transformed metabolic cost of transport (MCOT) and log-transformedVCO_2 in *D. ponderosae*. The plot shows predicted VCO_2 as a function of log-transformed MCOT (%/km), based on a linear mixed-effects model with random effects for mill and tree/bolt. The solid line represents predictions, and the shaded region shows the 95% confidence interval. Blue points are observed data with jitter to reduce overlap. The model suggests a negative relationship between MCOT and VCO_2.

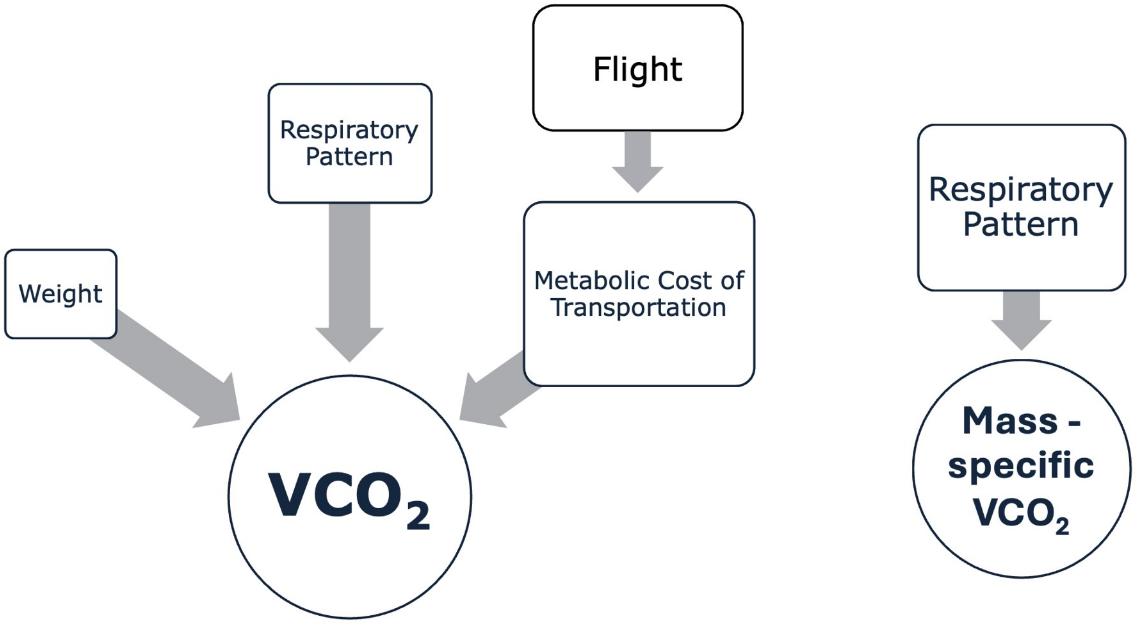

Figure 2.7. A cause-and-effect diagram illustrating the impact of multiple factors on post-flight VCO_2 levels in beetles. The map on the left highlights the influence of weight, flight, respiratory pattern, and the metabolic cost of transport (MCOT) on general VCO_2 levels. Specifically, weight and respiratory patterns directly affect VCO_2, while the flight treatment influences VCO_2 indirectly by impacting the metabolic cost of transport. The arrows indicate the relationships between these factors and their respective effects on post-flight VCO_2. On the right, the map focuses on mass-specific VCO_2, demonstrating how respiratory patterns are the primary factor influencing post-flight mass-specific metabolic rates in beetles. This diagram visualizes the complex interplay between these variables and their contributions to both total and mass-specific metabolic output.

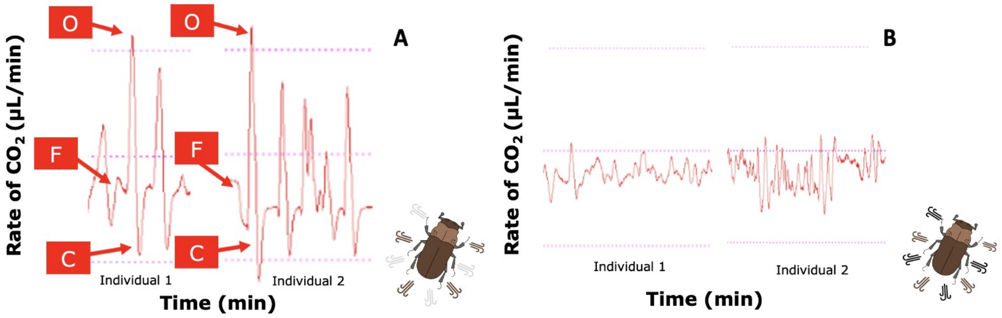

Figure 2.8. Respirometry recordings of adult *Dendroctonus ponderosae* demonstrating **(A)** discontinuous gas exchange with clear closed (C), flutter (F), and open (O) phases, and **(B)** continuous gas exchange. The rate of CO_2 output is shown over time, with discontinuous ventilation characterized by distinct phases: closed (C) periods with no gas exchange, flutter (F) periods marked by small peaks in CO_2 output due to spiracular fluttering, and open (O) periods where gas exchange occurs. The CO_2 output data were extracted using ExpeData software (V1.9.27, Sable Systems International, Las Vegas, NV). Continuous gas exchange in **(B)** shows a more steady CO_2 output without distinct phases.

Chapter 3: Effect of winter conditions and duration on subsequent life history traits of adult *Dendroctonus ponderosae*

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3.1 Abstract

The mountain pine beetle (*Dendroctonus ponderosae*) poses a significant ecological threat to North American pine forests. This study investigates the effects of overwintering severity and duration on subsequent metabolism, flight behavior, and host colonization of adult beetles. We exposed larvae-infested bolts to two winter conditions (ambient outdoor conditions and a controlled 5°C environment) for varying durations. Our findings show that natural cold conditions experienced by beetles overwintering outside facilitated better synchronization of emergence, with consistent metabolic rates across early, peak, and late emergence periods. In contrast, beetles overwintered indoors displayed a positive relationship between body weight and metabolic rate, while those overwintered outside exhibited a slight decrease in metabolic rate with increased body weight. Winter exposure duration did not significantly influence post-winter metabolic rates. However, early emerging beetles showed higher post-winter metabolic rates than those emerging later, especially for beetles kept in controlled 5°C conditions, underscoring the importance of natural cold in synchronizing emergence patterns. Additionally, pre-flight body weight significantly influenced flight propensity and flight distance, with heavier beetles more likely to fly and fly further. While winter treatment did not affect flight propensity, winter duration impacted flight distance, with longer exposure reducing post-winter flight performance. Overwintering treatment did not significantly affect post-winter host entry rates, but beetle weight strongly influenced the likelihood of post-winter host entry within three days, with heavier beetles being more successful.

Key Words: overwinter exposure, post-winter metabolic rate, synchronization, flow-through respirometry, post-winter flight capacity, flight bioassay, dispersal potential, flight behavior, post-winter host entry

3.2 Introduction

The mountain pine beetle (*Dendroctonus ponderosae*) is one of the most significant pests of mature pine forests in western North America, causing large-scale ecological and economic damage (Reid 1962; Carroll et al. 2004). The cold tolerance strategy of *D. ponderosae* shapes its distribution and abundance, especially as the beetle faces increasingly severe winters in its expanded range. The beetle's northern limit has been correlated with the -40°C isotherm, yet since the early 2000s, *D. ponderosae* has expanded eastward across the Rocky Mountains, encountering even harsher winter conditions (Thompson, 2022; Safranyik et al. 2010). Understanding how the beetle tolerates cold is crucial for predicting its persistence in these new environments and its potential for further range expansion into Canada's boreal forests (Bleiker 2019).

Dendroctonus ponderosae commonly exhibits a one-year life cycle, and late larval instars are the most successful overwintering stage (DeLeon et al. 1934; Reid, 1963; Amman, 1973; Langor, 1989; Bentz et al. 2001; Rosenberger et al. 2017). *Dendroctonus ponderosae* use a freeze-avoidance strategy to endure harsh overwinter conditions by actively avoiding body tissue freezing (Régnière and Bentz, 2007; Rosenberger et al. 2017). The larvae undergo physiological changes to enhance cold tolerance (Bentz and Mullins 1999; Bonnett et al. 2012; Robert et al. 2016). Rather than going through a hormonally controlled diapause over winter (Merivee, 1978; Sømme, 1982; Hodkova and Hodek, 1994), *D. ponderosae* prevent cold-related mortality through the production of cryoprotectants, mainly polyols (Sømme, 1964; Storey and Storey, 1983; Gehrken, 1984; Graham et al. 2007), which modify the freezing characteristics of their body fluids during cold spells (Bleiker et al. 2017; Thompson et al. 2020). Glycerol is the predominant antifreeze polyol found in *D. ponderosae* (Sømme, 1964; Bentz and Mullins, 1999). Some larvae can supercool to temperatures near -40°C (Wygant, 1940; Yuill, 1941; Sømme, 1982; Safranyik and Linton 1991; Bentz and Mullins, 1999; Cooke, 2009). Régnière and Bentz (2007) found that the supercooling point of *D. ponderosae* is affected by daily temperature-dependent mechanisms, that includes an adjustment of the metabolic rate. *D. ponderosae* adults exhibit discontinuous gas exchange with reduced gas exchange at low temperatures (Lester and Irwin, 2012). This adjustment involves shifts in the proportion of beetles between different physiological states: a non-cold-hardened feeding state, an intermediate state with reduced ice-nucleating agents, and a fully cold-hardened state with maximal cryoprotectant accumulation like glycerol. These shifts enable the beetles to optimize their cold tolerance throughout the seasonal and daily temperature fluctuations they experience. The cold tolerance of xylophagous insects, including *D. ponderosae*, varies significantly over winter (Merivee, 1978; Sømme, 1982) and is affected by winter duration (Bentz and Mullins, 1999; Régnière and Bentz, 2007).

Synchronization of beetle emergence is important for successful dispersal and colonization (Bentz and Mullins, 1999; Safranyik and Carroll, 2006; Régnière and Bentz, 2007). The natural cold conditions that beetles experience during winter help to synchronize their emergence, ensuring they come out in groups. This coordinated timing makes it easier for beetles to launch mass attacks on host trees, which is needed to overcome the trees' defenses. Although Rosenberger et al. (2017) found that beetles emerging early often have higher metabolic rates, which may aid in flying farther and entering host trees more easily, my data did not show a significant difference in VCO_2 levels based on emergence timing. Synchronization plays a key role in maintaining beetle populations and helps them survive in different environments.

Cold-induced mortality plays a crucial role in *D. ponderosae* population dynamics, with studies (Yuill, 1941; Reid, 1963; Amman, 1973; Safranyik, 1978; Cole, 1981) showing that effective acclimation to low temperatures significantly improves the insect cold hardiness and survival. During overwintering, *D. ponderosae* encounter abiotic environmental stress, such as low temperatures, water scarcity, and limited oxygen (Hahn and Denlinger, 2011; Overgaard and MacMillan, 2017).

Beetles cannot escape from the phloem and lack cold protection from the host tree bark. The metabolic activity and winter survival of *D. ponderosae* are affected by environmental factors, particularly temperature fluctuations and cold exposure duration (Yuill, 1941; Reid, 1963; Amman, 1973; Safranyik, 1978; Cole, 1981; Fraser et al. 2017). Remaining in subzero temperatures for too long puts stress on the cold hardiness strategies of *D. ponderosae* (Safranyik and Linton, 1998). Recent findings by Andersen et al. (2024) demonstrate that the freeze-avoiding *D. ponderosae* can survive prolonged exposure to stressful cold by mitigating ionoregulatory collapse. This adaptation allows *D. ponderosae* to better manage ionic balance during extended periods of cold, contributing to their resilience in harsh winter conditions. Little is known, however, about the sub-lethal effects of winter and duration on subsequent adult life-history traits. The body condition of adult beetles that emerge from natal hosts dictates the dispersal capacity (Evenden et al. 2014), and host colonization behaviors (Latty and Reid, 2010; Jones et al. 2020) of *D. ponderosae*.

The objective of this experiment is to investigate how winter condition and duration influence the subsequent metabolic rate, flight capacity, and host colonization behaviors of adult *D. ponderosae*. Specifically, I aim to test the following hypotheses: (1) beetles exposed to natural winter conditions will exhibit differences in post-winter metabolic rates, with beetle body weight playing a significant role in this response; (2) winter conditions and duration will interact with beetle body weight to influence subsequent dispersal capacity, with heavier beetles showing greater flight propensity and distance capacity; and (3) beetle body weight will significantly influence post-winter host colonization behaviors, with heavier beetles being more likely to enter host trees within three days.

3.3 Materials and methods

Beetle Collection and Overwinter Periods

In July 2022, six lodgepole pine trees with a diameter >25 cm, were baited with commercially available *D. ponderosae* baits (Synergy Semiochemicals, Delta, BC) to acquire *D. ponderosae*-infested material at two field sites in central Alberta. Infested trees were harvested in October 2022, and bolts from each tree were obtained from 1 m above the soil surface and cut into two-50 cm sections. Seven bolts from Hinton (53°24′40″N, 117°33′46″W) and fifteen bolts from Cynthia (53°16′58″N, 115°25′22″W), Alberta, Canada, were used in my experiment. The cut ends were sealed with paraffin wax (parowax®) to prevent desiccation.

Bolts (N = 22) were initially stored at 5°C for approximately 30 days until 31 October 2022. After this period, they were assigned to one of two winter treatments:

- **OW or rooftop:** Bolts were placed outside under ambient conditions, where beetles experienced natural winter conditions.
- **Control or cooler:** Bolts were kept inside a temperature-controlled cooler room maintained at ~5°C, serving as the control group for the winter manipulation experiment.

Bolts held outside on the rooftop were placed individually in heavy-duty plastic bags (Husky Contractor Clean-Up Bags, 158 L Capacity) and positioned on the rooftop of the Biological Sciences Building at the University of Alberta in Edmonton. Environmental conditions were monitored using HOBO MX Bluetooth Weatherproof Temperature and Humidity Loggers (Item# MX2301A, Omega Sensing Solutions ULC, https://www.omega.ca/en) with HOBO probes placed outside under ambient conditions and under the bark of six selected bolts to measure the temperature that beetles experienced.

Bolts were retrieved from the winter treatments at four different time points (Fig. 3.1) to assess the effects of varying durations of exposure:

- November 2022 (Timepoint 0)
- December 2022 (Timepoint 1)
- January 2023 (Timepoint 2)
- February 2023 (Timepoint 3)

Each retrieval marked a different stage of beetle exposure to the winter treatments for subsequent analysis.

Removal of bolts to supply beetles for the various experiments was staggered. Bolts retrieved from the various winter treatments were carefully placed individually into 121-liter opaque plastic containers to ensure isolation and prevent cross-contamination of any external environmental factors. These containers were maintained at a controlled temperature of approximately 22°C to simulate standard laboratory conditions, ensuring consistency in the beetles' post-winter treatment environment. The containers were also kept under a 16:8 light-to-dark photoperiod, which mimicked natural light cycles to support the beetles' circadian rhythms. This setup allowed researchers to monitor the beetles' emergence and behavior under standardized, controlled conditions following their winter treatments, minimizing external variables that could affect the results. Each container was equipped with a glass emergence jar. Bolts were checked daily for beetle emergence. Beetles were separated by sex, weighed, and placed into 1.5 mL microcentrifuge tubes with a small piece of paper and stored at 4°C until use in the bioassay. Based on the resulting emergence calendar (Fig. 3.2), beetles were randomly selected for use in each bioassay from the early, peak, and late emergence periods. To ensure no bias in selection, the entire group of emerged beetles was placed in a Petri dish, and individual beetles were arbitrarily chosen without considering factors such as sex, size, or color. This random selection process ensured that beetles from each emergence period were represented without any intentional preference or bias.

Post-overwinter Metabolic Rate Assay

The timeline of bolt removal and testing for metabolic rate is illustrated in Fig. 3.3. At each designated timepoint, one bolt was moved from the indoor 5°C cooler room to the growth room, while multiple bolts were transferred from the rooftop conditions to the growth room to facilitate beetle emergence. Emerged *D. ponderosae* adults were 3-5 d old when used in respirometry trials. In each respirometry trial, beetles were randomly selected by placing the entire group into a Petri dish, and then beetles were arbitrarily chosen without considering factors like sex, color, or body size, ensuring an unbiased sample for the experiment. They were placed individually in separate chambers (46 × 7 mm diameter) and were allowed to freely move about the activity chamber. The metabolic rate (VCO_2 in µL/min) of beetles from each winter treatment and duration combination was measured using a Multiple Animal Versatile Energetics Flow Through system (MAVEn-FT) respirometer system (Sable Systems International, Las Vegas, NV), with a constant flow rate of 30 mL/sec following the method described by Brophy & Luong (2021) (Fig. 3.4). In our respirometry setup, a lab-grade compressed air cylinder equipped with a series of filters provided a constant flow of clean, dry air to the chambers where beetles were placed. The air was filtered to remove moisture and CO_2, ensuring accurate measurement of the beetle’s metabolic rate (Lighton 2008). After passing through the respirometry chamber, the air was analyzed for changes in CO_2 concentration, which reflects the beetle's metabolic activity. The airflow rate was controlled to ensure a stable environment inside the chamber, preventing any external fluctuations from affecting the measurements. Respiration data was automatically recorded by the system for 2 min from the baseline chamber, 7 min from the experimental chamber, followed by another 2 min of baseline (Lighton 2008). Zeroing in respirometry was done by running the clean, filtered air through the system without any beetles in the chamber and recording the baseline CO_2 and O_2 levels. This ensures that any detected changes in gas concentrations after introducing the beetle are solely due to the beetle's metabolic activity. Respiration rates were measured over two temporal blocks; in the interim, the respirometer tubing was modified to create a more stable flow through the SS-4 Sub-Sampler Pump (Sable Systems International, Las Vegas, NV). The behavioral activity of each beetle within the chamber (in an average level) was simultaneously monitored alongside its metabolic rate using the MAVEn-FT system. This system allows for precise tracking of both physiological and behavioral responses during the experiment. The MAVEn-FT system’s activity monitor is equipped with a proximity detector that operates by emitting a wide cone of infrared light, which is reflected off the beetle's body. The system then detects the intensity and frequency of the reflected light to determine the beetle's movements. The respirometry measurements in this study focus on Standard Metabolic Rate (SMR) rather than Basal Metabolic Rate (BMR) (Chown & Nicolson, 2004; Lighton 2008).

All respirometry data transformations and calculations were performed in the Expedata-P Software (V1.9.27, Sable Systems International, Las Vegas, NV) using the formula for the rate of CO_2 production (µL/min) after overwintering:

$$VCO_2 = FR_i\ (F'_eCO_2 - F_iCO_2)$$

Here, VCO_2 stands for the rate of carbon dioxide production (Lighton 2008), FR_i is the flow rate of incoming air, and F'_eCO_2 and F_iCO_2 are excurrent fractional concentration and incurrent fractional concentration of CO_2, respectively. The excurrent fractional concentration of CO_2 is equal to the experimental chamber measurement minus the baseline measurement. Water vapor was dropped from calculations as inflow and outflow water vapor was scrubbed from the system and hence equals zero. Since the incoming air was scrubbed of CO_2 ($F_iCO_2 = 0$) (Lighton 2008), the formula was further converted for convenience:

$$VCO_2 = FR_i \times F'_eCO_2$$

Given the importance of body weight on the beetles' subsequent metabolic rate, we included mass-specific VCO_2 as a parameter to analyze the results. The formula is:

$$\text{Mass-specific } VCO_2 = \frac{V\,CO2}{pre-respirometry\ body\ weight}$$

The Gas Exchange Patterns

Based on the measurement of VCO_2, beetles were divided into groups based on their respiratory pattern (Lighton 1996). Beetles that exhibited continuous gas exchange were characterized by a steady release of CO_2 throughout their activity cycles. In contrast, beetles conducting discontinuous gas exchange showed intermittent bursts of CO_2 release, where periods of zero gas exchange could be observed within a single breathing cycle.

The gas exchange patterns were categorized based on the shape and timing of CO_2 peaks (refer to Fig.s 3.21A & 21B) using ExpeData software (V1.9.27, Sable Systems International, Las Vegas, NV). To get the rates of each gas exchange pattern, we used Expedata-P Data Analysis Software (V1.9.27, Sable Systems International, Las Vegas, NV. Website: https://www.sablesys.com), which processes the CO_2 data collected from respirometry trials from the MAVEn-FT chambers. We identified and quantified the distinct phases of gas exchange—closed, flutter, and open for discontinuous respiration, and continuous CO_2 output for the continuous pattern—using Expedata-P. A detailed method for differentiating these gas exchange patterns visually is based on the approach described by Lighton (1996). By analyzing the timing and amplitude of CO_2 peaks, Expedata-P provides direct measurements of the rate at which CO_2 is released during each trial, giving metabolic rate estimates for both gas exchange patterns.

Post-overwinter Flight Mill Bioassay

The timeline of bolt removal and testing for flight capacity is illustrated in Fig. 6. Beetles aged 3-5 days were weighed to ± 0.01 mg (Mettler Toledo XPE205 Microbalance, Columbus, OH, USA). Beetles were randomly assigned to one of two energetic manipulation treatments by placing the entire group into a Petri dish, from which beetles were arbitrarily selected without consideration of factors such as sex, color, or body size etc., ensuring an unbiased sample for the experiment.

The two manipulation treatments include:

- **Flown or flew**: a 23-hour session on the computer-linked flight mills was used to measure the total flight distance, serving as a representative metric of flight capacity.
- **Control or non-flown**: a 23-hour duration without the opportunity for flight, serving as a control group for the manipulation experiment.

Flown/flew beetles were tethered using 0.3 mm aluminum wire with a small loop at one end and a 2 cm straight segment perpendicular to the loop, secured with Press-Tite Contact Cement (LePage, Mississauga, Ontario) to allow unrestricted elytra movement. These tethered beetles were subsequently transferred to a specially designated flight mill room, where environmental conditions were tightly regulated to ensure consistency across all trials. The room was maintained at a constant temperature of 24°C, creating an optimal environment for beetle flight performance while minimizing the influence of external temperature fluctuations. In addition, the light-dark cycle was set to a 16:8 photoperiod, simulating natural day-night conditions and aligning with the beetles' circadian rhythms to avoid any disruptions in their natural behavior. Each beetle was individually affixed to a flight mill arm by inserting the tether into wire insulation at the arm's distal end, positioned approximately 100° from the arm's axis. Flight treatments commenced 4 h into the photoperiod and lasted for 23 h.

Control beetles were housed in perforated 1.5 mL microcentrifuge tubes with paper in the flight mill room during the treatment period. The flight mill room was illuminated with high flicker frequency fluorescent lights (550 lux) during the photophase and maintained in complete darkness (0 lux) during the scotophase (Evenden et al. 2014). A small magnetic transmitter, located on the flight mill arm, detected the arm's rotation driven by the flight of the beetles. This transmitter sent the signal to the connected computer system. LabView software (National Instruments Corporation, Austin, TX, USA) measured each revolution of the flight mill arm, which had a circumference of 94.4 cm. The recorded data included the duration of flight and the number of revolutions completed during each flight burst initiated by the beetles. Key flight parameters such as total flight distance, flight duration, flight velocity, and the number of flight bursts were computed from this recorded information. In this experiment, only the total flight distance was used for analysis.

After the 23 h treatment period, the tethers were removed from the flown beetles, and both the flown and control beetles were weighed to the nearest 0.01 mg to determine the weight loss following the treatment. Beetles that died or became detached from their tether during the flight treatment were excluded from subsequent measurements and statistical analyses.

Post-overwinter Host Colonization Experiment

This experiment investigated whether the behavior of female *D. ponderosae* during host colonization is influenced by the severity and duration of overwintering conditions. Host colonization was assessed based on two key factors: (1) the female beetle's capacity to penetrate lodgepole pine bolts, and (2) the time taken for successful host entry following (Jones et al. 2020). Uninfested lodgepole pine bolts were harvested from Hinton (53°24′40″N, 117°33′46″W) in October 2022. The cut ends of the bolts were covered with paraffin wax and bolts were stored at 5°C until use in the experiment. On each bolt, ten 30 mL clear plastic cups were positioned approximately 10 cm from the bolt's base and secured with an Arrow Heavy-Duty T-50 Staple Gun (all-purpose model: #T5-0), with a mesh skirt to eliminate gaps (Figs 3.7A & B).

Female beetles that emerged from each winter condition and duration combination (Fig. 3.1) were weighed and individual beetles were placed in a cup on a lodgepole pine bolt. Host entry activity was closely monitored after placement, or until they entered the host, as indicated by the presence of boring dust within the cup, or died (Figs 3.7A, C & D). Data pertaining to beetles that escaped from the cup were excluded from the analysis. The beetles were given up to 72 h to initiate entry into the host material, allowing them time to explore and bore into the substrate, simulating natural colonization behavior. This standardized period ensured consistent exposure across all trials.

I recorded host entry status every 24 h intervals as:

- **Escape with no dust:** when the beetle was not visible in the cup, suggesting it may have escaped.
- **Dust:** when the beetle was not visible, but some dust was observed in the cup, indicating it may have entered the host material.

- **Entered:** when the beetle was not visible, and a significant amount of dust had accumulated in the cup. Status was also confirmed after 72 h by peeling back the bark and finding to reveal the parental gallery.

Data analyses

All statistical analyses were conducted in R (R Core Team, 2023) using RStudio (RStudio Team 2023). Data processing and cleaning were carried out using packages from the *tidyverse* collection (Wickham and Grolemund, 2016; Wickham et al. 2019). To evaluate the statistical model fit, I used the *DHARMa* package (*simulateResiduals* ; Hartig 2022) to create scaled residuals and q-q plots. Additionally, I conducted the Kolmogorov-Smirnov test for overdispersion, the outlier test, and the Shapiro-Wilk test to assess the normality of residuals and determine if model assumptions were met. Final models were compared to a null model using likelihood ratio tests, and only those models that showed significant differences from the null model were interpreted. Model summaries included Chi-squared test and adjusted R^2 values for the overall models, and ANOVA tables were generated using the *car* package (Fox and Weisberg, 2019). Figures displaying raw data and fitted values of linear models were created using the *visreg* (Breheny and Burchett, 2017) and *ggplot2* (Wickham, 2016) packages.

Metabolic Rate Assay

The respirometry trials were conducted between 11:00 AM and 12:30 PM each experimental day to ensure consistent timing, accounting for natural daily variations. In preliminary analyses, I used linear mixed-effects models (*lmer*, Kuznetsova et al. 2017) to assess relationships. I modeled the logarithm of VCO_2 ($\log(VCO_2)$) against predictors include respiration pattern, overwintering treatment, and their interactions. The rate of CO_2 production from overwintering experiments was analyzed with a generalized linear mixed-effects model (*lme4* package, Bates et al. 2015); fixed effects included beetle body weight, winter duration, winter treatment, and respiration pattern along with a random factor (i.e., respirometry chambers as the ‘replicate block’) (Table 3.4). I included beetle body weight as a fixed effect to control for its significant influence on metabolic rates, ensuring the control for the variation in metabolic rates that is attributable to differences in body weight. In presenting the results, since the effect of body weight on the beetles' subsequent metabolic rate was only observed in the cooler group and not in the rooftop group, we included mass-specific VCO_2 as an independent variable in Table 3.4 to compare it with the general VCO_2 of beetles subjected to different treatments.

We also evaluated whether the average activity of beetles within the respirometry chamber influenced both the general and mass-specific VCO_2. Log-transformed general and mass-specific VCO_2 was modeled against average activity, with respirometry trials nested within chambers, and bolts nested within trees to account for variability. This hierarchical structure was implemented to ensure proper handling of nested data. Despite this, both models did not pass diagnostic tests, including normality and overdispersion checks, which precluded further analysis of the effect of average activity on VCO_2 levels.

All model assumptions were checked using diagnostic plots and statistical tests (*Anova*). I used *emmeans* (Lenth, 2022) to compute estimated marginal means and conduct pairwise comparisons for the fixed effects in the linear mixed-effects models using ANOVA statistics. Backwards model selection was implemented to arrive at the minimal model in which nonsignificant variables (χ^2 test, $p > 0.05$) were removed from subsequent models. Deviance was reported - equivalent to sum of squares of residuals. P-values for each independent variable were generated with ANOVA tables of the linear mixed effects models.

Flight Mill Bioassay

To examine the effect of overwintering duration and treatment on flight propensity, I used only beetles that were given the flight treatment (flight propensity - flew or did not fly) and excluded those from timepoint 0, as overwintering did not occur at this timepoint. For the analysis of flight propensity, I employed generalized linear mixed-effects models (*GLMMs*, Brooks et al. 2017) with a binomial family and *logit* link function (Dobson and Barnett, 2018). The response variable, flight propensity (flew or did not fly), was modeled as a function of predictors including pre-flight weight, overwintering treatment (OW vs. control), overwintering timepoint (tp), and their interactions. To avoid confounding factors, proportional weight loss was analyzed as an explanatory variable in a separate model, independent of body weight (Table 3.3). Random effects accounted for variability due to individual beetle flight mill number and nested structures within trees and bolts. ANOVA tables of test statistics and p-values for the fixed effects in the linear mixed effects models were generated using *car* (Fox and Weisberg, 2019) and pairwise means comparisons were performed using *lmerTest* (Kuznetsova et al. 2017). In my dataset, five outliers for proportional weight loss were identified by the functions *testOutliers*() and *outliers*() from *DHARMa* and so were removed. We identified five outliers in the timepoint 2 room group. During the flight bioassay, the room temperature of the flight mill was approximately 27-28 °C, higher than the normal temperature of 24 °C. This abnormal temperature likely affected beetle flight activity, as beetles are ectotherms and lose more energy when kept at a higher temperature compared to lower temperatures (Chubaty et al. 2014).

To assess the impact of overwinter timepoint on the flight distance of beetles that overwintered indoors, the flight distance data were square root transformed to satisfy normality assumptions and enhance model fit. Linear mixed-effects models (*L MMs*) were utilized, incorporating predictors such as pre-flight weight, winter duration, and their interactions (refer to Table 3.3). Random effects accounted for individual variability and the nested structure within trees and bolts. The model's adequacy was verified using ANOVA tests to evaluate the significance of fixed effects, and the model fit was checked by plotting residuals using *simulateResiduals* (Hartig 2022). To examine the proportion of weight lost by beetles across different winter duration periods (timepoints 0 to 3) and flight assay treatments (flown vs. non-flown), a *beta* regression model with a *logit* link function was applied (Dobson and Barnett, 2018). This analysis also included random effects to manage variability among individuals and nested structures within trees and bolts. Model effectiveness was assessed through ANOVA tests, evaluating the significance of fixed effects, and model fit was confirmed by plotting residuals using *simulateResiduals* (Hartig, 2022).

To visualize the effects of pre-flight weight, overwinter treatment and timepoint on the flight propensity as well as distance flown by beetles, I used the *ggpredict* function (Lüdecke, 2018) to generate predicted values from the linear mixed-effects model. The predicted values from the linear mixed-effects model of the impact of proportional weight loss were tested in a separate model to assess their effect on flight propensity and distance. I plotted these predictions using *ggplot2* (Wickham, 2016), setting terms to include the fixed variables. The fit lines of each plot represent the predicted values of linear models on the response (back-transformed) scale.

Host Colonization Experiment

The successful entry rate of the beetles into lodgepole pine bolts following exposure to different overwinter conditions and durations was converted into a binary dataset. Beetles that entered into the bolt after 72 h were coded as 1, and those that did not enter were coded as 0. I employed a generalized linear mixed-effects model using the *glmer* function to analyze the likelihood of beetle entry into bolts over a 72-h period. The model (*lme4* package, Bates et al. 2015) included fixed effects for beetle weight, overwintering duration, and overwintering treatment, as well as their interactions. The random effects were specified to account for nested data, with (1| tree/bolt) indicating random intercepts for both tree and bolt within tree (Table 3.3). The model was fitted assuming a binomial distribution of errors and a *logit* link function (Dobson and Barnett, 2018) to estimate probabilities of entry. To visualize the impact of weight on beetles' host entry, I used the ggpredict function (Lüdecke, 2018) to generate predicted values from the linear mixed-effects model. I plotted these plots using ggplot2 (Wickham, 2016), specifying terms that incorporate the fixed variables. The fit lines in each plot depict the predicted values of linear models transformed back to the response scale.

To maintain consistency, all significant figures are reported to three decimal places throughout the thesis.

3.4 Results

Fig. 3.8 shows the average temperature experienced by bolts placed on the rooftop throughout the experiment. Readings were taken at ambient temperature as well as under the bark. The highest temperature recorded under the bark reached ~ 30°C, while the lowest dropped to ~-30°C. The winter of 2022 to 2023 experienced four instances of extreme temperature drops, with the most severe occurring around mid-December. Bolts retrieved from winter conditions at timepoints 1, 2, and 3 would all have experienced this extreme temperature drop.

Metabolic Rate Assay

In the post-winter metabolism experiment (Table 3.1), a total of 206 beetles were used. Of these, 49% (101 beetles) were from the control (or the cooler) group, while 51% (105 beetles) were from the winter treatment group.

Neither winter treatment alone ($X^2 = 0.140$, df = 1, p = 0.711) nor weight alone ($X^2 = 0.240$, df = 1, p = 0.620) significantly affected post-winter VCO_2 levels. This contrasts with Chapter 2, where subsequent VCO_2 levels increased with beetle weight but did not experience real winter cold condition.

However, an interaction between overwinter treatment and beetle weight significantly affected post-winter VCO_2 levels (Estimate = -0.076, SE = 0.024, p = 0.001). For beetles overwintered in a controlled cooler condition at 5°C (shown by the red line in Fig. 3.9), a higher weight is associated with increased metabolic rates. Beetles overwintered outdoors under natural conditions exhibit a slight reduction in metabolic rate as their body weight increases (blue line in Fig. 3.9; rooftop: Estimate = -0.036, SE = 0.106).

Despite recognizing the importance of weight on post-winter VCO_2 levels in beetles kept in cooler conditions, we found no significant effect of winter treatment on post-winter mass-specific VCO_2 ($X^2 = 1.083$, df = 1, p = 0.298).

Winter exposure duration (timepoints) did not significantly affect post-winter VCO_2 levels ($X^2 = 0.404$, df = 2, $p = 0.817$), as well as the post-winter mass-specific VCO_2 ($X^2 = 2.902$, df = 2, $p = 0.234$) in beetles overwintered indoors at 5°C. Neither weight nor timepoints significantly affected post-winter VCO_2 levels in beetles held outdoors as well, as shown by non-significant main effects and interaction (all $p > 0.1$). The estimated marginal means suggest that post-winter VCO_2 levels at 1 month of winter exposure (timepoint 1) are slightly higher compared to beetles held for two (timepoint 2, Estimate = -0.094, SE = 0.151, t = -0.624) or three months (timepoint 3, Estimate = -0.071, SE = 0.167, t = -0.427), with no difference between timepoint 2 and timepoint 3 (Fig. 3.10). Although the effect of winter exposure duration on VCO_2 levels was not statistically significant, Fig. 3.10 illustrates a noticeable decline in VCO_2 levels with increasing periods of cold exposure in the control group.

There was no significant main effect of emergence time on post-winter VCO_2 levels ($X^2 = 2.281$, df = 2, $p = 0.320$) and post-winter mass-specific VCO_2 ($X^2 = 1.201$, df = 2, $p = 0.548$).

However, the interaction between emergence time and winter treatment had a significant influence on the only post-winter VCO_2 levels ($X^2 = 16.716$, df = 2, $p = 0.0002$), but not post-winter mass-specific VCO_2 levels ($X^2 = 5.78$ df = 2, $p = 0.06$) . Early-emerging beetles exhibited higher post-winter VCO_2 levels (mean = 16.8 μL/min) compared to those emerging during peak periods (mean = 7.84 μL/min, $p = 0.0001$) and late emergence periods (mean = 7.09 μL/min, $p < 0.001$) when overwintered indoors at 5°C (Fig. 3.11, control). Beetles overwintered outside (Fig. 3.11, overwintered) exhibited similar post-winter VCO_2 profiles among early (mean = 13.7 μL/min), peak (mean = 14.3 μL/min), and late (mean = 11.9 μL/min) emergence periods. Early emerging beetles that overwintered at 5°C, have higher metabolic rates compared to later-emerging individuals, whereas beetles that overwintered at natural temperatures maintained similar post-winter VCO_2 levels throughout the adult emergence period.

Also, there is a significant interaction between emergence status and overwinter timepoints ($X^2 = 10.266$, df = 4, $p = 0.036$) that affects post-winter VCO_2 levels. Post hoc comparisons (Fig. 3.12) showed varied post-winter VCO_2 patterns across the emergence period for beetles emerging from bolts: (1) early-stage emergers showed no significant differences across overwinter timepoints, (2) peak-stage emergers showed peak VCO_2 levels after timepoint 1 and decreased levels with longer winter exposure, while (3) late-stage emergers showed the lowest VCO_2 levels after timepoint 1 and a non-significant trend for increased levels with longer winter exposure. Fig. 3.12 shows a clear trend in the overwintering group exposed to only one month of cold, where VCO_2 levels decrease from early- and peak- stage emergers to late emergers. However, in beetle groups overwintered for two and three months, VCO_2 levels remain constant, suggesting a potential mechanism facilitating synchronization across emergence times.

For post-winter VCO_2 levels, within rooftop beetles, the interaction effect between gas exchange pattern and weight significantly influenced the VCO_2 levels ($\chi^2 = 4.241$, df = 1, $p = 0.039$). Beetles applying cyclic or discontinuous gas exchange patterns showed a positive relationship between fresh weight and VCO_2 level, whereas beetles with a continuous gas exchange pattern did not exhibit such variation (Fig. 3.13). For post-winter mass-specific VCO_2 levels, results show that respiratory pattern significantly affects mass-specific VCO_2 levels ($X^2 = 15.267$, df = 1, $p < 0.001$). Similarly, beetles that exhibited discontinuous gas exchange patterns produced higher mass-specific VCO_2 levels compared to those with a continuous gas exchange pattern.

To assess whether the average activity of beetles in the respirometry chamber affected post-winter VCO_2 levels, as well as mass-specific VCO_2 levels, we conducted an analysis using data from both winter treatment groups. However, these models did not pass the necessary diagnostics tests, including assessments for normality and overdispersion, preventing further interpretation of the results.

Flight Assay

In the flight experiment (Table 3.2), a total of 731 beetles were tested. Across all timepoints, 61% (449 beetles) were subjected to the flown treatment, and 39% (282 beetles) were in the room treatment. Of the flown beetles, 57% (257 beetles) successfully flew. At timepoint 0, 65% (79 beetles) were flown, of which 76% (60 beetles) successfully flew. In contrast, at timepoint 1, 49% (51 beetles) were flown, with 55% (28 beetles) achieving flight. Timepoint 2 had 48% (39 beetles) flown, with 56% (22 beetles) succeeding, while at timepoint 3, 53% (29 beetles) were flown, and 55% (16 beetles) flew successfully.

Flight Propensity

Our analysis revealed that pre-flight weight was the only factor that significantly influenced post-winter flight propensity ($\chi^2 = 25.642$, df = 1, $p < 0.0001$, Fig. 3.14). In contrast, neither winter treatment ($\chi^2 = 0.970$, df = 2, $p = 0.616$) nor overwinter duration ($\chi^2 = 0.268$, df = 1, $p = 0.605$) had significant effects. Although the interaction between winter treatment and pre-flight weight was not significant ($\chi^2 = 0.089$, df = 2, $p = 0.957$), Fig. 3.14 shows a clear trend where flight propensity decreases with longer winter exposure periods. This suggests that while the interaction effect is not statistically significant, the duration of winter exposure may still play a role in reducing flight propensity.

Flight Distance Analysis

Pre-flight weight positively influenced post-winter flight distance ($X^2 = 15.344$, $p < 0.0001$), and overwinter duration also had a significant effect on flight distance ($X^2 = 7.279$, $p = 0.026$). At timepoint 0, beetles with heavier body weight exhibited greater post-winter flight distances compared to timepoints 1 and 2. At timepoint 3, following the longest winter exposure, beetles showed exceptionally long post-winter flight distances, surpassing those at timepoint 0. Despite a significant interaction effect of pre-flight weight and winter exposure ($X^2 = 8.253$, df = 2, $p = 0.016$), no clear or explainable trend was evident across the timepoints (Fig. 3.15).

Energy Use During Flight

Flight status (flown or not) significantly affects the proportional weight loss of beetles ($\chi^2 = 195.611$, df = 1, $p < 0.01$), as does winter duration ($\chi^2 = 58.461$, df = 3, $p < 0.01$). However, the interaction between flight status and timepoint is not significant ($\chi^2 = 3.720$, df = 3, $p = 0.293$).

Beetles that flew exhibited significantly greater proportional weight loss compared to non-flying beetles (Estimate = 0.024, SE = 0.001, z = 14.62, $p < 0.01$). Among flying beetles, those at timepoint 0 showed the highest weight loss, whereas those at timepoints 1 (Estimate = 0.040, SE = 0.028), 2 (Estimate = -0.013, SE = 0.025), and 3 (Estimate = -0.038, SE = 0.024) exhibited lower weight loss. Similarly, beetles in controlled non-flown conditions at timepoint 0 demonstrated the highest weight loss, with decreasing weight loss observed at timepoints 1 (Estimate = 0.186, SE = 0.028), 2 (Estimate = -0.035, SE = 0.025), and 3 (Estimate = -0.053, SE = 0.024) (see Fig. 3.17).

Host Colonization Experiment

In the host entry experiment (Table 3.3), a total of 158 beetles were tested. Of these, 42% (66 beetles) were from the control group, and 58% (92 beetles) were from the winter treatment group. By the timepoint 1, the control group comprises 38% (12 beetles) and the winter group 62% (20 beetles). At timepoint 2, no beetles emerged from the control group due to unknown factors, while all 39 beetles (100%) emerged from the winter treatment. By timepoint 3, only 15% (6 beetles) were from the control group, compared to 85% (33 beetles) from the winter treatment group.

There was a marginally non-significant effect of overwintering duration on post-winter host entry (F = 2.169, $p = 0.091$). However, due to the limited number of female beetles used in this experiment, the impact may not be sufficiently robust to draw definitive conclusions. The main effect of overwintering treatment conditions did not significantly influence post-winter host entry by female beetles (F = 0.78, $p = 0.122$), indicating that where beetles overwintered (indoors vs. outdoors) did not impact their subsequent host entry behavior. The interaction effects between overwintering timepoint and weight ($\chi^2 = 3.624$, df = 3, p = 0.305), overwintering timepoint and overwintering treatment ($\chi^2 = 1.360$, df = 1, p = 0.240), weight and overwintering treatment ($\chi^2 = 0.173$, df = 1, p = 0.678), as well as timepoint, weight, and treatment combined ($\chi^2 = 0.193$, df = 1, p = 0.660) were not statistically significant.

Beetle weight alone significantly affected the likelihood of post-winter host entry within three days ($\chi^2 = 11.29$, p = 0.0008). Heavier female beetles had a higher probability of entering host trees (Fig. 3.18).

3.5 Discussion

Insects, including beetles, are highly sensitive to environmental temperature changes, which significantly influence their metabolic activity and post-winter traits (Bale, 1987; Bale and Hayward, 2010; Harvey et al. 2020; Bodlah et al. 2023). For *D. ponderosae*, winter conditions shape physiological responses that affect their metabolic rates and behaviors upon emergence (Andersen et al. 2024). While many studies have focused on cold adaptations like cryoprotectants and membrane fluidity adjustments (Overgaard and MacMillan, 2017; Rozsypal, 2022), my research examines how these adaptations influence post-winter traits such as metabolic rates, flight ability, and host colonization. Natural cold exposure synchronizes beetle emergence and stabilizes metabolic rates, supporting previous findings (Bentz and Mullins, 1999; Lester and Irwin, 2012).

Beetles exposed to real winter conditions exhibited more stable post-winter metabolic rates across varying body weights compared to those kept in controlled environments, suggesting that natural winter conditions foster physiological adaptations crucial for synchronized emergence and post-winter behaviors (Bentz and Mullins, 1999; Safranyik and Carroll, 2006; Régnière and Bentz, 2007). This synchronization is essential for species *like D. ponderosae*, as it ensures that large populations emerge simultaneously, enabling coordinated mass attacks on host trees, which increases their likelihood of overcoming tree defenses. The adaptations developed under real winter conditions likely include improved cold tolerance mechanisms, such as enhanced energy conservation strategies and metabolic regulation, which are vital for surviving harsh winter environments.

We are the first to incorporate a beetle emergence calendar and examine how the timing of emergence affects life traits. Early-emerging beetles displayed higher post-winter metabolic rates and greater body weight, likely reflecting a physiological readiness for energetically demanding activities like flight and host colonization (Reid et al. 2011; Rosenberger et al. 2017). This readiness could be linked to their ability to rapidly recover from cold stress and mobilize stored energy more efficiently than later-emerging beetles. However, my study did not find significant differences in emergence timing alone, suggesting that additional factors, such as the specific intensity and duration of cold exposure, individual variation in energy reserves, or genetic differences in cold tolerance, may also influence post-winter metabolic rates and emergence behavior.

The respiratory patterns observed in my study likely play a significant role in *D . ponderosae*'s adaptation. We found that beetles applying discontinuous gas exchange had significantly higher post-winter VCO_2 levels compared to those with continuous respiratory patterns. This aligns with findings in species like *Tribolium confusum* and *Schistocerca gregaria*, where discontinuous gas exchange has been shown to reduce water loss and increase metabolic efficiency (Groenewald et al. 2012). Discontinuous gas exchange may serve a critical function in enabling beetles to conserve water, particularly in natural cold and dry environments where dehydration poses a substantial threat. Given that *D. ponderosae* might not have experienced such cold and dry environments in my experiment during overwintering, the role of this respiratory pattern in water conservation could be less evident in the lab setting but may play a far more critical role in natural conditions where dehydration is a more significant challenge. Furthermore, several experimental limitations must be considered. The absence of literature on the optimal baseline duration for respirometry trials creates uncertainty regarding whether the 2-minute baseline period was sufficient for beetles to exhibit their true respiratory patterns, or whether the 7-minute period allowed adequate time for transitions between gas exchange patterns. To date, no studies have documented a full transition from one respiratory pattern to another within a single trial, leaving open questions about the temporal dynamics of these patterns. Also, storing the beetles in our lab fridge prior to the trials may have altered their physiological state, preventing an accurate assessment of the immediate metabolic effects of cold exposure. While no significant impact of activity levels on VCO_2 was detected—contrary to our original predictions—it is possible that we did not capture the full range of breathing strategies the beetles employ during overwintering. In particular, the artificial conditions of the respirometry chamber may not fully replicate the environmental stressors that *D. ponderosae* faces in the wild, potentially affecting their respiratory behavior. Future studies should investigate the role of discontinuous gas exchange in natural settings, particularly under the varying conditions of cold and dryness that beetles encounter during overwintering. Additionally, respiratory patterns were only identified visually using Expadata-P software, without applying a highly specific criterion for differentiation. A more refined approach to baseline settings in respirometry, alongside immediate post-winter trials and gas exchange pattern differentiation rule, could offer clearer insights into how these respiratory patterns contribute to the beetle's overall winter survival strategy.

Contrary to predictions, neither cold period duration nor real winter conditions significantly impacted *D. ponderosae*'s flight initiation. This differs from the cold tolerance observed in larvae, which can supercool to -40°C (Bentz and Mullins, 1999; Bonnett et al. 2012; Robert et al. 2016). While larvae are well-studied, the impact of these adaptations on adult dispersal post-winter remains underexplored. In other bark beetles, such as *D. frontalis* and *Ips pini*, lipid reserves are key for flight readiness (Moser, 1986; Lombardero et al. 2000). Winter metabolic rates are affected more by energy reserves than external temperatures (Gaylord et al. 2014, 2016), and prolonged cold can suppress flight capacity in some species (Kukal et al. 1991; Denlinger and Lee, 2010; Lalouette et al. 2011). This might be due to the experimental design, where beetles were stored in a lab fridge for 3-5 days after winter treatment rather than being tested immediately, potentially missing the immediate physiological effects of cold exposure on flight behavior.

In contrast, pre-flight weight played a significant role in influencing both flight initiation and distance. Heavier *D. ponderosae* were more likely to initiate flight and sustain longer distances, showing the importance of physiological preparedness (Evenden et al. 2014). Body weight, which reflects the energy reserves stored before and/or during overwintering, is critical for powering flight muscles and meeting the high metabolic demands of flight (Bretscher and O’Connor, 2020). Beetles with greater energy reserves are not only better equipped for long-distance dispersal but also more likely to succeed in colonizing new host trees, aligning with the findings of Jones et al. (2019). These energy reserves also influence beetle responses to semiochemicals, which are essential for finding suitable hosts and initiating mass attacks, further showing how winter conditions indirectly shape population dynamics and range expansion (Petro et al. unpublished). It is important to note, however, that in this study we used pre-flight weight rather than body fat or body volume, which are commonly used in the literature to assess beetle condition more precisely. As a result, while our findings highlight the importance of weight, further research is needed to better understand how these more specific measures of body condition, such as fat reserves or body volume, impact flight performance and dispersal capacity (Evenden et al. 2014; Wijerathna and Evenden 2019). This will offer clearer insights into how real body condition influences beetles' physiological readiness and their ability to thrive after overwintering.

Beetles exposed to extended cold exhibited shorter flight distances, likely because of the downregulation of genetic pathways related to muscle function and energy production (Clark and Worland, 2008; Hedges et al. 2019). Prolonged cold exposure has been shown to decrease ATP synthase expression, which impairs the beetles' ability to produce and utilize energy for flight (Storey and Storey, 2012). This reduction in energy metabolism, combined with the depletion of energy reserves during extended cold periods, presents a challenge to sustaining flight (Isobe et al. 2013; Enriquez and Visser, 2023). Furthermore, the formation of ice crystals within flight muscles during freezing conditions may cause structural damage, compromising muscle function and further diminishing flight capacity (Bale, 2002; MacMillan and Sinclair, 2011). Together, these physiological limitations highlight the substantial issue that cold exposure takes on the beetles' ability to engage in the energetically demanding task of flight, which is crucial for dispersal and survival.

Flight depletes energy reserves, impacting reproduction (Wijerathna and Evenden, 2019). My study showed that *D. ponderosae* in flight assays lost more weight than non-flown beetles, and longer winters led to higher weight loss during flight due to cold-stressed metabolism (Bonnett et al. 2012). Even though beetles resume feeding post-winter, accumulated energy deficits can hinder their ability to forage effectively, delaying recovery (Tussey, 2017). Beetles likely adjust their metabolism during prolonged winters to conserve energy for survival and reproduction (Elkin and Reid, 2005; Raffa et al. 2015).

The severity of winter also affects the preparatory stages critical for dispersal and host colonization. Cold stress, metabolic adjustments, and energy accumulation are essential for successful dispersal (Bale and Hayward, 2010; Mellanby, 1939; Marshall and Sinclair, 2012; Lillis et al. 2023). I found no effect of overwintering on host entry rates, possibly due to *D. ponderosae*'s adaptive mechanisms like cryoprotectant production and ion regulation (Storey and Storey, 2005; Régnière and Bentz, 2007; Sinclair, 1999). Furthermore, the host entry experiment did not allow females to choose their host or experience flight beforehand—factors that influence host entry behavior (Jones et al. 2020). Females use volatile, visual and gustatory cues to assess potential hosts (Byers, 2004; Raffa et al. 2013), abandoning unsuitable trees that lack the right chemical signals or offer poor-quality phloem (Safranyik and Carroll, 2006. The absence of these factors may have caused the beetles not to exhibit normal host entry behaviour after the winter.

Finally, my findings indicate that weight plays a critical role in post-winter host entry, with heavier females more likely to enter host trees. Increased body mass correlates with higher fat reserves, essential for energy-intensive activities like host colonization (Jones et al. 2020). Energy reserves are vital for survival and adaptability, influencing population dynamics and expansion (Latty and Reid, 2010). This highlights the importance of post-winter nutritional status for *D. ponderosae* survival and reproductive success.

Overall, this study provides valuable insights into how winter conditions influence the physiological traits and behaviors of *D. ponderosae* post-winter. Exposure to natural cold plays a critical role in synchronizing emergence and stabilizing metabolic rates, which are essential for successful flight and host colonization. Body weight, influenced by energy reserves accumulated during overwintering, emerges as a key factor determining flight capacity and host entry success. While cold tolerance adaptations, such as cryoprotectant production, allow *D. ponderosae* to survive harsh winters, the study highlights the trade-offs between cold exposure, energy expenditure, and post-winter behavior. These findings deepen our understanding of the beetle’s adaptive strategies and offer a framework for predicting its population dynamics and range expansion under changing winter conditions. Further research on the interactions between winter severity, physiological adaptations, and post-winter life traits will be necessary for predicting *D. ponderosae* in the face of climate change.

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Figures

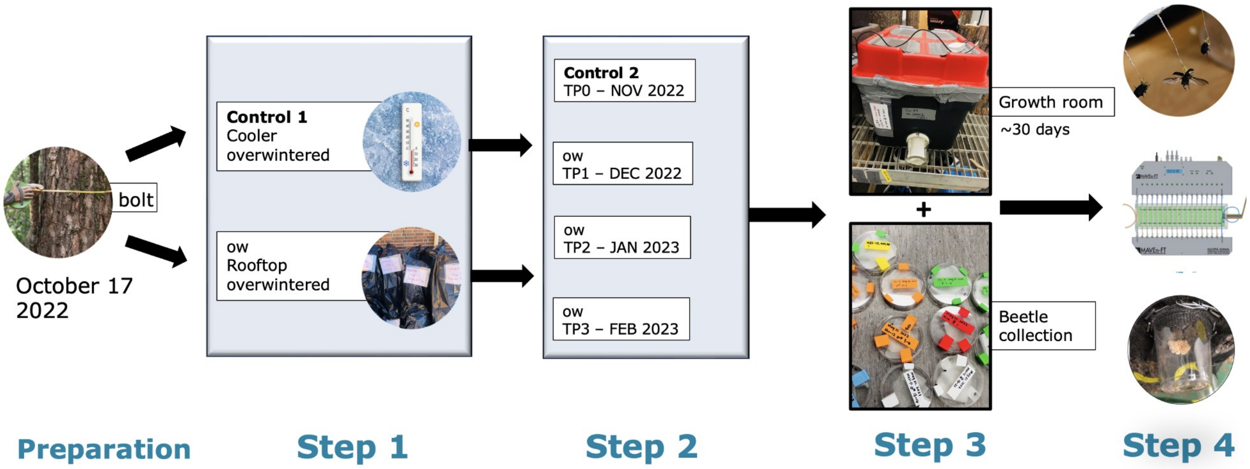
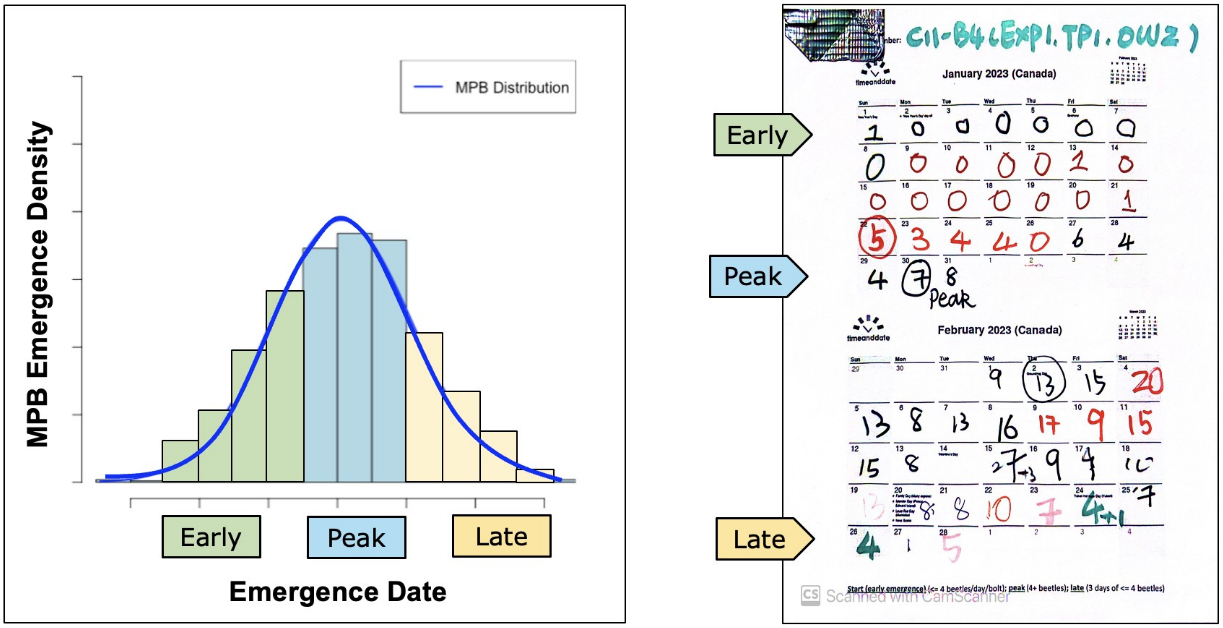


Figure 3.1. Experimental workflow for assessing the effects of overwintering conditions on *Dendroctonus ponderosae* metabolism, flight capacity, and host colonization. In Step 1, bolts were collected and assigned to two overwintering treatments: (1) a cold room at ~5°C (Control 1), and (2) a rooftop under ambient conditions (RW: Rooftop overwintered). In Step 2, the overwintering period was divided into four timepoints (TP0 to TP3), representing different durations of cold exposure: November 2022 (TP0), December 2022 (TP1), January 2023 (TP2), and February 2023 (TP3). At each timepoint, bolts from both treatments were transferred to a growth room at 22°C to allow beetle development and emergence (Step 3). In Step 4, the emerged beetles were tested for metabolic rates, flight capacity, and host colonization behavior to evaluate the impact of varying overwintering durations and conditions.



ber: C11-B4 (EXP1.TP1.OW2)

January 2023 (Canada)

Sun	Mon	Tue	Wed	Thu	Fri	Sat
1 1	2 0	3 0	4 0	5 0	6 0	7 0
8 0	9 0	10 0	11 0	12 0	13 1	14 0
15 0	16 0	17 0	18 0	19 0	20 0	21 1
22 5	23 3	24 4	25 4	26 0	27 6	28 4
29 4	30 7	31 8 Peak	1	2	3	4

February 2023 (Canada)

Sun	Mon	Tue	Wed	Thu	Fri	Sat
29	30	31	1 9	2 13	3 15	4 20
5 13	6 8	7 13	8 16	9 17	10 9	11 15
12 15	13 8	14	15 27+3	16 9	17 4	18 10
19 13	20 8	21 8	22 10	23 7	24 4+1	25 7
26 4	27 1	28 5	1	2	3	4

Start (early emergence) (<= 4 beetles/day/bolt); peak (4+ beetles); late (3 days of <= 4 beetles)

Scanned with CamScanner

A sample calendar for one of the bolts

Figure 3.2. A sample emergence calendar for adult mountain pine beetles (*Dendroctonus ponderosae*) illustrating the distribution of beetles emerging during the early, peak, and late phases. The 'Early' phase is defined by less than 20% emergence per day for at least 3 consecutive days. The 'Peak' phase occurs when over 60% of the beetles emerge, indicating the highest density period. The 'Late' phase is characterized by a consistent emergence rate of less than 20% per day for at least 3 days. This calendar provides a visual representation of the emergence dynamics for one of the bolts used in the study.

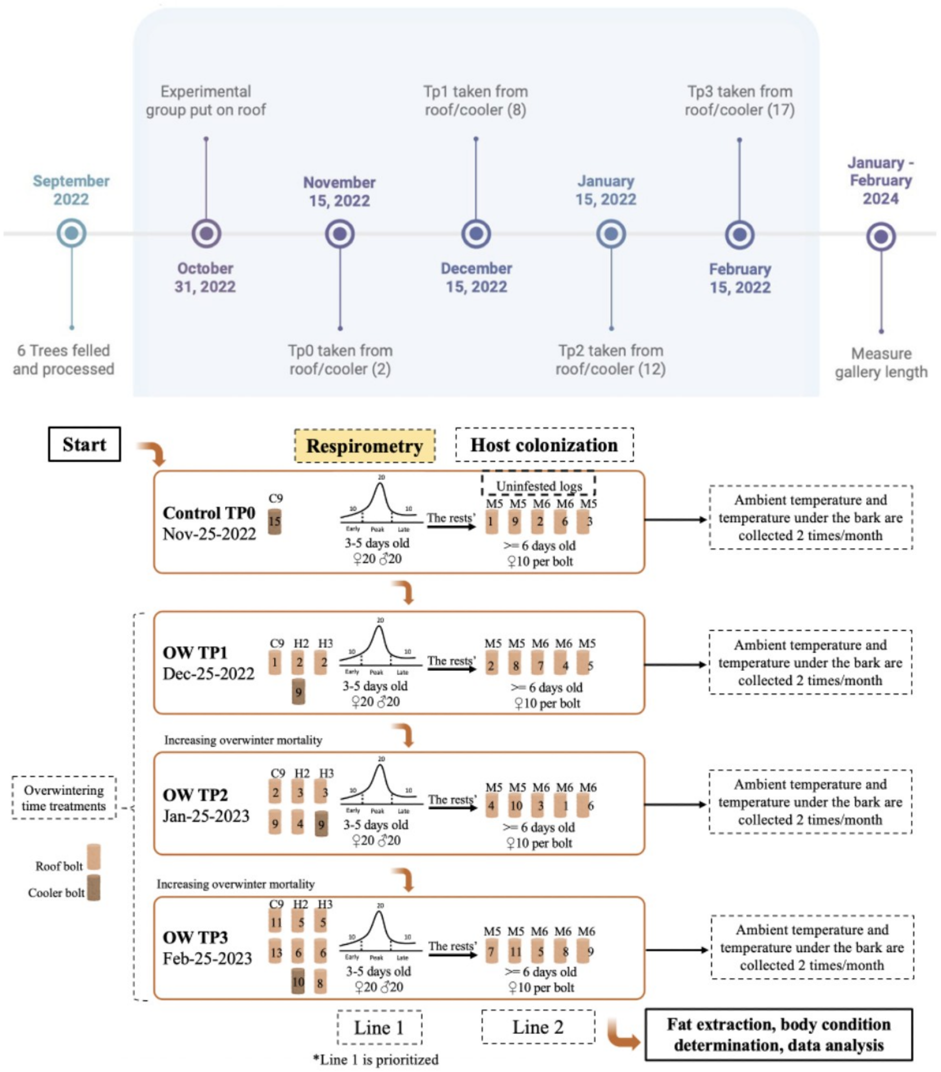


Figure 3.3. Experimental timeline and schematic outlining the placement and retrieval of bolts from overwintering conditions either indoors in a cooler (5°C) or under ambient conditions on the rooftop of the Biological Sciences Building. Six trees were felled and processed in September 2022, with bolts placed for overwintering in October. Bolts were retrieved at four timepoints: November 2022 (TP0), December 2022 (OW TP1), January 2023 (OW TP2), and February 2023 (OW TP3). Adult beetles emerging from these bolts were subjected to metabolic rate measurements using flow-through respirometry and host colonization assays. Overwintering conditions and timepoints were examined to assess the effects of cold exposure on beetle physiology and behavior, with subsequent data analysis.

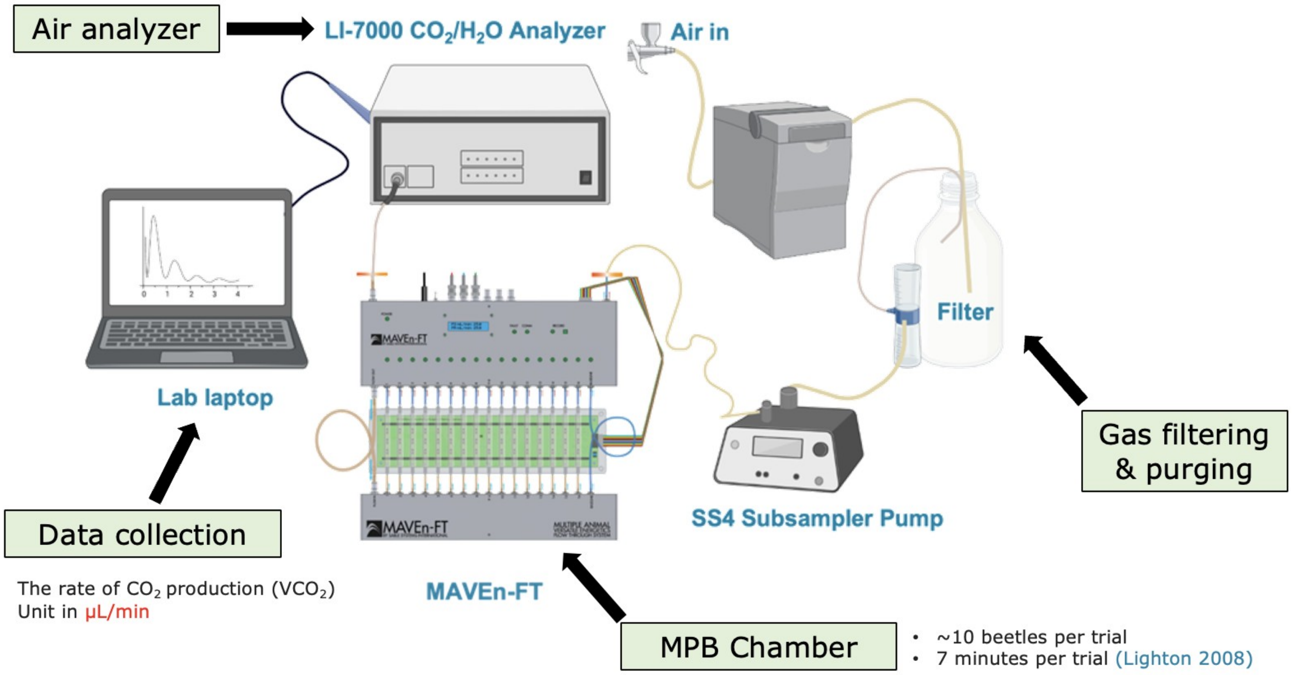


Figure 3.4. A flow-through respirometry setup designed to directly measure the metabolic rate and respiratory characteristics of tested beetles. The system includes a MAVEn-FT (Multiple Animal Versatile Energetics Flow-Through) system, which regulates airflow and houses the beetles during trials. Air is drawn into the system from a lab-grade compressed air cylinder, which supplies clean, dry air after passing through a gas filtration and moisture removal process. The air then flows through the respirometry chambers, where beetles are placed for measurement. An SS4 Subsampler Pump controls the airflow, ensuring consistent flow rates for accurate data collection. CO_2 concentrations in the outflow air are measured by an LI-7000 CO_2/H_2O Analyzer, which quantifies the beetles' metabolic rate (VCO_2) in μL/min. A filter is used to remove water vapor before the gas enters the analyzer. Data collected from the analyzer is processed and recorded on a laboratory laptop for further analysis. Each trial includes approximately 10 beetles, with a measurement duration of around 7 minutes per beetle (Lighton 2008).

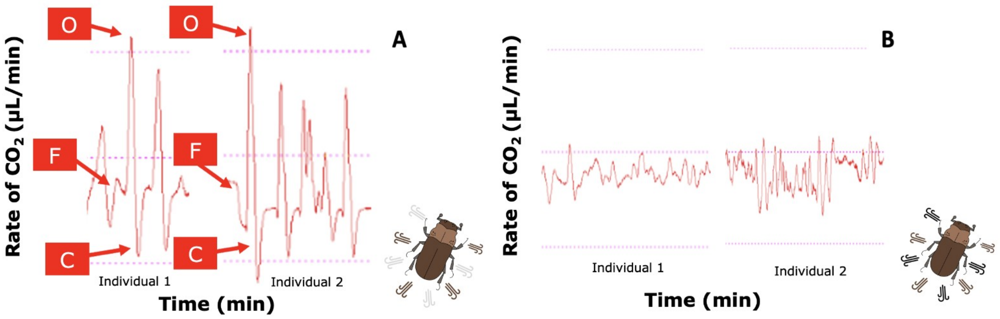


Figure 3.5. Respirometry recordings of adult *Dendroctonus ponderosae* demonstrating **(A)** discontinuous gas exchange with clear closed (C), flutter (F), and open (O) phases, and **(B)** continuous gas exchange. The rate of CO_2 output is shown over time, with discontinuous ventilation characterized by distinct phases: closed (C) periods with no gas exchange, flutter (F) periods marked by small peaks in CO_2 output due to spiracular fluttering, and open (O) periods where gas exchange occurs. The CO_2 output data were extracted using ExpeData software (V1.9.27, Sable Systems International, Las Vegas, NV). Continuous gas exchange in **(B)** shows a steadier CO_2 output without distinct phases.

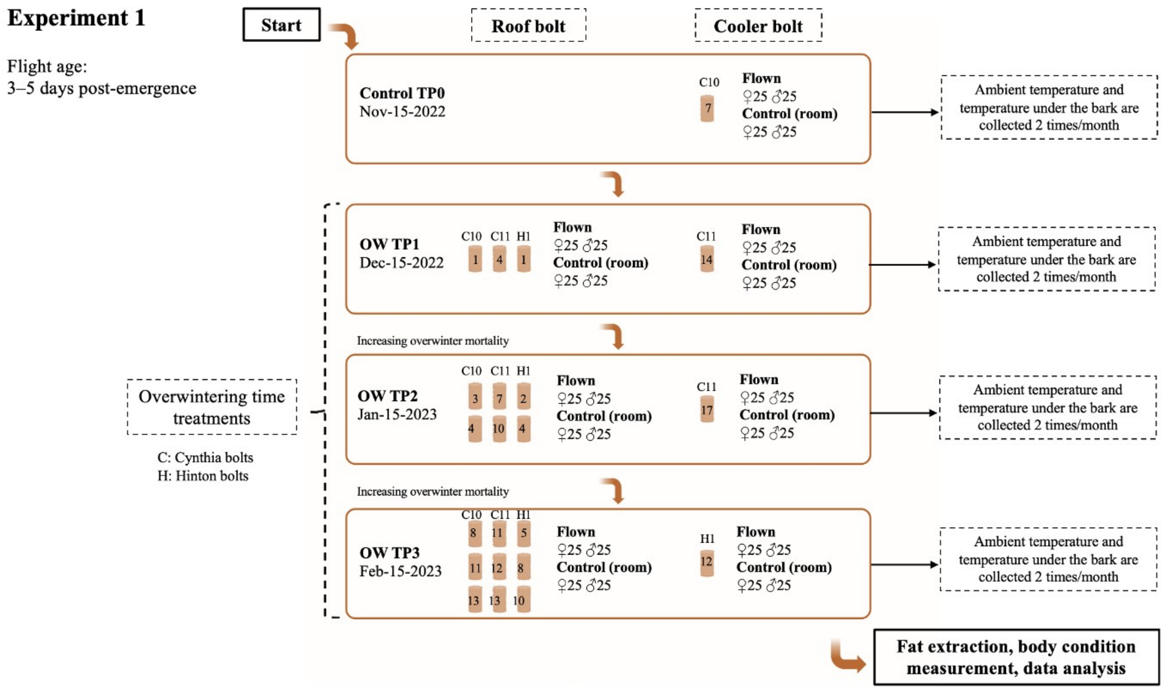


Figure 3.6. Experimental design illustrating the placement and retrieval of bolts from overwintering conditions, either indoors in a cooler (5°C) or under ambient conditions on the rooftop of the Biological Sciences Building. Bolts were removed at four timepoints: November 2022 (Control TP0), December 2022 (OW TP1), January 2023 (OW TP2), and February 2023 (OW TP3). Adult beetles emerging from these bolts were subjected to flight mill bioassays 3–5 days post-emergence. Overwintering treatments (cooler vs. rooftop) and flight mill experiments were conducted to assess the impact of cold exposure on beetle flight performance.

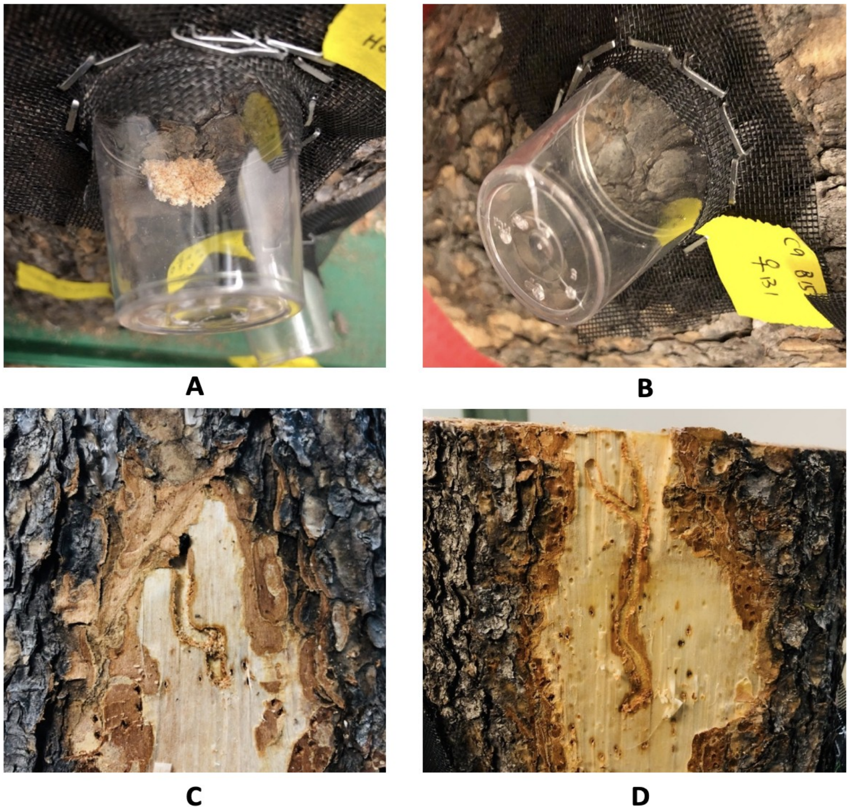


Figure 3.7. Host colonization bioassay adapted from Jones et al. (2020). **(A)** Evidence of successful host colonization by a female *D. ponderosae*, indicated by the accumulation of wood dust around the entrance hole excavated by the beetle. **(B)** An unsuccessful colonization attempt, potentially due to beetles escaping or perishing during the experiment. **(C)** Confirmation of successful colonization through the initiation of a parental gallery 72 hours post-inoculation. **(D)** Further confirmation of colonization, with no beetle visible on the surface as it tunnels within the newly formed parental gallery.

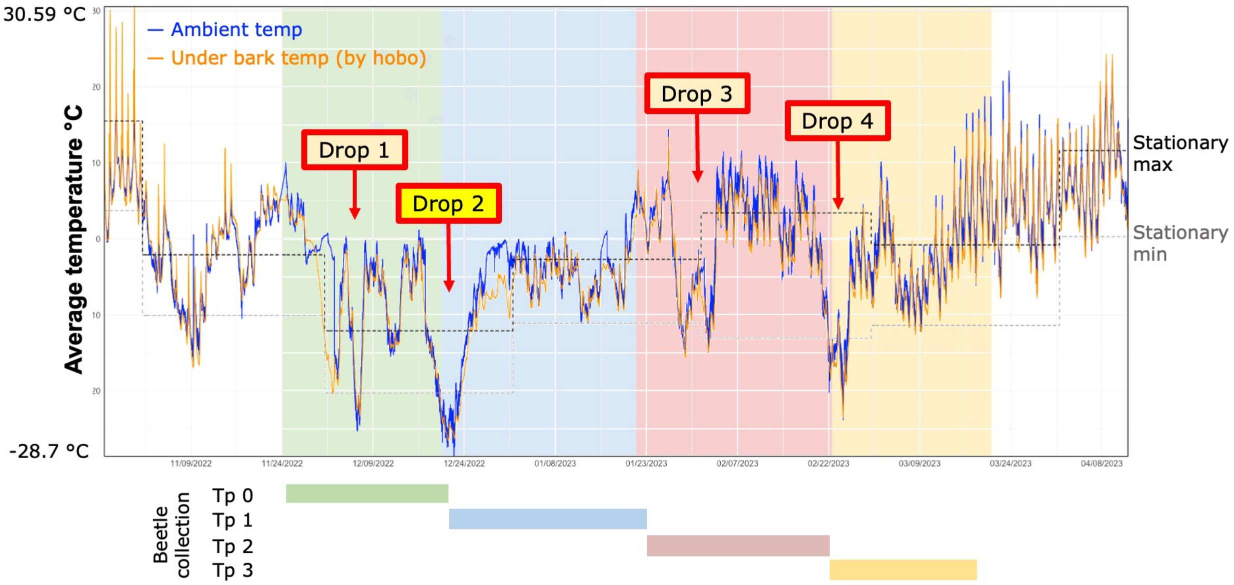


Figure 3.8. Variation in temperature experienced by the bolts on the rooftop of the Biological Sciences Building at the University of Alberta from 15 November 2022 to 12 February 2023. Experimental timeline indicates the duration of winter experienced by bolts retrieved at Timepoint 0-3 (i.e., length of cold exposure 0 month to 3 months) with measurements of ambient (blue line) and underbark temperature (orange line) overlaid. These data points were gathered using HOBO MX Bluetooth Weatherproof Temperature and Humidity Loggers (Item# MX2301A, Omega Sensing Solutions ULC, https://www.omega.ca/en). Dashed lines denote the minimum (dark grey) and maximum (black) temperatures recorded by the monitoring station (Historical Data - Climate - Environment and Climate Change Canada, https://climate.weather.gc.ca/historical_data/search_historic_data_e.html, Current Station Operator: ECCC – MSC, 53°34'23.008" N, 113°31'00.010" W, Blatchford, Edmonton, AB) during the same period. It serves as background information to provide context about the local climate conditions during the study period.

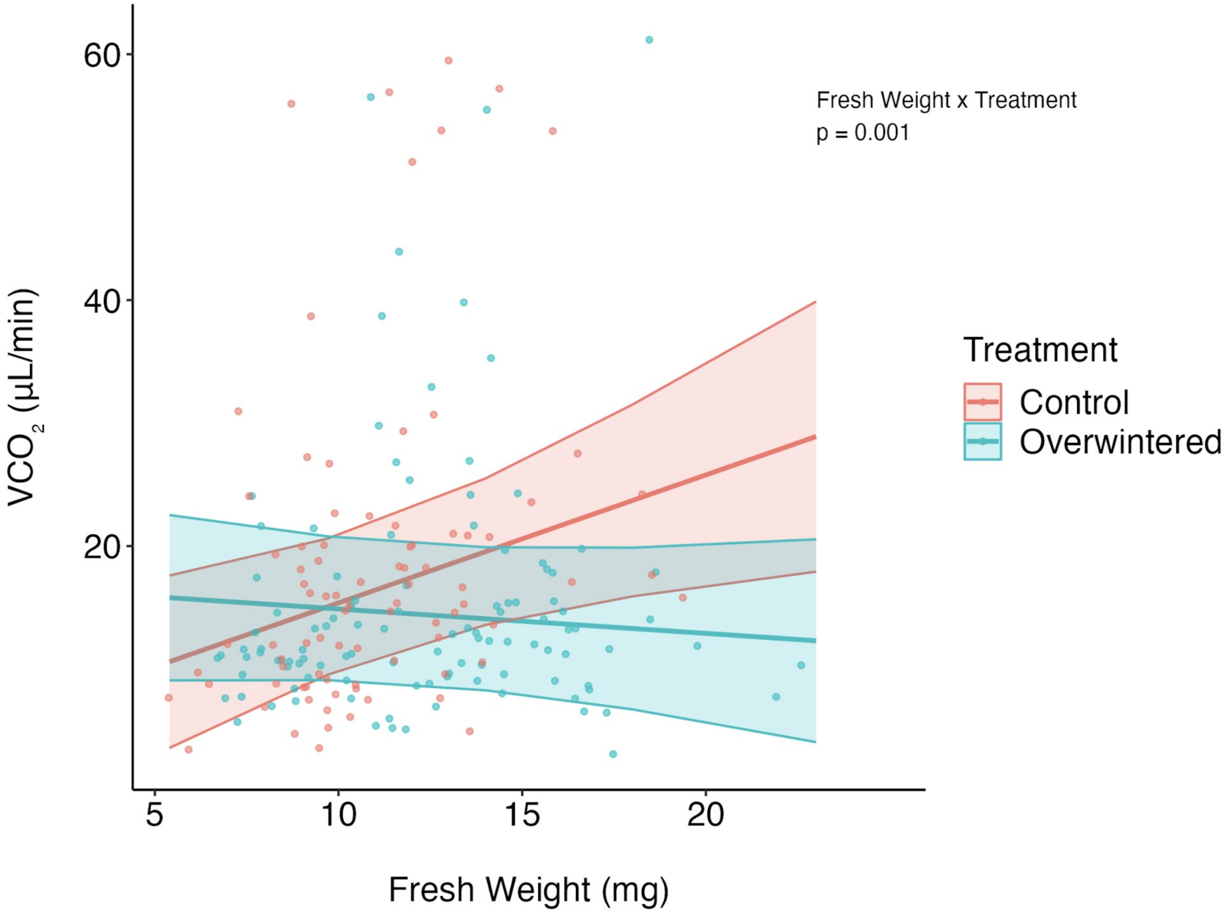


Figure 3.9. The relationship between beetle fresh weight (mg) and VCO_2 (µL/min) across two winter treatments: Control (indoors at 5°C, N = 85) and Overwintered (outdoors under ambient conditions, N = 110). The plot shows model results with back-transformed (exponential) predictors, illustrating how body weight influences metabolic rate (VCO_2) differently across treatments. The x-axis represents fresh weight, while the y-axis shows VCO_2. The shaded areas around each line represent the 95% confidence intervals. A significant interaction between fresh weight and treatment ($p = 0.001$) is highlighted, indicating that the effect of body weight on metabolic rate varies based on overwintering conditions.

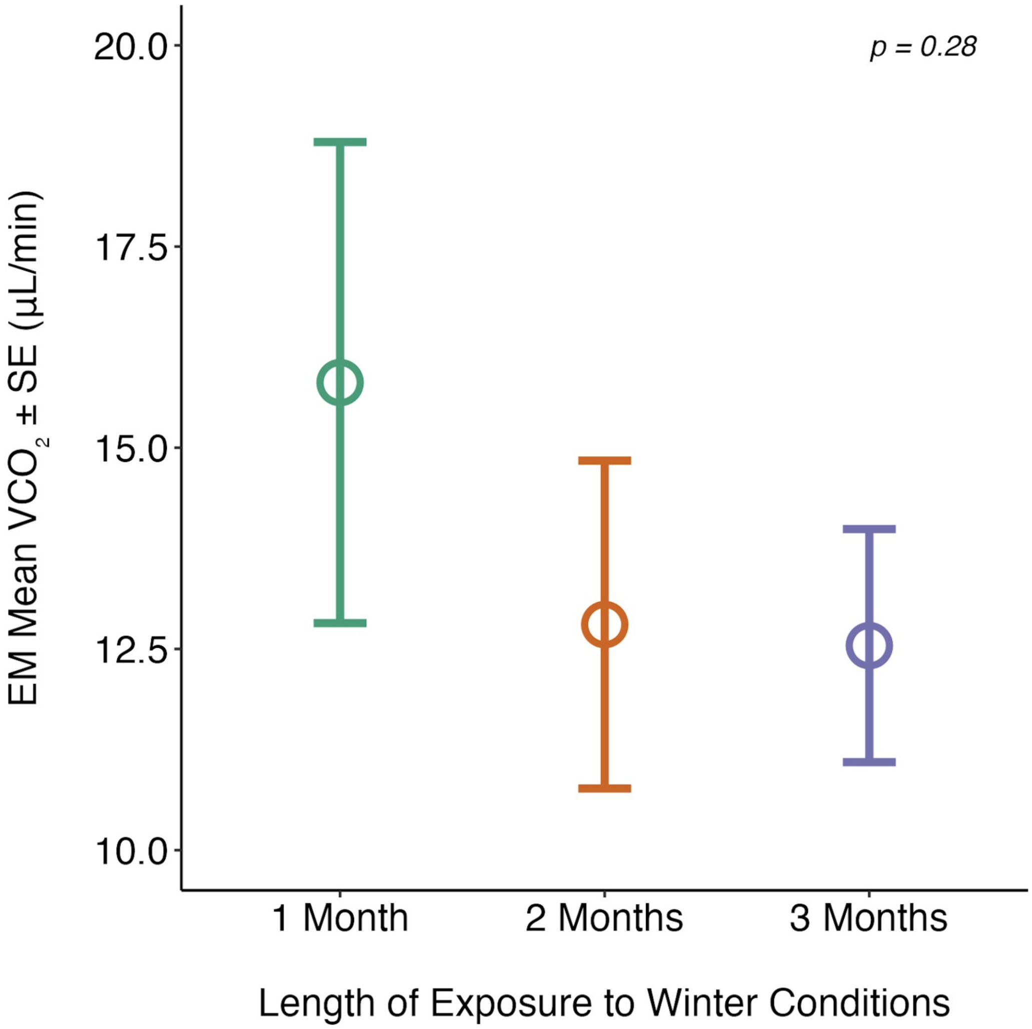


Figure 3.10. Estimated marginal mean (EM Mean) VCO_2 (μL/min) ± SE for beetles overwintered indoors at 5°C, plotted across different lengths of exposure to winter conditions (1 month, 2 months, and 3 months). Each point represents the mean VCO_2 value, with error bars indicating the standard error (SE). The x-axis shows the duration of exposure to winter conditions, while the y-axis represents the estimated marginal mean VCO_2. The p-value from the ANOVA test ($p = 0.28$) is displayed, indicating no statistically significant difference in VCO_2 across the different exposure durations.

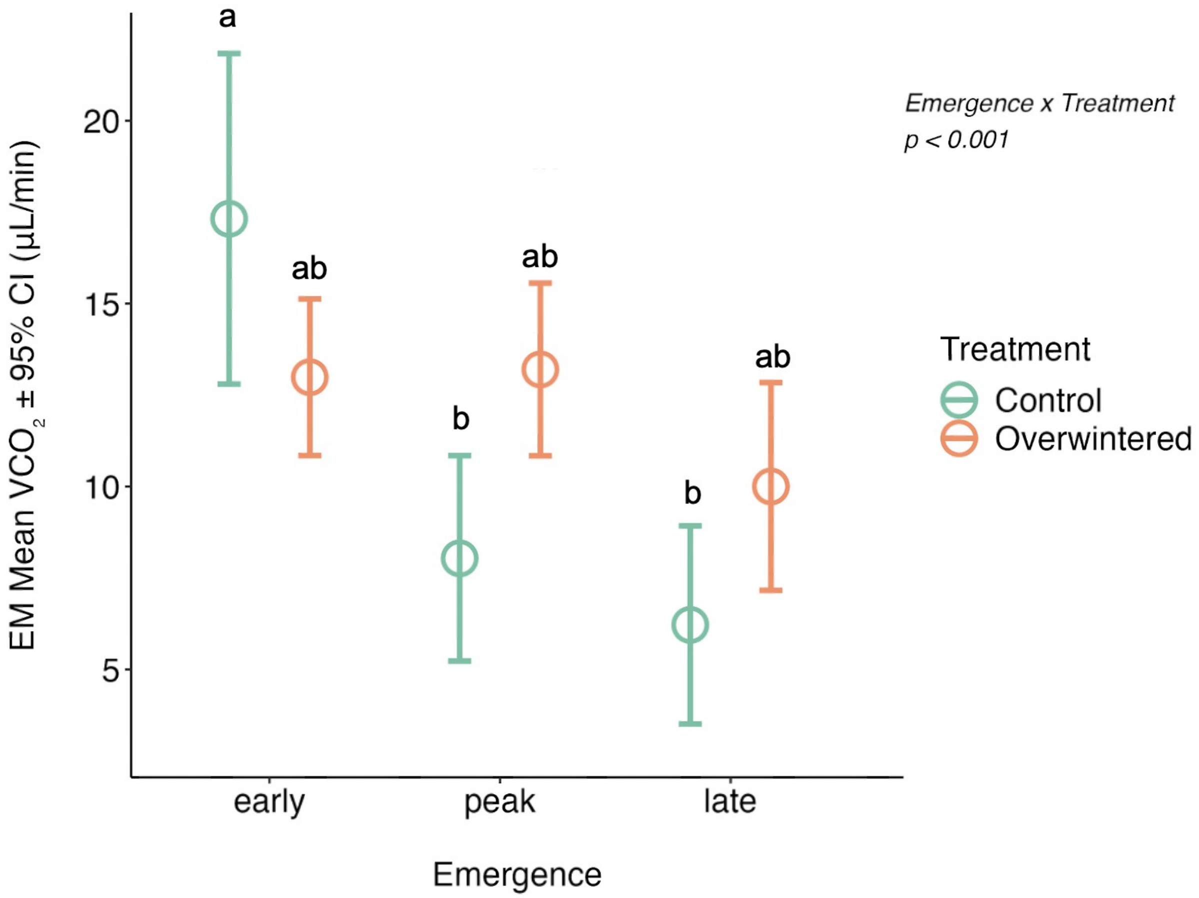


Figure 3.11. Estimated marginal mean (EM Mean) VCO_2 (μL/min) ± 95% CI for beetles across different emergence periods (early, peak, and late) and winter treatments (Control: indoors at 5°C; Overwintered: outside under ambient conditions). Points with different letters (a, ab, b) indicate significant differences between groups based on Tukey HSD post-hoc tests. The interaction between emergence time and treatment was significant ($p < 0.001$), as indicated by the annotation. P-values for each variable were generated using ANOVA tables from linear mixed effects models. The x-axis represents the emergence periods, while the y-axis shows the estimated marginal mean VCO_2 values.

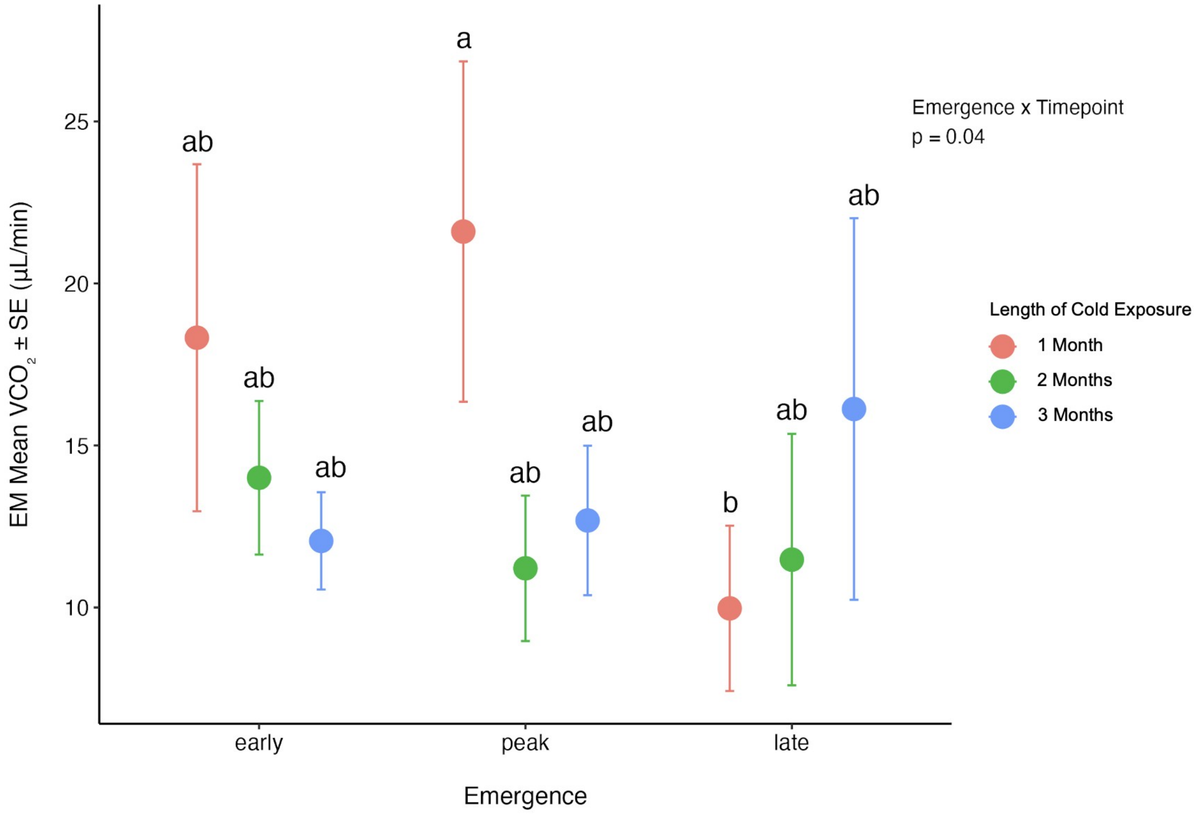


Figure 3.12. Estimated marginal mean (EM Mean) VCO_2 (µL/min) ± SE for beetles across different emergence periods (early, peak, and late) and varying lengths of cold exposure (1 month, 2 months, and 3 months). Points represent mean VCO_2 values with error bars indicating standard errors. Emergence time and the length of cold exposure (both in the cooler at 5°C and outside under ambient conditions) were considered. Points labeled with different letters (a, ab, b) are significantly different based on Tukey HSD post-hoc tests. The interaction between emergence time and timepoint was significant ($p = 0.04$). P-values were generated using ANOVA tables from linear mixed effects models. The x-axis represents emergence periods, while the y-axis shows the estimated marginal mean VCO_2 values.

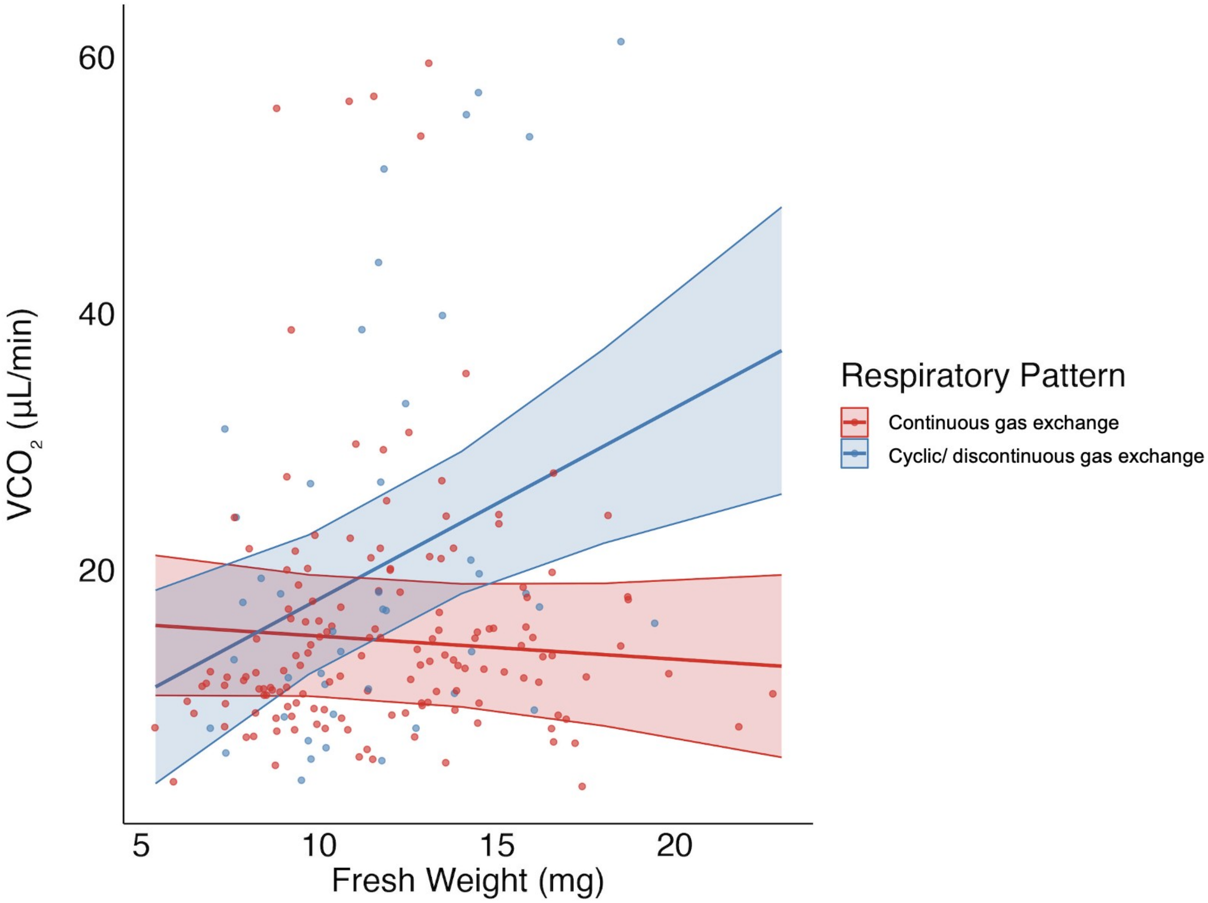


Figure 3.13. Relationship between body weight and VCO_2 (μL/min) across different respiratory patterns in beetles overwintered indoors at 5°C. The interaction between gas exchange pattern and body weight significantly influenced VCO_2 levels (ANOVA, p = 0.039). Individual VCO_2 measurements are represented by points, with colors distinguishing between continuous gas exchange and cyclic/discontinuous gas exchange patterns. The model results are shown with back-transformed (exponential) predictors overlaid. Beetles exhibiting cyclic or discontinuous gas exchange (N = 44) produced higher VCO_2 levels as body weight increased, whereas beetles with a continuous gas exchange pattern (N = 151) showed no significant variation in VCO_2 levels with increasing body weight.

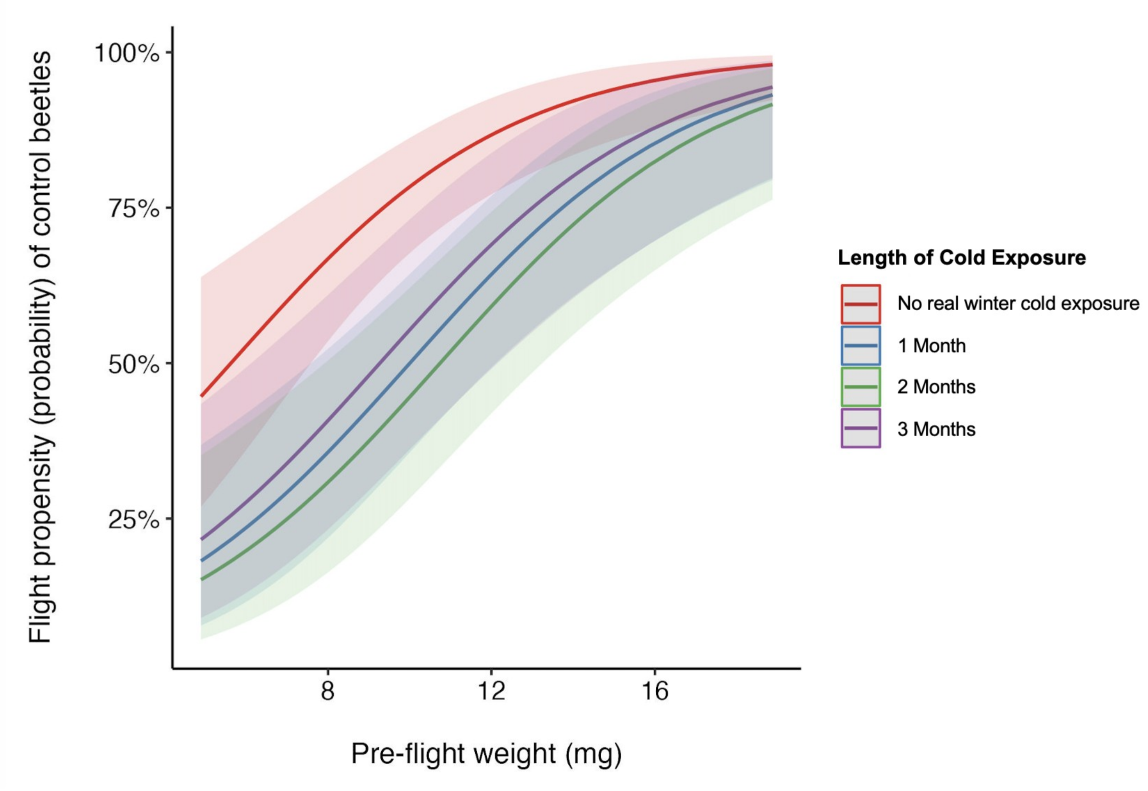


Figure 3.14. Predicted flight propensity (probability) of beetles overwintered in a cooler at 5°C as a function of pre-flight weight and the length of cold exposure (0 to 3 months). The plot shows the results from the generalized linear mixed model (GLMM), with fit lines representing the predicted values on the back-transformed response scale. Different colors indicate the length of cold exposure: no real winter cold exposure (0 months), 1 month, 2 months, and 3 months. The x-axis represents pre-flight weight (mg), and the y-axis shows the predicted probability of flight. Beetles exposed to longer durations of cold showed reduced flight propensity at higher weights compared to those without real winter exposure.

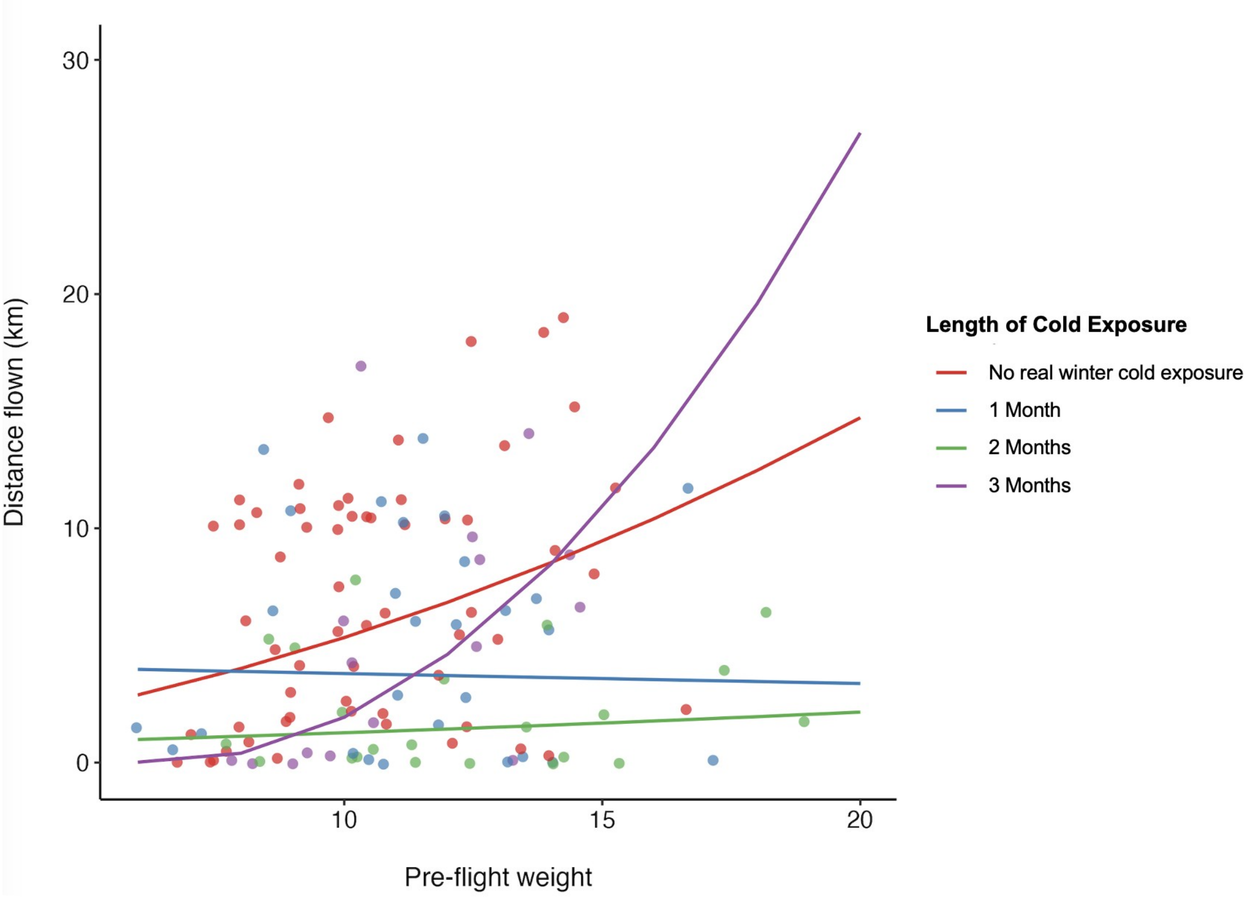


Figure 3.15. Distance flown (km) by beetles overwintered in a cooler at 5°C (N = 126) as a function of pre-flight weight and the length of cold exposure (0 to 3 months). The plot displays results from the generalized linear mixed model (GLMM), with back-transformed predictions. Different colors represent varying lengths of cold exposure: no real winter cold exposure (0 months), 1 month, 2 months, and 3 months. A notable exponential increase in flight distance is observed in beetles with higher pre-flight weights after three months of cold exposure. The x-axis represents pre-flight weight (mg), while the y-axis indicates the distance flown (km).

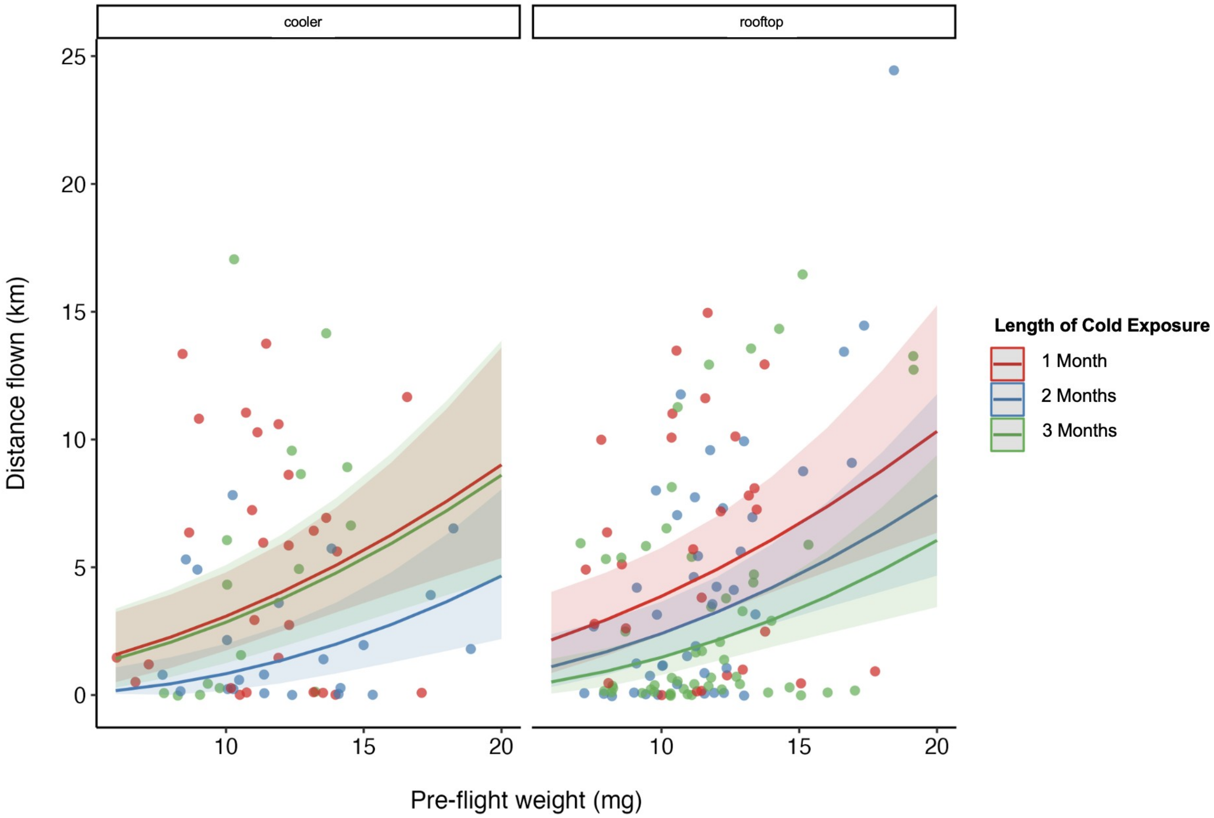


Figure 3.16. Distance flown (km) by beetles across two winter treatments: cooler (indoors at 5°C, N = 119) and rooftop (ambient conditions, N = 210), as a function of pre-flight weight and the length of cold exposure (1 to 3 months). The plot displays results from the generalized linear mixed model (GLMM), with back-transformed y-axes. Pre-flight weight was positively correlated with flight distance ($X^2 = 15.344$, $p < 0.0001$), indicating that heavier beetles flew longer distances. Winter treatment ($X^2 = 0.520$, $p = 0.471$) and the interaction between winter duration and treatment ($X^2 = 4.193$, $p = 0.122$) did not show significant effects on flight distance. The shaded areas represent 95% confidence intervals for the predicted flight distances.

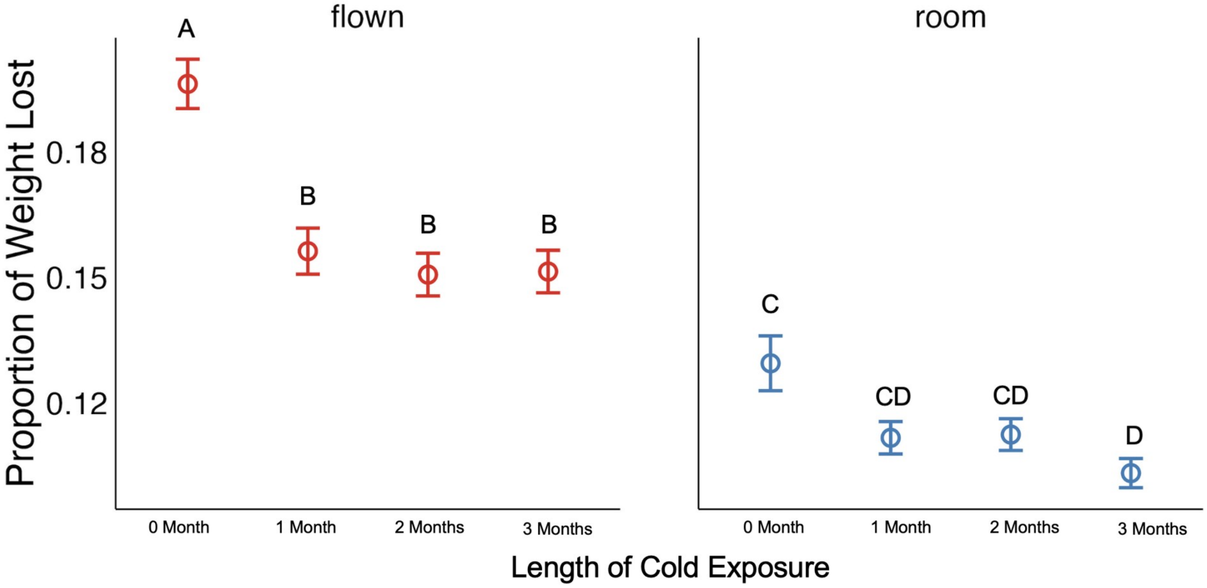


Figure 3.17. Proportion of weight lost by beetles across different winter duration periods (0 to 3 months) and flight assay treatments (flown vs. non-flown). Each point represents the mean proportion of weight lost, with error bars indicating standard errors. The flight treatment (flown) is represented in red, while the room treatment (non-flown) is shown in blue. Points marked with different letters (A, B, C, D) are significantly different based on Tukey HSD post-hoc tests. Data were analyzed using a beta regression model with a logit link function, accounting for nested experimental structures. P-values for independent variables were generated using ANOVA tables from the linear mixed effects models.

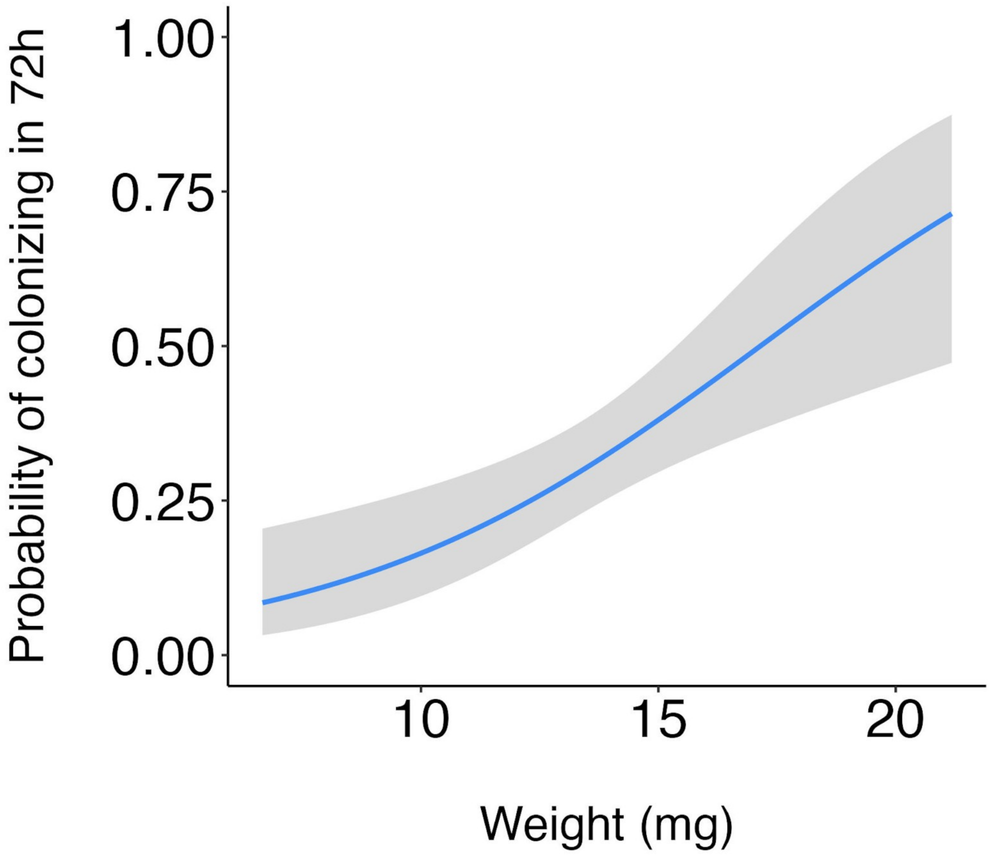


Figure 3.18. Probability of colonizing lodgepole pine bolts within 72 hours as a function of female beetle weight (mg) (N = 158, across four overwintering timepoints). The response variable is the binary probability of host entry initiation within three days. The analysis reveals a significant positive relationship between fresh weight and colonization probability (ANOVA, $\chi^2 = 11.29$, df = 1, $p = 0.0008$). The shaded area represents the 95% confidence intervals around the predicted probabilities.

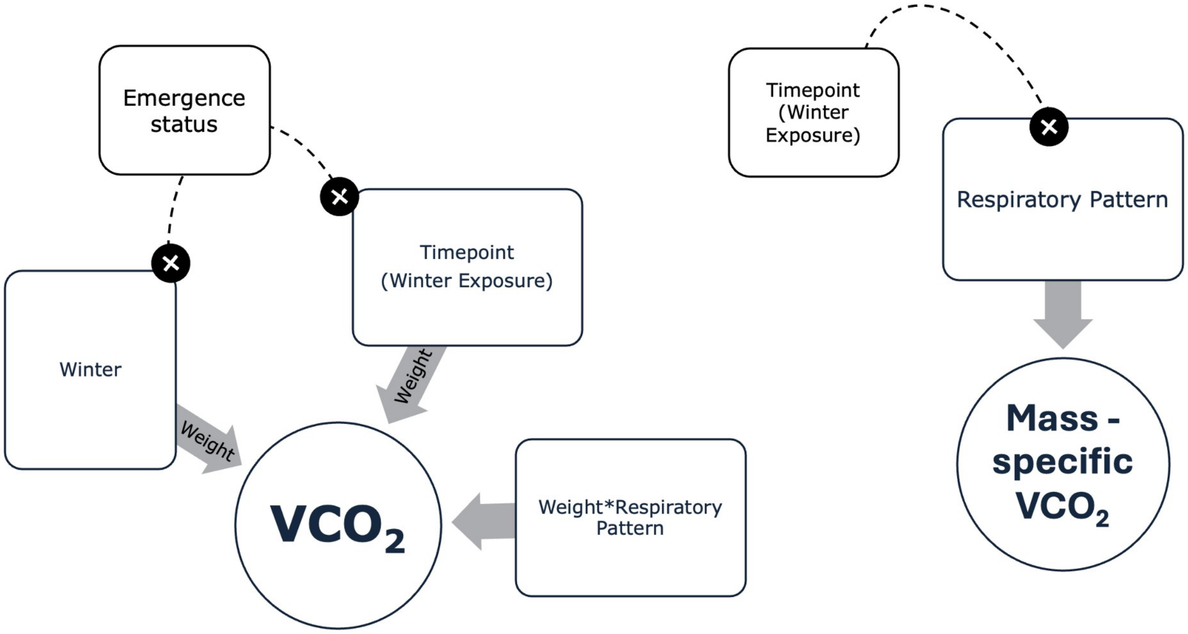


Figure 3.19. Cause and effect map illustrating the impact of various factors on VCO_2 levels in *D. ponderosae*. The left map shows the influence of weight, winter treatment, emergence status, respiratory pattern and winter timepoint on general VCO_2 levels, while the right map focuses on mass-specific VCO_2, highlighting the effects of respiratory pattern and winter timepoint. The arrows represent the relationships between the causes and their respective effects on VCO_2 and mass-specific VCO_2 levels. When analyzing the effect of body weight on beetle VCO_2 levels, considering winter treatment and timepoints (the duration of exposure to winter cold), we found a significant interaction effect. This indicates that winter conditions, in combination with timepoints, influence VCO_2 levels through their impact on body weight. However, when using mass-specific VCO_2, where body weight is incorporated into the response variable, the effects of winter treatment and timepoints become non-significant. This implies that, once weight is controlled for, winter treatment and timepoints don't have a direct impact on VCO_2 levels, indicating that their primary influence on metabolism comes through changes in body weight. In other words, the metabolic changes observed under winter conditions are largely mediated by the beetles' body weight, with winter influencing VCO_2 levels indirectly via its effect on weight.

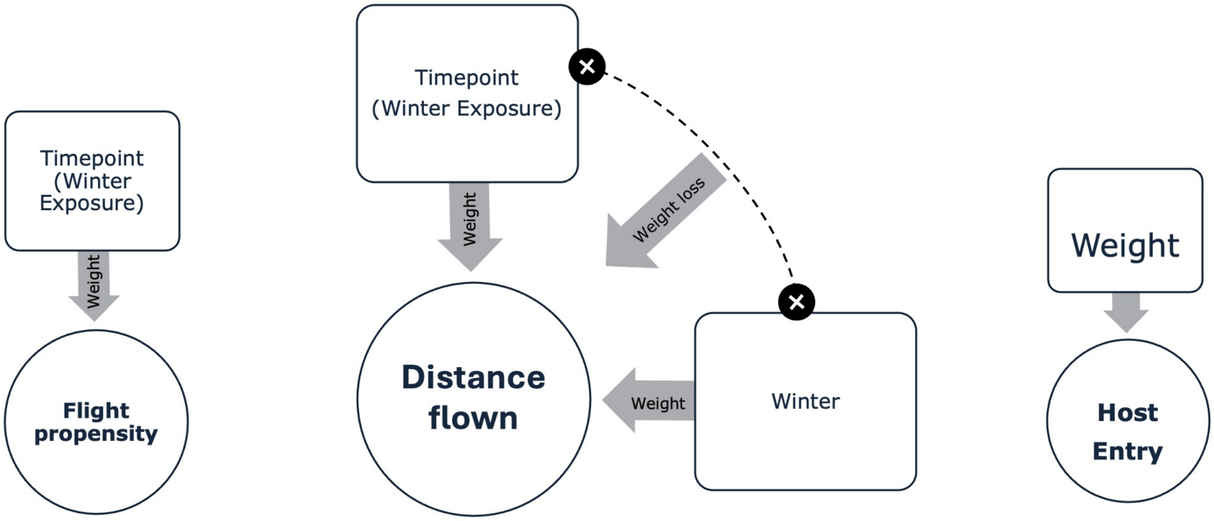


Figure 3.20. Cause and effect map illustrating the influence of various factors on flight propensity, flight distance, and host entry in beetles. The left section shows that flight propensity is influenced by the timepoint of winter exposure. The middle section highlights the impact of winter exposure and the interaction between timepoint and weight loss on the distance flown. The right section emphasizes the role of body weight in determining host entry success. Across all sections, beetle body weight is a critical factor influencing physiological behaviors such as flight and host entry, demonstrating its central role in post-winter beetle activities.

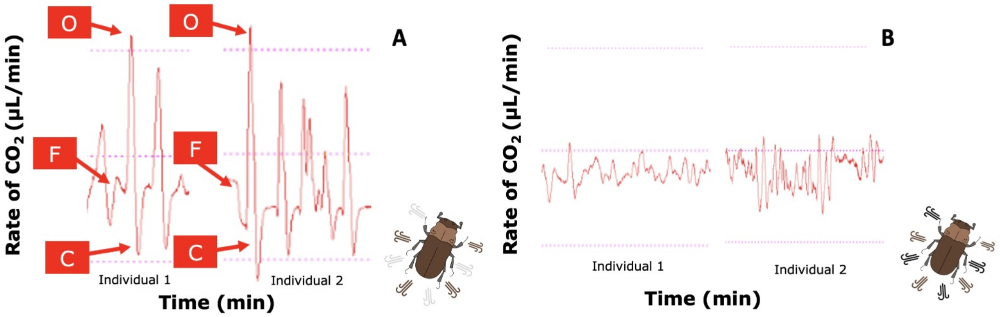


Figure 3.21. Respirometry recordings of adult *Dendroctonus ponderosae* illustrating **(A)** discontinuous, cyclic, and **(B)** continuous gas exchange patterns. The CO_2 output graph panels were extracted from the ExpeData software (V1.9.27, Sable Systems International, Las Vegas, NV). Note the closed (*C*), flutter (*F*) and open (*O*) periods in each cycle of discontinuous ventilation. The small peaks in the O_2 and CO_2 traces during the (*F*) period represent spiracular fluttering.

Tables

Table 3.1. Summary of beetle counts by overwintering treatment (OW Treatment) and overwintering timepoints (Timepoint) in the metabolism experiment. The table shows the number of beetles (n) in each category.

Timepoint	OW Treatment	N
0	Control	39
1	Control	34
	Winter	36
2	Control	3
	Winter	43
3	Control	9
	Winter	42
	Total	206

Table 3.2. Summary of beetles' flight responses across timepoints and treatment conditions.

Timepoint	OW Treatment	Flight Treatment	N	N (flew)
0	Control	Flown	79	60
	Control	Room	42	
1	Control	Flown	51	28
	Control	Room	52	
	Winter	Flown	53	28
	Winter	Room	36	
2	Control	Flown	39	22
	Control	Room	42	
	Winter	Flown	68	42
	Winter	Room	51	
3	Control	Flown	29	16
	Control	Room	26	
	Winter	Flown	89	53
	Winter	Room	74	
		Total	731	

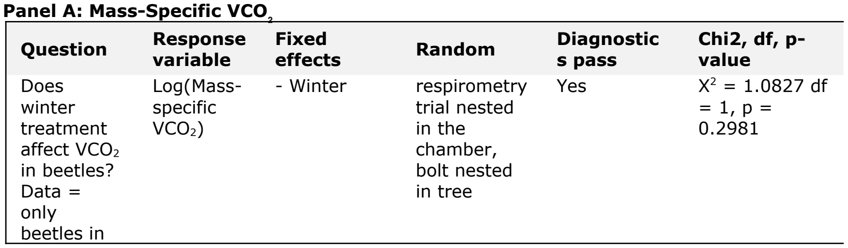
Table 3.3. Summary of beetle counts by overwintering treatment (OW Treatment) and overwintering timepoints (Timepoint) in the host entry experiment. The table shows the number of beetles (n) in each category.

Timepoint	OW Treatment	N
0	Control	48
1	Control	12
	Winter	20
2	Control	0 *
	Winter	39
3	Control	6
	Winter	33
	Total	158

* Due to unknown factors, likely attributed to heavy wood boring and mold, bolts assigned to the timepoint 2 control group did not yield any beetles.

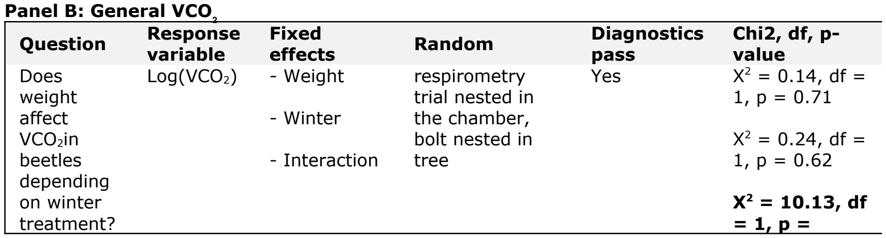
Table 3.4. Comparison of factors influencing VCO_2 levels in beetles. **Panel A** focuses on environmental factors such as winter treatment, timepoint, and emergence on the log transformed mass-specific VCO_2 levels in either the cooler beetles or all of the beetles, while **Panel B** analyzes environmental factors including weight, respiratory pattern, and interactions on the log transformed VCO_2 levels in beetles. Weight refers to the pre-respirometry measurement of the beetles' weight. Significant results, highlighted by X^2 tests, are presented for each analysis.

Panel A: Mass-Specific VCO_2



Question	Response variable	Fixed effects	Random	Diagnostics pass	Chi2, df, p-value
Does winter treatment affect VCO_2 in beetles? Data = only beetles in	Log(Mass-specific VCO_2)	- Winter	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 1.0827$ df = 1, p = 0.2981

Panel B: General VCO_2



Question	Response variable	Fixed effects	Random	Diagnostics pass	Chi2, df, p-value
Does weight affect VCO_2in beetles depending on winter treatment?	Log(VCO_2)	- Weight - Winter - Interaction	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 0.14$, df = 1, p = 0.71 $X^2 = 0.24$, df = 1, p = 0.62 **$X^2 = 10.13$, df = 1, p =**

control group					
Does the winter timepoint and treatment affect VCO_2 in beetles? Data = all beetles in tp 1, 2, and 3	Log(Mass-specific VCO_2)	- Winter - Timepoint - Interaction	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 0.0424$, df = 1, p = 0.8369 $X^2 = 0.0424$, df = 3, p = 4.5556 $X^2 = 3.5149$, df = 2, p = 0.1725
Does the winter timepoint affect VCO_2? Data = only beetles in **control** group	Log(Mass-specific VCO_2)	- Timepoint	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 2.9018$, df = 2, p = 0.2344
Does stage of emergence affect VCO_2? Data = only beetles in **control** group	Log(Mass-specific VCO_2)	- Emergence	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 1.201$, df = 2, p = 0.548
Does the average activity in the chamber affect VCO_2? Data = only beetles in **control** group	Log(Mass-specific VCO_2)	- Average activity in the respirometry chamber	respirometry trial nested in the chamber, bolt nested in tree	No	
Does winter treatment and stage of	Log(Mass-specific VCO_2)	- Emergence, - Winter	respirometry trial nested in the chamber,	Yes	$X^2 = 5.78$ df = 2, p = 0.06
Data = all beetles					**0.001***
Does weight and time exposed to winter conditions affect VCO_2? Data = only beetles in ow group	Log(VCO_2)	- Timepoint - Weight - Interaction	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 0.6408$, df = 2, p = 0.7259 $X^2 = 0.0278$, df = 1, p = 0.8676 $X^2 = 4.0324$, df = 2, p = 0.1332
Does weight and respiratory pattern affect VCO_2 in control beetles? Data = only beetles in control group	Log(VCO_2)	- Respiratory pattern - Weight - Interaction	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 16.0154$, df = 1, p < 0.001* $X^2 = 0.0973$, df = 1, p = 0.75515 $X^2 = 4.2413$, df = 1, p = 0.03945*
Does timepoint and stage of emergence affect VCO_2? Data = all beetles at tp 1-3 (excluding tp = 0 where no ow beetles exist)	Log(VCO_2)	- Emergence - Timepoint - Interaction	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 4.40$, df = 2, p = 0.11 $X^2 = 5.00$, df = 2, p = 0.08 $X^2 = 10.27$, df = 4, p = 0.04*
Does treatment and stage of emergence affect VCO_2? Data = all beetles at tp 1-3 (excluding	Log(VCO_2)	- Emergence - Treatment - Interaction (Fig. 11)	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 5.78$, df = 2, p = 0.06. $X^2 = 1.58$, df = 1, p = 0.21. $X^2 = 16.72$, df = 2, p < 0.001*

emergence affect VCO_2? Data = all beetles		- Interaction	bolt nested in tree		
Does respiratory pattern affect VCO2 in control beetles? Data = only beetles in **control** group	Log(Mass-specific VCO_2)	- Respiratory pattern	respirometry trial nested in the chamber, bolt nested in tree	Yes	$X^2 = 15.267$ df = 1, $p < 0.001$*
Does treatment and stage of emergence affect VCO_2? Data = all beetles at tp 1-3 (excluding tp = 0 where no ow beetles exist)	Log(Mass-specific VCO_2)	- Emergence - Timepoint - Interaction	respirometry trial nested in the chamber, bolt nested in tree	No [a]	

tp = 0 where no ow beetles exist)					
Does weight, timepoint and treatment affect VCO_2? Data = all beetles	Log(VCO_2)	- Weight - Timepoint - Interactions	respirometry trial nested in the chamber, bolt nested in tree	No [a]	
Does weight, timepoint and treatment affect VCO_2 in beetles? Data = all beetles in tp 1, 2, and 3	Log(VCO_2)	- Treatment - Timepoint - Weight - Interactions	respirometry trial nested in the chamber, bolt nested in tree	No [a]	
Does weight, timepoint and stage of emergence affect VCO_2? Data = all beetles at tp 1-3 (excluding tp = 0 where no ow beetles exist)	Log(VCO_2)	- Emergence - Timepoint - Weight - Interactions	respirometry trial nested in the chamber, bolt nested in tree	No [a]	
Does weight, timepoint and respiratory pattern affect VCO_2 in control beetles? Data = only beetles in	Log(VCO_2)	- Respiratory pattern - Timepoint - Weight - Interactions	respirometry trial nested in the chamber, bolt nested in tree	No [a]	

control group

[a] fails equal variance between groups (Levenes) and within group deviations from uniformity, reduction in sample size for control group in tp = 2 and 3 causes change in variance.

Table 3.5. Summary of statistical analyses examining factors influencing flight propensity, flight distance, proportional weight loss, and host entry in beetles. The table presents results from generalized and linear mixed effects models, focusing on the impact of pre-flight weight, timepoint, treatment, and interactions on various response variables. Significant effects are highlighted by X² tests with corresponding p-values, indicating the influence of these factors on beetle behavior.

Flight experiment				
Question:	**Response variable**	**Fixed effects**	**Diagnostics pass**	**Chi2, df, p-value**
Does pre-flight weight affect flight propensity? Generalized linear mixed effects model (binomial)	Flew (yes or no)	• Pre-flight weight Random: mill number, bolt nested in tree	Yes	$X^2 = 58397$, df = 1, $p < 0.001$*
Does timepoint affect flight propensity of control beetles (kept in cooler)? Generalized linear mixed effects model (binomial)	Flew (yes or no)	• Timepoint • Pre-flight weight (Fig. 14) Random: mill number, bolt nested in tree	Yes	$X^2 = 14.05$, df = 3, $p = 0.003$* $X^2 = 18.38$, df = 1, $p < 0.001$*

Does timepoint and treatment affect flight propensity of beetles from tp 1-3? Generalized linear mixed effects model (binomial)	Flew (yes or no)	• Timepoint • Treatment • Pre-flight weight • Timepoint * treatment Random: mill number, bolt nested in tree	Yes	Best model only includes pre-flight weight. M4_ow_propensity in script.
Does timepoint and pre-flight weight affect flight distance of control beetles (kept in cooler)? Linear mixed effects model	Square root distance flown (km)	• Timepoint • Pre-flight weight • Timepoint * Pre-flight weight (Fig. 15) Random: mill number, bolt nested in tree	Yes	X^2 = 24.05, df = 3, p < 0.001 X^2 = 6.71, df = 1, p = 0.01* X^2 = 7.92, df = 3, p = 0.048*
Does timepoint, treatment, and pre-flight weight affect flight distance of beetles from tp-1-3? Linear mixed effects model	Square root distance flown (km)	• Timepoint • Treatment • Pre-flight weight • Timepoint * treatment (Fig. 16) Random: mill number, bolt nested in tree	Yes	X^2 = 9.1674, df = 2, p = 0.01* X^2 = 0.742, df = 1, p = 0.389 X^2 = 15.34, df = 3, p < 0.001* X^2 = 6.27, df = 2, p = 0.044*
Does flight, timepoint and affect proportional weight loss of beetles from tp-0-3? Linear mixed effects model	Proportional weight loss	• Flight (flown or not) • Timepoint (Fig.17) • Flight * Timepoint Random: mill number, bolt nested in tree	Yes	X^2 = 195.611, df = 1, p < 0.01* X^2 = 58.461, df = 3, p < 0.01* X^2 = 3.720, df = 3, p = 0.2933
Host Entry experiment				
Question:	**Response variable**	**Fixed effects**	**Diagnostics pass**	**Chi2, df, p-value**
Does weight affect host entry? Generalized linear mixed effects model (binomial)	Entry (yes or no)	• Weight (Fig. 18) Random: bolt nested in tree	Yes	X^2 = 11.29, df = 1, p < 0.001*
Does timepoint affect host entry? Generalized linear mixed effects model (binomial)	Entry (yes or no)	• Timepoint • Weight Random: bolt nested in tree	Yes	X^2 = 1.865, df = 3, p = 0.601 X^2 = 12.01, df = 1, p < 0.001*
Does timepoint and treatment affect host entry? Generalized linear mixed effects model (binomial)	Entry (yes or no)	• Timepoint • Treatment • Treatment * Timepoint • Weight	Yes	Best model only includes weight (M5 below).

		Random: bolt nested in tree		

[a] fails equal variance between groups (Levenes) and within group deviations from uniformity, reduction in sample size for control group in tp = 2 and 3 causes change in variance.

Table 3.6 Summary of linear mixed effects models analyzing factors influencing square root of total distance flown and host entry in beetles.

This table summarizes the results from several linear mixed effects models investigating the influence of pre-flight weight (preflyweight) in the flight capacity experiment, weight in the host entry experiment, timepoint (tp), and treatment (treat) on the square root of total distance flown, as well as host entry (Host entry) by beetles. Model formulas, statistical results (Chi-square tests with degrees of freedom and p-values), and the Akaike Information Criterion (AIC) are provided for each model.

Flight Capacity			
Model	**Formula**	**Results**	**AIC**
M1	Sqrt(total distance) ~ preflyweight*tp*treat + (1|mill num) + (1|tree/bolt)	pre_fly_weight: chisq = 16.6281, df = 1, p < 0.001* tp: chisq = 9.8456, df = 2, p = 0.007279* treat: chisq = 0.8445, df = 1, p = 0.358101 pre_fly_weight:tp chisq = 7.8740, df = 2, p = 0.019507* pre_fly_weight:treat chisq = 0.8829, df = 1, p = 0.347406 tp:treat chisq = 7.0812, df =2, p = 0.028995* pre_fly_weight:tp:treat chisq = 10.3834, df = 2, p = 0.005563*	618.78
M2*	Sqrt(total distance) ~ preflyweight + tp*treat + (1|mill num) + (1|tree/bolt)	pre_fly_weight chisq = 15.3444, df = 1, p < 0.001* tp chisq = 9.1674, df = 2, p = 0.01022 * treat chisq = 0.7416, df = 1, p = 0.38916 tp:treat chisq = 6.2688, df = 2, p =	614.15 * the best fit model

<table>
<tr><td></td><td></td><td>0.04353*</td><td></td></tr>
<tr><td>M3</td><td>Sqrt(total distance) ~ tp*treat + (1|mill num) + (1|tree/bolt)</td><td>tp
chisq = 7.2791, df = 2, p = 0.02626*
treat
chisq = 0.5202, df = 1, p = 0.47074
tp:treat
chisq = 4.1928, df = 2, p = 0.12290</td><td>621.90</td></tr>
<tr><td>M4</td><td>Sqrt(total distance) ~ preflyweight + tp + (1|mill num) + (1|tree/bolt)</td><td>tp
chisq = 7.6804, df = 2, p = 0.0214895*
pre_fly_weight
chisq = 13.3405, df = 1, p < 0.001*</td><td>613.53</td></tr>
<tr><td>Null</td><td>Sqrt(total distance) ~ (1| mill num) + (1|tree/bolt)</td><td></td><td>618.22</td></tr>
<tr><td colspan="4">Host entry without preceding flight behaviours</td></tr>
<tr><td>M5</td><td>Host entry ~ weight*tp*treat + (1|tree/bolt)</td><td>tp:
Chisq = 1.2601, df = 3, p = 0.738632
weight:
Chisq = 9.1858, df = 1, p = 0.002439*
treat:
Chisq = 1.1788, df = 1, p = 0.277596
tp:weight
Chisq = 3.6240, df = 3, p = 0.305036
tp:treat
Chisq = 1.3588, df = 1, p = 0.243743
weight:treat
Chisq = 0.1727, df = 1, p = 0.677709
tp:weight:treat
Chisq = 0.1931, df = 1, p = 0.660353</td><td>199.81</td></tr>
<tr><td>M6</td><td>Host entry ~ weight + tp*treat + (1|tree/bolt)</td><td>weight:
Chisq = 12.6416, df = 1, p = 0.0004*
tp:
Chisq = 2.4089, df = 3, p = 0.4919836
treat:
Chisq = 2.1809, df = 1, p =</td><td>195.36</td></tr>
</table>

		0.1397357 tp:treat: Chisq = 1.2254, df = 1, p = 0.2683077	
M7	Host entry ~ tp*treat + (1|tree/bolt)	tp: Chisq = 1.5838, df = 3, p = 0.6631 treat: Chisq = 1.3960, df = 1, p = 0.2374 tp:treat Chisq = 1.2492, df = 1, p = 0.2637	207.86
M8	Host entry ~ weight + tp + (1|tree/bolt)	tp chisq = 1.865, df = 3, p = 0.600895 weight chisq = 12.014, df = 1, p = 0.0005*	195.02
M9*	Host entry ~ weight + (1|tree/bolt)	weight chisq = 11.29, df = 1, p = 0.00078*	190.98 * the best fit model
Null	Host entry ~ (1|tree/bolt)		201.67

Chapter 4: General Conclusion

The mountain pine beetle (*Dendroctonus ponderosae*) represents a significant ecological and economic threat across North American forests due to its ability to exploit host trees in a range of environmental conditions (Carroll et al. 2006b). Its recent range expansion underscores its adaptive capacity, particularly in responding to new and diverse climatic challenges. Understanding the metabolic adaptations of *D. ponderosae* is essential for predicting its survival, reproduction, and broader ecological impacts on forest ecosystems.

In Chapter 2, my study examined the effects of flight on the metabolic rate of *D. ponderosae*. Surprisingly, flight did not significantly elevate metabolic rates across manipulated energy conditions (flown, room, or fridge). However, beetle weight emerged as a key determinant, significantly influencing metabolic rates across all conditions. This supports previous findings that larger beetles are generally more successful at dispersing and colonizing new hosts. The lack of interaction between weight and flight treatment suggests that the metabolic cost of flight is independent of the energy manipulation applied. The role of respiratory patterns, especially the advantage of cyclic or discontinuous breathing, may provide adaptive benefits for *D. ponderosae* living under tree bark, where oxygen availability fluctuates. Lighton (1996) highlighted how discontinuous gas exchange could minimize respiratory water loss, which likely enhances survival in the dry inner bark environment. The significant interactions between flight distance and proportional weight loss also influenced metabolic rates, underscoring their importance in understanding the dispersal and colonization abilities of *D. ponderosae*. The energy expended during flight, as reflected in post-flight weight loss, significantly impacts beetles' success in locating and colonizing new hosts. Previous research (Evenden et al. 2014; Wijerathna et al. 2019) has shown that beetles traveling longer distances experience reduced lipid reserves, potentially affecting their survival and reproductive success in new environments.

Chapter 3 of my thesis demonstrates that neither overwinter treatment alone nor beetle weight significantly affects VCO_2 levels, contrasting with Chapter 2’s findings where non-winter conditions showed a positive correlation between VCO_2 and beetle weight. However, the significant interaction between overwinter treatment and weight highlights how environmental conditions modulate beetles’ metabolic responses. This interaction is consistent with research showing that climatic factors influence *D. ponderosae*'s flight capacity and energy allocation, which are crucial for synchronized emergence, reproduction, and colonization (Elkin and Reid, 2005; Evenden et al. 2014). The absence of significant changes in VCO_2 levels across different durations of winter exposure suggests that once beetles acclimate to stable low temperatures, their metabolic rates stabilize. This finding supports previous studies indicating that beetles adapt their metabolic rates in constant environments during overwintering (Harrison et al. 2012; Williams et al. 2016; Bleiker and Smith, 2019). Early-emerging beetles at 5°C exhibited higher metabolic activity, reflecting their readiness for synchronized dispersal and reproductive activities (Irwin and Lester, 2000). Synchronization is vital for *D. ponderosae* populations, as it enhances the timing of mass attacks on host trees, improving their success in colonization. Additionally, the positive correlation between body mass and flight propensity underscores the importance of size in locomotor activity, consistent with studies on metabolic rate and body mass correlations in *D. ponderosae* (Overgaard and MacMillan, 2017). Moreover, *D. ponderosae*'s ability to maintain ion balance during cold exposure, avoiding ionoregulatory collapse, suggests that larger beetles with greater internal reserves may better endure prolonged activity and stressful conditions, thereby enhancing their capacity for sustained dispersal after overwintering (MacMillan, 2019; Overgaard et al. 2021). This relationship between size, metabolic stability, and synchronized emergence is crucial for understanding the species' dispersal and survival strategies in cold environments.

Overall, my thesis provides a comprehensive analysis of the effects of flight and winter conditions on adult life history traits of *D. ponderosae*. Key findings reveal that neither overwinter treatment nor beetle weight alone significantly influenced VCO_2 levels, underscoring the complexity of environmental interactions. The significant interaction between overwinter treatment and beetle weight highlights how these factors are interdependent and influenced by environmental conditions. Moreover, my research confirms the importance of beetle weight in influencing both flight propensity and metabolic rates, suggesting that larger beetles, with greater internal reserves, are better equipped for prolonged activity and survival under harsh conditions. This study also suggests that metabolic suppression during overwintering facilitates synchronized emergence, which is critical for coordinated mass attacks on new host trees. By examining these interactions, my thesis contributes valuable insights into the adaptability and survival strategies of *D. ponderosae*, enhancing our understanding of its potential impact on forest ecosystems.

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