Factors Influencing Mountain Pine Beetle (Coleoptera: Curculionidae: Scolytinae) Dispersal by Flight and Subsequent Trade-off Between Beetle Flight and Reproduction

by

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Abstract

The mountain pine beetle, *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae), has an obligatory dispersal phase before selection of a suitable host for brood production. Flight during the dispersal phase is affected by both beetle physiology and environmental factors. Mountain pine beetle utilizes energy obtained during larval development and adult feeding before emergence from the natal tree host to fuel flight. Energy expenditure on flight may have consequences for subsequent reproduction. Mountain pine beetles were flown on computer-linked flight mills to establish how beetles utilize energy during flight and to determine how environmental conditions affect flight capacity. Mountain pine beetles utilize both lipids and carbohydrates to fuel the flight. Neutral lipids are the major fuel for flight. Beetles held at lower temperatures were more likely to fly and flew for longer distances than beetles held at warmer temperatures. Beetles increase flight distance and duration with day length. Beetles fly against wind speeds of 0-2 m/s in a wind tunnel. The effect of flight on subsequent reproduction was tested by introducing beetles flown on flight mills and un-flown control beetles into bolts of either lodgepole, *Pinus contorta* Douglas. ex. Loud. var. *latifolia*), or jack, *Pinus banksiana* Dougl., pine to reproduce. There is a trade-off between flight and reproduction in the mountain pine beetle as control beetles that did not fly produced a significantly higher number of offspring than flown beetles. The pine host that offspring were reared in affected the number of offspring and their condition, as more offspring emerged from jack pine but higher quality offspring emerged from lodgepole pine. Offspring from flown parents have a slightly lower body condition compared to the offspring from control adults. The study reveals physiological and environmental factors that influence dispersal capacity of mountain pine beetle and a trade-off between flight and reproduction in mountain pine beetle.

Preface

This document presents three studies (Chapter 2, Chapter 3 and Chapter 4) intended for publication and is a collaborative work led by Dr. Maya Evenden of the University of Alberta. I was responsible for data collection and analysis, literature review and manuscript composition. Dr. Evenden was involved with concept formation and manuscript composition throughout the work. Dr. Heather Proctor provided manuscript edits. Additionally, Caroline M. Whitehouse of the University of Alberta collected data used in Chapter 4. All research presented in this thesis was conducted in accordance with all applicable laws and rules set forth by provincial and federal governments and the University of Alberta and all necessary permits were in-hand when the research was conducted.

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Chapter 1 : Introduction

Dispersal is a basic animal life history trait that allows individuals to change their habitat rather than confined to a habitat, and can increase individual fitness (Bowler and Benton 2005). Dispersal refers to the movement of individuals from the natal site to the site of reproduction or movement between successive sites of reproduction (Clobert et al. 2012). Animal movement ranges from small movement in search for foraging and/or breeding sites to long distance multi-generational migrations spurred by seasonal changes (Roff and Fairbairn 2001). Classification of individuals on the basis of movement includes two major categories: movement within the individual's home range and movement beyond the home range (Dingle 1996). Individuals move within the home range to obtain resources for survival and reproduction, and this movement ceases when the resource is encountered. Movement beyond the home range occurs either by ranging (movement over an area to explore and locate new area of residence) or by migration (Dingle 2001). Animals disperse in search of food (Loxdale and Lushai 1999, Dingle 2001), mates (Rudinsky 1962, Real 1990, Stamps 2001, Dingle and Drake 2007) or to escape from deteriorating habitats (Dingle 2001, Weins 2001) and from predatory risks (Weisser 2001). Dispersal is driven by a combination of individual characteristics (physiological condition, sex, age, reproductive stage) and environmental effects (temperature, day lengths, wind) (Lambin et al. 2001, Bowler and Benton 2005, Benard and McCauley 2008). The costs (predation, starvation, less suitable habitats) and consequences of dispersal may alter with these factors (Clobert et al. 2012). The study of population dynamics is important in relation to dispersal, because population density can affect the relationship between habitat quality and individual fitness (Loxdale and Lushai 1999, Benton et al. 2005, Clobert et al. 2012). Insect dispersal includes adult movement by flight (Robertson et al. 2007, Roff and Fairbairn 2007) and by wind (Torres-Vila et al. 1997, Jackson et al. 2008) or movement of flightless juvenile or adult individuals (Hardie et al. 2001). Dispersal can be affected by both exogenous (environmental conditions, habitat quality, predators etc.) and endogenous factors (physiological condition, sex, age etc.) which make it difficult to study (Diss et al. 1996, McCauley and Rowe 2010, Suhonen et al. 2010).

Climatic conditions can affect energy metabolism and movement capacities of insects (Chubaty et al. 2014, Atkins 1960, Rudinsky and Vite 1956). The daily movements of the lesser marsh grasshopper, *Chorthippus albomarginatus* De Geer (Acrididae: Orthoptera), are enhanced in warmer climates, which may result in faster spread rates under climate warming (Walters et al. 2006). Brown marmorated stink bug nymphs, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), reduce their walking distance with increased surface temperature (Lee et al. 2014). Dispersal of migratory aphids (Homoptera: Aphididae) is a combination of passive movements by wind and active movements during take-off and flight phases (Reynolds and Reynolds 2009).

Physiological condition has direct effects on insect dispersal. The flight distance of *Monochamus galloprovincialis* Olivier (Coleoptera: Cerambycidae) is positively associated with energy allocation to thoracic reserves in terms of thorax weight, which increases with beetle age (David et al. 2015). Insect age (Ishiguri and Shirai 2004, Elliott and Evenden 2009, Arnold et al. 2016) and sex (Hughes et al. 2004, Elliott and Evenden 2009, Arnold et al. 2016) have varying effects on flight in different insects. In the moth *Choristoneura conflictana* Walker (Lepidoptera: Tortricidae), lipid stores influence flight in a way that depends on sex, mating status, and age (Elliott and Evenden 2009). Reproductive status of insects affects dispersal and its effect is dependent on sex (Schumacher et al. 1997, Yanagi and Miyatake 2003, Elliott and Evenden 2009).

Trade-offs between flight capability and reproduction are common in insects in which flight is the main mode of dispersal. Flight is one of the most energy demanding activities conducted by insects (Candy et al. 1997). High investment in flight activity (Shirai 1995, Zhao and Zera 2002) and flight muscle development and maintenance (Marden 2000) is required for active dispersal between suitable habitats. Due to the high energetic cost of active dispersal (Dudley 2001), dispersal may result in an energetic trade-off with other life history traits such as survival and reproduction (Johnson 1969, Boggs 1992). Loss of energy during dispersal can have adverse effects on capital breeders that store energy to use later in reproduction (Stearns 1992). The oogenesis-flight syndrome predicts a trade- off between female reproduction and flight (Johnson 1969). Trade–offs between flight and reproduction occur in wing-polymorphic insects in which the female flying morphs have reduced reproductive capacity (Guerra 2011) and short-winged females with low flight capacity produce larger eggs than fully winged females (Steenman et al. 2013). In wing-monomorphic species, energy use during flight can decrease subsequent reproductive output (Isaacs and Byrne 1998, Zhang et al. 2009, Gibbs and van Dyck 2010, Elliott and Evenden 2012, Duthie et al. 2014).

Bark beetles (Coleoptera: Curculionidae: Scolytinae) are a species-rich group of insects that specialize in feeding on sub-cortical tissue of host plants. Irruptive outbreaks of some bark beetles such as *Ips* and *Dendroctonus* can cause ecological (Kurz et al. 2008, Bunnell et al. 2011, Dhar et al. 2015) and economical (Grégoire and Evans 2004) damage by killing large tracts of forest (Raffa et al. 2008, Lausch et al. 2013). Majority of tree-killing bark beetles complete their life cycle under the bark except a short adult flight period. Adults have an obligatory dispersal phase in which emerging beetles must fly from the depleted natal habitat in search of a suitable host for brood production (Wood 1982). Most species of bark beetle emerge from brood galleries in spring or summer and seek a new host and a mate. Bark beetles use several mechanisms of dispersal including within-stand dispersal by flight within and between infested patches, and to un-infested patches in the same stand (Safranyik et al. 1992, Robertson et al. 2007). Alternatively, long-range, wind-aided dispersal can carry beetles for hundreds of kilometres (Nilssen 1984, Jackson et al. 2008, de la Giroday et al. 2011, 2012, Samarasekera et al. 2012) from the original habitat. The effective dispersal of bark beetles by flight is often no more than a few hundred meters (Salom and McLean 1989, Safranyik et al. 1992, Zumr 1992) within which most successful attacks tend to occur (Wichmann and Ravn 2001). Bark beetles are able to fly longer distances, however, as illustrated in both laboratory flight mill studies (Jactel 1993, Erbilgin et al. 2014) and field studies (Jactel and Gaillard 1991, Yan et al. 2005). Bark beetle dispersal distance varies among and within species, with beetle condition, distribution of susceptible hosts and environmental conditions (Franklin and Grégoire 1999, Franklin et al. 2000). Bark beetle host colonization after dispersal is dictated by elaborate chemical communication, in which pioneer beetles use aggregation pheromones to attract enough individuals to overcome the host tree defenses (Pitman 1968, Pitman and Vite 1968). After successful host colonization, beetles release anti-aggregation pheromones to discourage further colonization (Rudinsky et al. 1974). Bark beetle flight during the dispersal phase is an important aspect of their life cycle that allows them to reach high quality hosts even at high population density.

Dendroctonus (Coleoptera: Curculionidae: Scolytinae) is a small genus that includes most economically and ecologically important conifer-killing bark beetles of the world. The genus contains 19 species in total with 17 species primarily distributed in North and Central America, one species in China and one species in Eurasia (Six and Bracewell 2015). *Dendroctonus* dispersal is linked to beetle physiology (Atkins 1966, Atkins 1969, Thompson and Bennett 1971, Kinn et al. 1994, Williams and Robertson 2008, Chen et al. 2011, Erbilgin et al. 2014), the number and distribution of suitable host species (Robertson et al. 2007), environmental conditions (Atkins, 1959, McCambridge 1971) and host and/or attractive pheromone stimuli (Wood 1982). In the absence of pheromones, the initial flights by newly emerged beetles tend to distribute them widely throughout the forest environment (Raffa and Berryman 1980). Flight capacity and flight patterns of beetles are variable across the genus *Dendroctonus*. Flight has been studied in *D. armandi* Tsai and Li (Chen et al. 2010), *D. frontalis* Zimmermann (Kinn et al. 1994), *D. pseudotsugae* Hopkins (Williams and Robertson 2008), *D. valens* LeConte (Liu et al. 2014), *D. simplex* LeConte (Langor 1987), *D. brevicomis* LeConte (Fettig et al. 2004), *D. rufinnis* Kirby (Gary and Dyer 1972) and *D. ponderosae* Hopkins (Evenden et al. 2014). Both field (Wang et al. 2010) and laboratory studies using flight mills (Kinn et al. 1994, Williams and Robertson 2008, Chen et al. 2010) have examined aspects of beetle flight capacity, including distance, duration, velocity and beetle propensity to fly. The average flight distance of *Dendroctonus* beetles lies between 0.28 km to 5.95 km under laboratory conditions (Kinn et al. 1994, Chen et al. 2010, Evenden et al. 2014), but active flight distances are lower in field studies (Safranyik et al. 1992, Wang et al. 2010). The average flight duration and average flight velocity vary between 0.01-3.3 h and 1.3 - 2.38 km/h, respectively, under controlled laboratory conditions (Chen et al. 2010, Kinn et al. 1994). The Chinese white pine beetle, *Dendroctonus armandi* shows three different flight behaviours; intermittent, short-burst and sustained flight, with short bursts being the predominant behaviour (Chen et al. 2010). Females are capable of more sustained flight than males (Chen et al. 2011).

The mountain pine beetle (MPB), *Dendroctonus ponderosae* is native to western North America. Mountain pine beetle distribution within Canada has historically been restricted to British Columbia, though it has recently invaded higher latitudes and new habitats east of Rocky Mountains (Cullingham et al. 2011). The primary hosts of MPB are lodgepole pine (*Pinus contorta* Douglas ex. Loud. var. *latifolia*) and ponderosa pine (*Pinus ponderosa* Douglas ex P. Laws &. C. Laws). In its recently expanded range, MPB has attacked the novel host jack pine (*Pinus banksiana* Lamb.) (Cullingham et al. 2011, de la Giroday et al. 2012) and hybrids of lodgepole and jack pine (Cullingham et al. 2011). Epidemic MPB populations have killed approximately 18.3 million ha of lodgepole pine in British Columbia during the recent outbreak (www.for.gov.bc.ca). In addition to extensive forest losses, MPB outbreaks may increase fuel loading, affect watershed quality, wildlife composition and recreational values (Safranyik et al. 1974, McGregor 1985). Due to the high economic and ecological impact of MPB on pine in Canadian forests, and the potential for increased range expansion through the boreal forest, insight into MPB dispersal will increase our understanding of its population dynamics and impacts on forests in its expanded range.

Mountain pine beetles have an obligatory dispersal phase in which adult beetles find a new host for brood production. Females are the pioneers in host finding. On reaching a suitable host tree, females release the aggregation pheromone *trans*-verbenol, which attracts both sexes to initiate the mass attack (Pitman 1968). Arriving males release the aggregation pheromone *exo*-brevicomin, which further attracts females to overwhelm tree-defenses in the mass attack (Pureswaran et al. 2000). Both sexes produce anti-aggregation pheromones, frontalin and verbenone, to discourage further aggregation at the optimal beetle density (Pureswaran and Borden 2003). Despite the extensive economic and ecological damage caused by MPB, dispersal of adult beetles remains a poorly understood aspect of this species' ecology. Mountain pine beetle dispersal includes stand-level dispersal (Safranyik et al. 1992, Williams and Robertson 2008) and long- distance dispersal above the canopy aided by wind (Jackson et al. 2008, de la Giroday et al. 2011, 2012). Individual MPBs are capable of flying over 24 km under lab conditions and flight velocity range from 1.55 to 1.93 km/h (Evenden et al. 2014). In the field, however, MPB fly for shorter distances compared to the laboratory studies. A larger proportion of MPB were trapped within 30 m of the release site within 3 days of release in a mark-recapture experiment compared to the beetles that captured beyond 30 m range (Safranyik et al. 1992). Mountain pine beetle can fly against wind at wind speeds up to 2 m/s under natural conditions (Safranyik 1989). Bigger MPB are more likely to fly and can fly longer and further than smaller beetles. Despite the presence of sexual size dimorphism in this species (Lyon 1958), beetle sex has no effect on MPB flight capacity. Flight capacity decreases with beetle age post emergence from the natal habitat. Older beetles (9-11 d post emergence) fly shorter distances than both young (1-3 d post emergence) and middle-aged (5-7 d post emergence) beetles (Evenden et al. 2014). Beetle physiology and abiotic factors (temperature, day length, wind speed) may affect the dispersal capacity of MPB. The energy condition of MPB affects host location (Elkin and Reid 2010), colonization (Chubaty et al. 2009, 2014, Latty and Reid 2010) and reproductive success (Elkin and Reid 2005). Energy expenditure during flight may lead to a trade-off between flight and reproduction which may differ with tree host species. Knowledge of factors that influence MPB dispersal capacity and potential trade-offs between beetle flight and reproduction has implications for understanding the population dynamics of MPB and will be important in modeling of MPB dispersal.

Mountain pine beetles metabolize lipids during flight. Lipid content is lower in beetles that have been flown on flight mills than in un-flown control beetles, and flight distance is negatively correlated with the beetle post-flight lipid content (Evenden et al. 2014). It is not known if MPB utilize carbohydrates during dispersal. Energy metabolism during flight affects subsequent host selection behaviour of MPB. Individual MPB with low energy reserves are less selective and accept poorer quality host trees sooner than do individuals with high-energy reserves (Chubaty et al. 2009, 2014, Latty and Reid 2010). Individuals with higher lipid content are expected to survive longer (Safranyik 1976) and to have a greater flight capacity (Evenden et al. 2014). Young adult MPBs that are about to fly have larger metathoracic muscles compared to those at the reproductive stage post host colonization (Reid 1958). Mountain pine beetle flight may lead to a trade- off between flight and reproduction as male beetles synthesize aggregation pheromone, *exo*-brevicomin, in the fat body (Song et al. 2014). Low fat reserves after the flight may negatively affect the male pheromone production which, may lower the host colonization and reproduction. Starved female MPB produce smaller eggs compared to fed females (Elkin and Reid 2005) indicating adverse effects of low energy on reproduction.

Climatic factors can affect flight propensity and flight capacity of MPB. Frequency of take-offs from the natal host are affected by temperature (Safranyik 1989). During a mark recapture study, the proportion of MPB recaptured was directly related to the flight threshold temperature of 16°C and recapture rates increased during warm, clear periods (Safranyik et al. 1992). High temperature results in lower quality MPB with low lipid content and reduces the distance travelled by beetles during a field study (Chubaty et al. 2014). Within the optimum flight temperature range, flight response increases with light intensity and humidity (Safranyik 1989). Newly emerged MPBs are photopositive and will orient to a spot source of light over diffuse light. Females orient more toward a single light source than males (Atkins 1966). Scolytids generally fly in warm-fair weather with upward convection currents (Chapman 1967). During a mark-recapture study, MPBs were recaptured both upwind and downwind from the release point. Initially, MPB disperse downwind in the absence of attractive chemicals and change their direction in response to the detection of pheromone plumes (Safranyik et al. 1992).

MPB range has recently expanded eastward across the Canadian Rocky Mountains into Alberta, where beetles have colonized the naïve host, jack pine (Cullingham et al. 2011, de la Giroday et al. 2012). Mountain pine beetle perform better in jack pine than in the historical host, lodgepole pine. (Erbilgin et al. 2014) and have higher reproductive success in naïve lodgepole pine populations in an area that has not previously been exposed to outbreaks (Cudmore et al. 2010). Long distance dispersal occurs by convection currents pushing beetles upward above the canopy and these beetles are carried by wind over long distances (Furniss and Furniss 1972): however, only 0.2% of marked MPB dispersed above the canopy during a mark-recapture study while a higher proportion was trapped below the canopy level (Safranyik et al. 1992). In addition, MPB range expansion has occurred into high-elevation stands of whitebark pine (*P. albicaulis* Englem) (Logan et al. 2010).

Objectives

Mountain pine beetle dispersal capacity is a seldom studied factor that could greatly affect population dynamics of the pest. Here, we assess the factors that may affect the dispersal of adult

MPB. In Chapter 2, we assess the total energy budget of MPB and the changes of four major metabolic compounds (proteins, lipids, free sugars and glycogen) with beetle flight using a flight mill system. In Chapter 3, we examine the effects of changing particular environmental conditions (temperature, day length, wind) on beetle flight capacity using a flight mill system and a wind tunnel flight bioassay. In Chapter 4, we test the hypothesis that there is a trade- off between flight and reproduction in MPB. Overall, we assess the impact that physiological and environmental parameters have on flight, and the consequences that energy expenditure may have on the fecundity and offspring production of this destructive forest pest.

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Chapter 2 : Energy use by the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) for dispersal by flight.

2.1 Introduction

Insect dispersal is linked to morphological and physiological traits of individuals. Flight capable insects have functional wings and flight muscles, and a high amount of fuel that can be used for flight (Clobert et al. 2012). Insect flight activity demands a lot of energy, during which the metabolic rate increases 50- to 100-fold (Beenakkers et al. 1984) over non-flight metabolism. Movement to and from foraging sites and to new habitats can cause energy allocation trade-offs between dispersal and reproductive output (Zhao and Zera 2002). Insect flight muscles contain small amounts of energy reserves and the energy required to fuel extended flight is provided by energy substrates circulating in the hemolymph, which are constantly replenished by the fat body (Beenakkers et al. 1984). The fat body is the major site for energy storage and intermediate metabolism (Keeley 1985). Insects store energy reserves in the form of glycogen and triglycerides in adipocytes in the fat body. Energy substrates (i.e. lipids, carbohydrates, proteins) that fuel insect flight exhibit high interspecific variation. Some insects use only one energy substrate to fuel flight while others exploit more than one source of energy during different stages of flight (Beenakkers et al. 1984, Arrese and Soulages 2010).

Carbohydrates are the major substrate for flight in most species of Diptera and Hymenoptera while many species of Lepidoptera and Orthoptera use both carbohydrates and lipids (Beenakkers et al. 1984). Lipids and carbohydrates are gained through larval (Coll and Yuval 2004, Arrese and Soulages 2010) or adult feeding (Hanski et al. 2006, Arrese and Soulages 2010).The carbohydrate trehalose is a general substrate for insect flight. Short-distance flyers such as cockroaches use trehalose as the major fuel for flight (Elliott et al. 1984) and long distance flyers (e.g. locusts, mosquitoes) use trehalose at the initiation of flight and switch to lipid reserves for longer flights (Van Der Horst et al. 1980, Kaufmann and Briegel 2004). Long distance flyers (e.g. Lepidoptera and Orthoptera) mostly use lipids as their main energy source (Beenakkers et al. 1985) and some insects indirectly use lipids as the fuel for flight through the use of proline as the major energy substrate in the flight muscles. Proline is synthesized in the fat body of insects (Arrese and Soulages 2010). Many insects utilize fat body lipids during flight

(Beenakkers et al. 1985, Ziegler and Schulz 1986, Canavoso et al. 2003, Gade and Auerswald 2003, Kaufmann and Briegel 2004, Elliott and Evenden 2009). More than 90% of the stored lipids are neutral lipids in the form of triglycerides (Bailey 1975, Canavoso et al. 2001).

Bark beetles (Curculionidae: Scolytinae) use both carbohydrates and lipids to fuel flight (Thompson and Bennett 1971, Byers and Liifqvist 1989, Kinn et al. 1994, Chen et al. 2010, Evenden et al. 2014), but lipids are the major source and most of these are in the form of triglycerides (Kinn et al. 1994). This seems the case in many species of *Dendroctonus* (Coleoptera: Curculionidae) (Atkins 1969, Thompson and Bennett 1971, Kinn et al. 1994, Chen et al. 2011) but carbohydrates are used by some species during flight initiation (Thompson and Bennett 1971, Chen et al. 2010). Flight capacity of *Dendroctonus* is related to body lipid content, in which individuals with higher lipid content fly for longer distances (Kinn et al. 1994, Williams and Robertson 2008, Chen et al. 2011, Evenden et al. 2014) and durations (Hodges and Barras 1974, Kinn et al. 1994, Williams and Robertson 2008, Chen et al. 2011) compared to beetles with low body lipid levels.

Mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins is a major native pest of pine (*Pinus* Linnaeus (Pinaceae)) in western North America. Over 18 million hectares of pine forest have been killed by MPB during the most recent outbreak which start in early 2000 in western North America (www.nrcan.gc.ca). Host selection by MPB is associated with an obligatory dispersal phase during which beetles fly in search of a suitable host. Females are the pioneers in host location and colonization. Upon reaching a suitable host, females release the aggregation pheromone *trans*-verbenol which attracts both sexes of beetle to initiate the mass attack of the tree (Pitman 1968). Arriving males produce another aggregation pheromone *exo*-brevicomin which attracts mostly females. As tree colonization progresses, both sexes produce the anti-aggregation pheromone verbenone and males produce frontalin to discourage further host colonization (Pureswaran et al. 2000). Mountain pine beetles use stored energy obtained from feeding in the natal habitat during this obligatory flight period before brood production (Bentz 2006). Lipids fuel MPB flight (Thompson and Bennett 1971, Evenden et al. 2014), however, it is still not known if MPB use additional energy sources during flight. In MPB, lipid content is lower in beetles that have been flown on flight mills (7% of body mass) than in un-flown control beetles (15%), and flight distance is negatively correlated with beetle lipid content remaining after flight (Evenden et al. 2014).

The metathoracic flight muscles operate the hind wings, which are essential for MPB flight. Flight muscle resorption may occur after flight in some insects as an energy saving mechanism for subsequent activities such as reproduction. A reduction of flight muscle occurs in ambrosia beetle, *Trypodendron lineatum* Oliv. (Coleoptera: Curculionidae) during adult activities without feeding (Chapman 1956). Flight muscles change after flight during brood establishment in bark beetles (Reid 1958, Atkins and Farris 1962, Gray et al. 1972) in which young, post-emergent adults that are about to fly have larger metathoracic muscles compared to those in the reproductive stage after host colonization (Atkins and Farris 1962).

We test the hypothesis that energy substrates in MPB will decrease due to energy metabolism during flight. Here, we compare the level of energy substrates, proteins, carbohydrates, and lipids of MPB flown on flight mills with un-flown beetles. We predict that beetles flown on flight mills will have lower substrate levels compared to un-flown beetles. We conduct a colourimetric method to measure the energy budget of each individual beetle. We assess if volume of flight muscle of MPB is correlated with flight speed, distance and duration. Beetles with bigger flight muscles volume are expected to have increased flight capacity compared to beetles with smaller flight muscle volume.

2.2 Methods

2.2.1 Biochemical Assay

2.2.1.1 Beetles

Mountain pine beetle-infested lodgepole pine bolts were obtained from five different sites (3 trees/site) near Grande Prairie, AB in October 2013. One 50 cm bolt from 1 m above the soil surface was cut from each tree. Bolts were transported to the laboratory at the University of Alberta where the ends were sealed with paraffin wax before storage at 5°C until use.

2.2.1.2 Beetle Flight Treatment

After removal from cold storage 4-6 months later, the infested bolts were placed at room temperature in separate 121 L bins made of opaque plastic and fitted with emergence jars. The emergent adult beetles were separated by sex (Lyon 1958). Beetles were stored at 4°C in microcentrifuge tubes (2.0 ml) with a piece of paper to provide a surface for beetles to cling to before flight (Evenden et al. 2014). Beetles 3-5 days post emergence were weighed to the nearest 0.0001 g (Mettler Toledo, XS105, Columbus, OH) and prepared for flight by attaching a tether of 0.03-mm-diameter aluminum wire with a 0.14-mm-diameter loop and a 2-cm straight portion of wire perpendicular to the loop to the beetle pronotum with Press-Tite Contact Cement (LePage, Mississauga, ON, Canada). Flight experiments were conducted in a controlled environmental chamber maintained at 24°C and 16L: 8D (621 lux during the photophase) photoperiod. Tethered beetles were attached to the distal end of each flight mill arm by inserting the 2 cm portion of the aluminum tether at an approximately 100° angle with the mill arm (Fig 2.1). The beetles were flown for 23 h. The flight assay was initiated 4 h after the beginning of the photophase. Males and females were flown on alternate days (n=3-15 per day) in order to avoid the influence of sensory cues from the opposite sex. As beetles propelled the mill arms, a magnetic sensor on each flight mill indicated the arm rotation of each mill to the computer. One revolution of the mill arm was 94.2 cm. The software (LabView, National Instruments Corporation, Austin, TX) output included number of revolutions, longest single flight and flight duration. The flight distance was calculated by multiplying number of revolutions by 94.2 cm.

A random sample of beetles was selected to serve as control beetles. Control beetles were tethered in the same manner, but the tether was then removed from the beetle and beetles were kept individually in perforated microcentrifuge tubes (2.0 ml) during the flight period in the same environmental chamber that housed the flight mills. Beetles were weighed and stored in 2-ml Eppendorf vials at -20°C immediately after the flight bioassay.

2.2.1.3 Biochemical Analysis

The colourimetric method was used to estimate the total energy budget of flown and control MPB (Foray et al. 2012). Carbohydrates (glucose, glycogen and trehalose), lipids (total lipids and triglycerides/neutral lipids) and protein content were measured for each individual in the assay. The Bradford assay was used to determine the protein content (Fig 2.2). Glucose and trehalose content were then determined using a hot anthrone reaction (Van Handel 1965, Van Handel 1985a). In the next step, the glycogen level was determined using another hot anthrone reaction. The total lipid in each insect was determined in accordance with the vanillin assay procedure (Van Handel 1985b) and total triglycerides were determined following the same method after removing polar lipids by binding with dry silicic acid.

Biochemical experiments were carried out on 104 flown (n_{female}= 32, n_{male}= 29) and un-flown (control) (n_{female}= 23, n_{male}= 20) beetles. To correct for a possible effect of body mass on energetic condition, each flown and control beetle was weighed to the nearest 0.01mg before the biochemical analysis. The standard curves were set up to check for linearity (Foray et al. 2012) in each type of assay (ranges of R^2: protein curves= 0.96, carbohydrates curves= 0.92-0.95, lipid curves=0.94-0.95). Mountain pine beetle weights before analyses ranged between 4 mg to 15.5 mg which permitted the use of similar volumes of solutions as Foray et al. (2012). Each insect was placed individually in a 2-ml Eppendorf tube containing a stainless steel bead and 180 µl of aqueous buffer solution (100 mm KH_2PO_4, 1mm dithiothreitol (DTT) and 1mm ethylenediaminetetraacetic acid (EDTA)). Each individual beetle was crushed by shaking the tube for 30 s at 25 Hz (BIO 101, SAVANT).

Bradford assay for protein content

Samples were centrifuged separately (180*g* at 4°C) (Eppendorf 5415) and 2.5 µl of each supernatant was transferred into a 96-well microplate without removing the lipid layer from the surface of the supernatant. Two hundred and fifty µl of Bradford micro-assay reagent (B6916: Sigma) was added to each well and incubated at room temperature for 15-20 min. Protein concentration was determined spectrophotometrically at 595 nm (Molecular Devices, ThermoMax, California) using a dilution series of bovine serum albumin dissolved into the same buffer as the standard.

Hot anthrone reaction for carbohydrate content

After the completion of the Bradford assay, 20 µl of 20% sodium sulphate solution (S421-500: Fisher) was added to the homogenate, to dissolve all the carbohydrates. Then 2.5 µl of the extraction buffer solution was added to reach a final solution of 0.2 ml of 2% Na_2SO_4 which was mixed with 1500 µl of a chloroform-methanol solution (1:2 v/v) to solubilize the total lipids and water soluble carbohydrates. Samples were vortexed and then centrifuged for 15 min at 180*g* and

4°C to separate glycogen from the supernatant. The supernatant was transferred into a new tube for subsequent analysis and the pellet was kept for determination of glycogen content. To determine glucose content, 150 µl of the supernatant was transferred into a different Eppendorf tube which was left to evaporate for approximately 50 min at room temperature until a volume of ~10 µl was reached. Two hundred and forty µl of 1.42 gl^{-} anthrone (AAA1911814: Fisher) reagent (Foray et al. 2012) was added to the tube and tubes were incubated for 15 min at room temperature. Tubes were further incubated at 90°C in a water bath for another 15 min. Absorbance of the solution was read at 625 nm by transferring 200 µl to a borosilicate microplate using D- glucose (D-16500: Fisher) as the standard. Similarly, 150 µl of supernatant was used to determine the trehalose content. 75 µl of 1N HCL was added to the tube and was incubated at 90°C for 7 min (van Handel 1985). After this 75 µl of 1N NaOH was added and incubated for another 7 min at 90°C in a water bath. Anthrone (500 µl) was added to the tubes and heated at 90°C in a water bath for another 17 min. Two hundred µl of the sample was used for the absorbance reading using D- trehalose (BP2687-10: Fisher) as the standard. Glycogen content of each insect was assayed by twice washing the pellets using 400 µl X 2 of 80% methanol. The washing steps included vortexing followed by centrifuging 5 min at 16000*g* and the supernatant was removed. Then 1 ml of anthrone was added to the pellet, followed by 15 min of incubation at 90°C. Samples were cooled on ice to stop the reaction and 200 µl was transferred to borosilicate microplate. Absorbance was read at 625 nm with glucose as the standard.

Vanillin assay for lipid content

First, 100 µl of the remaining supernatant was transferred to an Eppendorf tube and heated at 90°C until complete solvent evaporation. Ten µl of 98% sulphuric acid (SA818-1: Fisher) was added to the tube and was incubated at 90°C for 10 min in a water bath. After cooling, 190 µl of vanillin (1.2 g/l) (AC14082-1000) was added to the tube and incubated for 15 min at room temperature. Two hundred µl of the solution was then transferred to a borosilicate microplate and absorbance of total lipids was measured spectrophotometrically at 525 nm using triolein (44895-U: Sigma) as the standard. In a second step, the amount of neutral lipids/triglyceride present in the insect was assayed. Five hundred µl of the remaining supernatant was transferred into a new tube, which was then heated until complete evaporation at 90°C until complete evaporation of the solvent. One milliliter of chloroform was added followed by 200 mg of dry silicic acid (SILA200: Sigma). The mixture was shaken vigorously and centrifuged for 10 min at 180*g* and 4°C to remove silicic acid and the bound polar lipids. The neutral lipid content was then measured using 100 µl of the final supernatant following the same protocol for total lipids.

2.2.2 Flight Muscle Experiment

2.2.2.1 Beetles

Beetle-infested lodgepole pine bolts were obtained from five different sites near Grande Prairie, AB on October 2014 (Appendix I). Bolts were stored and emerging beetles were collected following the previously explained method.

2.2.2.2 Beetle Flight

Beetle flights were done following the previously explained method and beetles were stored at -20°C immediately after the flight bioassay.

2.2.2.3 Flight muscle dissections

Pronotum width and the body length of the beetles were measured using an ocular micrometer on a dissecting microscope (6.3 X magnification) to the nearest 0.001 mm. Body size of the individual beetles was determined by calculating the area of an ellipsoid (Knud Thompson Formula $S \approx 4\pi\ [a^p b^p + a^p c^p + b^p c^p]^{1/p}$) in which a=b=half the pronotum width, c=half the length of the beetle and p=1.6075 (Mori et al. 2011). Left and right indirect flight muscles (two pairs of dorso-longitudinal muscles (DLM) and two pairs of dorso-ventral muscles (DVM) of the metathoracic segment were selected for the muscle measurements. Beetles were placed in 70 percent ethanol and flight muscles were removed and placed in 70 percent ethanol for measurement. Measurements (muscle length, muscle width, muscle thickness) were made to the nearest (0.001 mm), using a dissecting microscope with ocular micrometer (40 X magnification). The width and thickness were recorded at ¼, ½, ¾ of the muscle length and the averages were calculated. Volume of each muscle was calculated using the volume of elliptical (πabh) shape

(Borden and Slater 1968). Total muscle volume was calculated by addition of DLM and DVM volumes.

2.2.3 Data Analysis

Data were analyzed using R v. 3.1.1 2014.07.10 (R Core Development Team 2014). Data were separately analyzed for the two experiments (biochemical assay and flight muscle experiment) using mixed effects models. Initial models contained all explanatory variables and interactions between all explanatory variables. In all analyses, model simplification was achieved by removal of nonsignificant factors based on ANOVA hypothesis testing ($p < 0.05$) for full and reduced models, until the most parsimonious model remained and using backward model selection.

A general linear model was used to analyze total energy budget of MPB in the biochemical assay. The metabolite content as proportion of body weight was the dependent variable of the model. The metabolite type (protein, total lipid, glucose, glycogen and trehalose), flight treatment and beetle sex were treated as fixed effects and tree bolt from which beetles emerged was treated as a random factor. We report the results of the minimal model; results are presented with F value and *p* value. The energetic condition of experimental beetles was analyzed using separate generalized mixed effects models. The content of total lipid, neutral lipid, proteins, and carbohydrates (glycogen, glucose, trehalose) were compared between flown and control beetles using separate models for each metabolite (Table 2.1). The models specified each metabolite as a proportion of body weight as the dependent variable. The fixed independent factors were flight treatment (flown, control) and beetle sex with tree bolt from which beetles emerged was specified as a random factor. Response variables were not normally distributed. For this reason, gamma models were used in each analysis. We report the results of minimal models; results are presented with chi-squared values and *p* values.

Beetle flight capacities were compared with the flight muscle volume using general mixed effects models. The flight distance, duration and flight speed were the dependent variables in each model. Dependent variables were transformed to fourth-root to maintain normality. A Shapiro test was conducted to test the normality of data and homogeneity of data were tested using a Levene test. Fixed factors in the full model were total muscle volume, beetle sex and body size, with tree bolt from which beetles emerged treated as a random factor. The pooled residuals of each model were tested for normality using histograms. We report the results of the minimal models; results are presented with F values and *p* values.

2.3 Results

2.3.1 Biochemical analyses

Mountain pine beetle total energy budget consist of significantly higher amount of protein and lipid per individual compared to carbohydrate (F=231.5, p <0.001) (Fig 2.3).

Table 2.1: Statistical models used for analyzing total energy budget, metabolite content and flight capacity of mountain pine beetles. Each model includes dependent variables, fixed factors, random factors and the interaction between fixed factors used in each test. Symbol * indicates interactions between fixed factors and symbol + indicates no interactions between fixed factors.

Experiment	**General mixed effects Models**
	General linear model
Total energy budget	M1= (metabolite weight as a fraction of body weight ~ metabolite type*flight treatment* sex, family= Gamma)
	Generalized mixes effects models
Metabolite content	M2= (protein ~ flight treatment* sex, random= Bolt, family= Gamma)
	M3= (male protein content ~ flight treatment, random= Bolt, family= Gamma)
	M4= (female protein content ~ flight treatment, random= Bolt, family= Gamma)
	M5= (glucose ~ flight treatment* sex, random= Bolt, family= Gamma)
	M6= (female glucose content ~ flight treatment, random= Bolt, family= Gamma)
	M7= (male glucose content ~ flight treatment, random= Bolt, family= Gamma)
	M8= (trehalose ~ flight treatment+ sex, random= Bolt, family= Gamma)
	M9= (glycogen ~ flight treatment+ sex, random= Bolt, family= Gamma)
	M10= (total lipid ~ flight treatment+ sex, random= Bolt, family= Gamma)
	M11= (neutral lipid ~ flight treatment+ sex, random= Bolt, family= Gamma)
	General mixed effects models
Flight muscle Volume	M12= (flight duration ~ muscle volume + sex + body size, random= Bolt, family= Gaussian)

M13= (flight distance ~ muscle volume + sex + body size, random= Bolt, family= Gaussian)

M14=(flight speed ~ muscle volume + sex + body size, random= Bolt, family= Gaussian)

Table 2.2 : Statistical results of biochemical assay and flight muscle experiment of mountain pine beetles. Symbol * indicates interactions between fixed factors.

Dependent variables	**Model**	**Independent variables**	**Statistical results**
Metabolite content			
Protein	M2	Flight treatment* sex	χ^2=7.6054, p=0.00582
	M3	Flight treatment (male only)	χ^2=6.8825, p=0.008704
	M4	Flight treatment (female only)	χ^2=0.4932, p=0.4825
Glucose	M5	Flight treatment * sex	χ^2=9.1793, p=0.002448
	M6	Flight treatment (female only)	χ^2=30.118, p=4.006e-08
	M7	Flight treatment (male only)	χ^2=0.5194, p=0.4711
Trehalose	M8	Sex	χ^2=0.3158, p=0.5741
		Flight treatment	χ^2=17.502, p=2.87e-05
Glycogen	M9	Sex	χ^2=2.8935, p=0.08894
		Flight treatment	χ^2=3.8057, p=0.05108
Total lipid	M10	Sex	χ^2=8.9477, p=0.002778
		Flight treatment	χ^2=11.957, p=0.0005444
Neutral lipid	M11	Sex	χ^2=2.576, p=0.1085
		Flight treatment	χ^2=25.373, p=4.725e-07
Flight muscle volume			
Flight distance	M12	Flight muscle volume	F=1.7487, df=40, p=0.1936
		Sex	F=0.0558, df=40, p=0.1237
		Body size	F=2.4722, df=40, p=0.1237
Flight duration	M13	Flight muscle volume	F=2.33229, df=40, p=0.1346

		Sex	F=0.07727, df=40, p=0.7825
		Body size	F=2.56507, df=40, p=0.1171
Flight speed	M14	Flight muscle volume	F=0.6724, df=40, p=0.4171
		Sex	F=0.5546, df=40, p=0.6851
		Body size	F=0.1668, df=40, p=0.6851

As there was a marginal interaction effect of flight treatment and sex on protein content (χ^2=7.6054, p= 0.00582) (Fig 2.4) (Table 2.2), we used simple models to analyze the effect of flight treatment on protein content in each sex. Flown males had lower protein content compared to un-flown control males (χ^2=6.8825, p= 0.008704), while protein content was similar between flown and un-flown control females (χ^2=0.4932, p= 0.4825). Flight treatment and sex had a significant interaction effect on beetle glucose content (χ^2=9.1793, p= 0.002448). Therefore, we used simple models to analyze the effect of flight treatment on glucose content in each sex. Flown females used more glucose to fuel flight than un-flown control females (χ^2=30.118, p= 4.006e-08) (Fig 2.5). Glucose content was similar between flown and control males (χ^2=0.5194, p= 0.4711). Trehalose content was not different between the sexes (χ^2=0.3158, p= 0.5741) but was higher in un-flown control beetles compared to flown beetles (χ^2=17.502, p= 2.87e-05) (Fig 2. 5). Glycogen content was not different between sexes (χ^2=2.8935, p= 0.08894) but was marginally affected by flight treatment (χ^2=3.8057, p= 0.05108) (Fig 2.5). Control beetles had more total lipids than flown beetles (χ^2=11.957, p= 0.0005444) and females had more total lipids than males (χ^2=8.9477, p= 0.002778) (Fig 2.6). Flown beetles had a low amount of neutral lipids remaining after flight compared to un-flown control beetles (χ^2=25.373, p= 4.725e-07), but it was not different between sexes (χ^2=2.576, p= 0.1085) (Fig 2.6).

2.3.2 Flight muscle dissections

Beetle flight capacity (duration, distance and speed) was not affected by beetle flight muscle volume, beetle sex or body size (Table 2.2) (Fig 2.7).

2.4 Discussion

This study reveals that the total energy budget of MPB is composed of more protein and lipid than carbohydrate. Both female and male MPBs utilize lipids and carbohydrates as energy sources during flight. Females and males, however, show variation in the energy substrates that they use for flight. Male MPBs utilize protein as a fuel for flight, while protein content is not different between flown and control females. In a previous study, male and female MPB had similar proportions of functional proteins before host colonization. It is known that there is a shift in protein composition following starvation in MPB (Pitt et al. 2014) but it remains to be tested if such a shift occurs as a result of flight. Males lost more protein during flight than did females in the current study. Males are known to use proteins in flight muscle during host colonization in order to access energy while females conserve protein resources for reproduction (Pitt et al. 2014).

Female MPBs flown on the flight mills have lower amounts of glucose compared to control females in the current study while, both males and females flown on the flight mills have lower amounts trehalose compared to control beetles. Glycogen content is similar between flown and control beetles and glucose and glycogen content does not differ between the sexes. Similarly, the glucose content in *D. armandi,* does not differ between the sexes but females have higher amounts of glycogen than males (Chen et al. 2011). Lipids are the primary source of flight fuel in MPB, with neutral lipids being the major component that powers flight. Current study reveals that MPB use both carbohydrates and lipid to fuel the flight. Similarly, other bark beetles are known to utilize both carbohydrates and lipids during flight (Thompson and Bennett 1971, Byers and Liifqvist 1989, Chen et al. 2011). Carbohydrates are the major energy materials for flight initiation (Freidman 1985), whereas lipids become the major energy material during sustained flight (Atkins 1969). *Dendroctonus armandi* Tsai and Li use carbohydrates in flight initiation, and flight performance decreases with starvation treatment (Chen et al. 2011). Males of *D. pseudotsugae* Hopkins use carbohydrates during the initial dispersal process. The current analysis, however cannot distinguish which portions of beetle flight are fueled by the different energy sources. Insects use trehalose at the initiation of flight and switch to lipids during sustained flight (Van der Horst et al. 1980, Kaufmann and Brown 2008). MPB might use trehalose for the initial flight and shift to lipids for sustained flight.

Lipid content of MPB flown on flight mills is lower compared to the un-flown control beetles in this and previous (Evenden et al. 2014) studies. The current study revealed that flown MPB have lower total and neutral lipids compared to flown beetles. MPB mostly metabolize neutral lipids during the flight as higher proportion of lipid used by beetles were neutral lipids. Bark beetles utilize lipids to power the flight and the majority of lipids come from neutral lipids. Triglycerides make up the majority of lipid content in *Dendroctonus frontalis* Zimmermann accounting for 83% of total lipids in females and 74% of total lipids in males (Kinn et al. 1994). Lipids are metabolized during flight in *D. frontalis* by the degradation of triglycerides into short chain fatty acids, a process that can occur between adult emergence and subsequent host colonization (Hodges and Barras 1974). The lipid content of *D. pseudotsugae* females flown on flight mills has been variously estimated to be reduced from 46% to 37% during a 3.5h flight (Atkins 1969), by 50% in 6 h flight (William and Robertson 2008) and from 15% to 5% after a 5 h flight (Thompson and Bennett 1971). Our study confirms earlier work (Evenden et al. 2014) that lipids are the major source of energy for flight of MPB. The energy level provided by lipids is much higher than carbohydrates (Chen et al. 2011) which may be why lipids are the major energy source used for flight in MPB. Similar with other studies, female MPBs have higher total lipid content than males (Evenden et al. 2014), but neutral lipid content is not different between the sexes. Higher lipid content of females may relate to their host finding behaviour. Females are the pioneers of host finding and colonization (Pitman 1968) in mountain pine beetle.

There is a link between bark beetle energy content and the dispersal capacity. Mountain pine beetle flight distance negatively correlates with MPB lipid content after flight (Evenden et al. 2014). In *D. armandi*, total flight distance and time spent flying decrease with a reduction in body lipid content but these flight parameters are not related to glucose or glycogen content (Chen et al. 2011). *Dendroctonus pseudotsugae* with high fat reserves fly further and longer than beetles with low initial fat reserves (Williams and Robertson 2008). The lipid content of male and female D. *frontalis* after flight is negatively correlated with beetle flight duration and distance (Hodges and Barras 1974, Kinn et al. 1994). These differences in energy metabolism during flight may affect the host finding behaviours (Atkins 1966, Chubaty et al. 2009, 2014) and subsequent reproduction (Elkin and Reid 2005) of bark beetles. Production of reproductive proteins by female MPB after encountering a suitable host after dispersal may be a mechanism to avoid protein use during flight, which may reduce the trade-off between dispersal and reproduction (Pitt et al. 2014). Individual MPBs with low lipid reserves are less selective and accept poorer quality host trees sooner than do individuals with high-lipid reserves (Chubaty et al. 2009, 2014, Latty and Reid 2010).

MPB flight capacity (duration, distance, speed) is not affected by flight muscle volume during the current study. Flight capacity of MPB during the current study is depend on energy substrates and most of the energy substrates in flight muscles are replenished by energy metabolism in the fat body (Beenakkers et al. 1984). Mountain pine beetle seems to be using these energy substrates regardless of the flight muscle volume.

This study reveals the total energy budget of MPB and what energy reserves MPB uses in flight. We observed that MPBs utilize lipids and carbohydrates during flight in a 23 h flight bioassay. Energy metabolism during flight under natural conditions may differ from this, due to differences in habitat quality and environmental variables which may change the flight capacity and energy expenditure of beetles. Knowledge on beetle energy metabolism during flight is important for modelling of dispersal because beetle flight capacity and flight propensity may differ with beetle energetic condition. Beetles raised in high quality habitats may be better competitors and/or better able to bear the cost of dispersal. Mountain pine beetle will experience new hosts and environmental conditions with the range expansion into the boreal forest of Canada (Cullingham et al. 2011), which may change energy metabolism during flight. Further studies are needed to explain how MPB use lipids and carbohydrates during different stages of flight. These differences of energy metabolism during dispersal may influence subsequent reproduction (Pitt et al. 2014, Elkin and Reid 2005) and might change host acceptance behaviours (Chubaty et al. 2009, 2014, Latty and Reid 2010) of MPB.

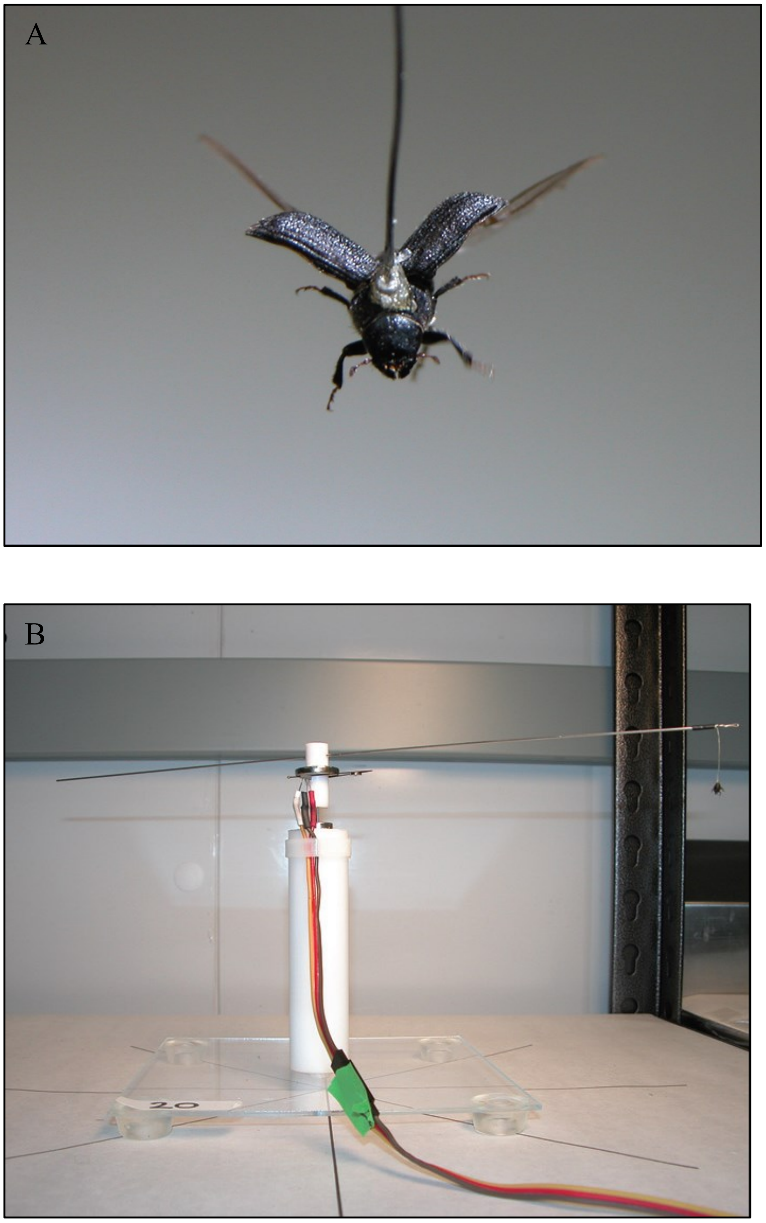

Figure 2.1: A) A mountain pine beetle on flight tether. B) A flight mill with tethered beetle attached by inserting the 2 cm portion of the aluminum tether.

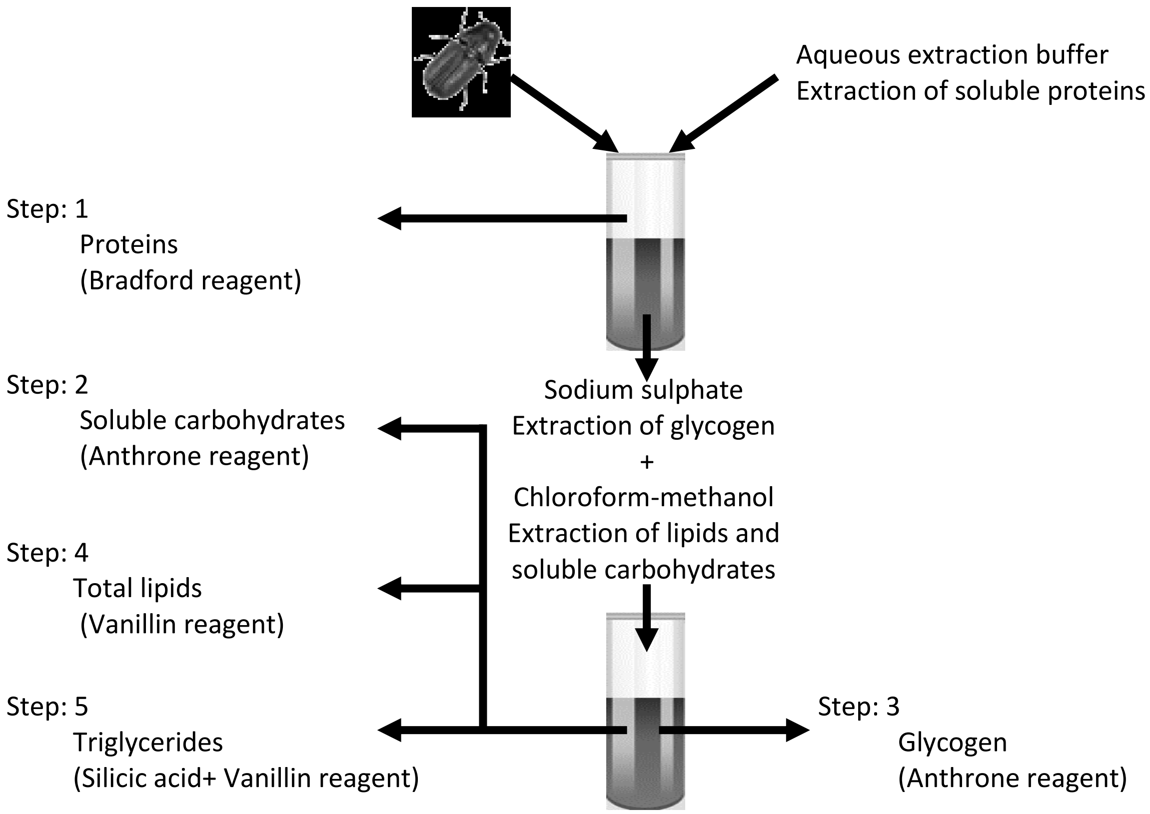

Figure 2.2: Successive steps used for extraction of proteins, soluble carbohydrates, glycogen, total lipids and triglycerides in a single individual mountain pine beetle. Reagents used for each extraction are given in parentheses. Method adapted from Foray et al. (2012).

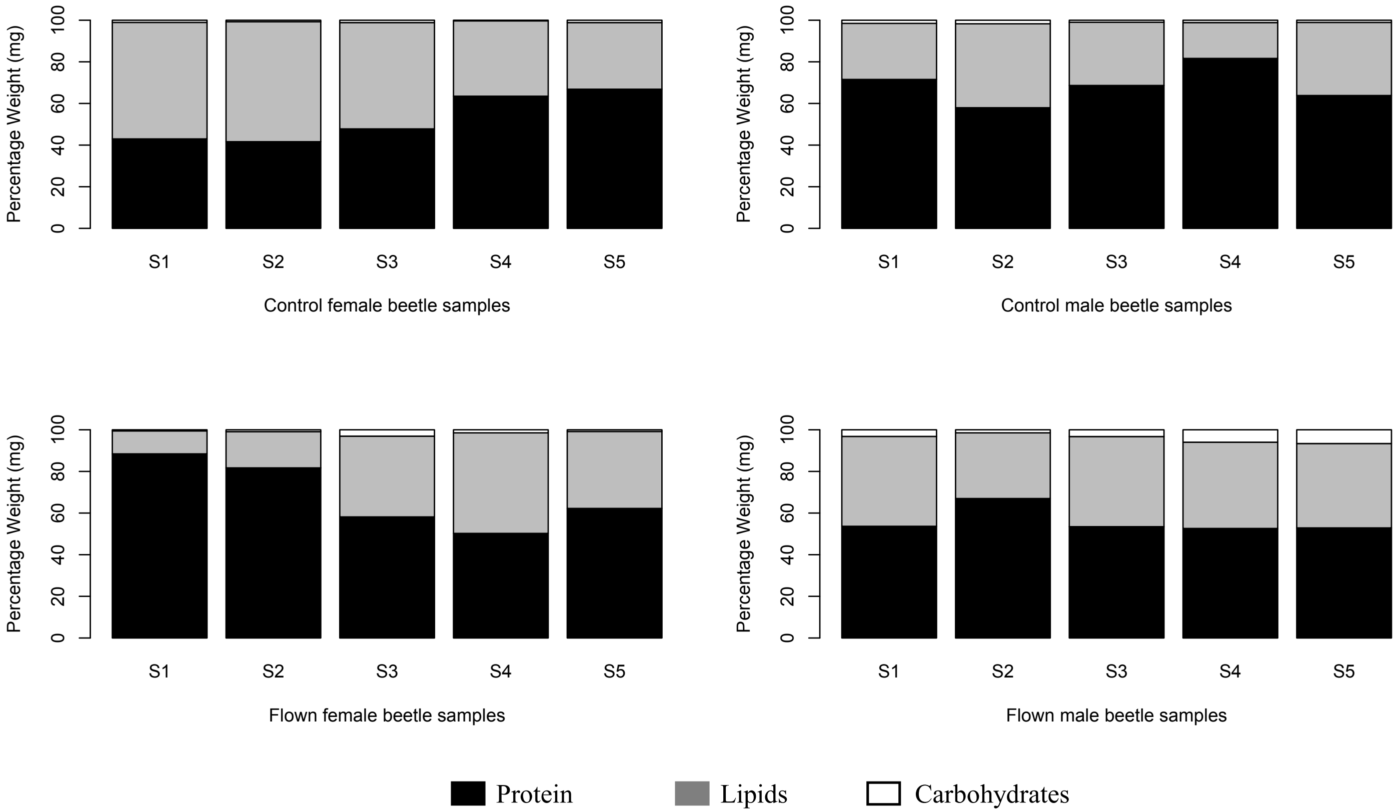

Figure 2.3: The proportional energy budget of randomly selected flown and un-flown (control) female and male mountain pine beetles. Proteins, lipids and carbohydrates were extracted from each individual beetle sample using a colourimetric method. Each panel includes five individual representative samples (S1-S5) indicating protein, total lipids and total carbohydrates weight as a percentage of the total energy budget. Data were analyzed using generalized mixed effects model.

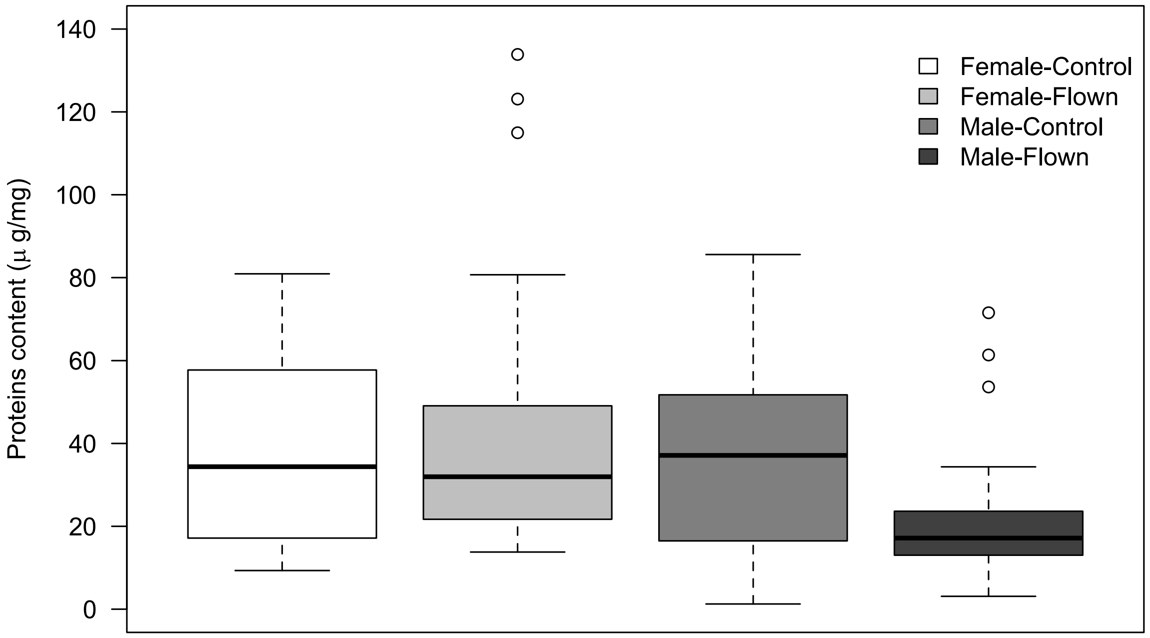

Figure 2.4: Protein content as a proportion of individual body weight, determined for female and male mountain pine beetle (n=20-32) in accordance with flown and control flight conditions. Mountain pine beetles were flown on flight mills for 23 h. The proteins were extracted from flown and un-flown (control) beetles using a Bradford assay. Protein content of beetles was compared with the known contents of bovine serum albumin as the standard. Data were analyzed using a generalized mixed effects model. Raw data are plotted.

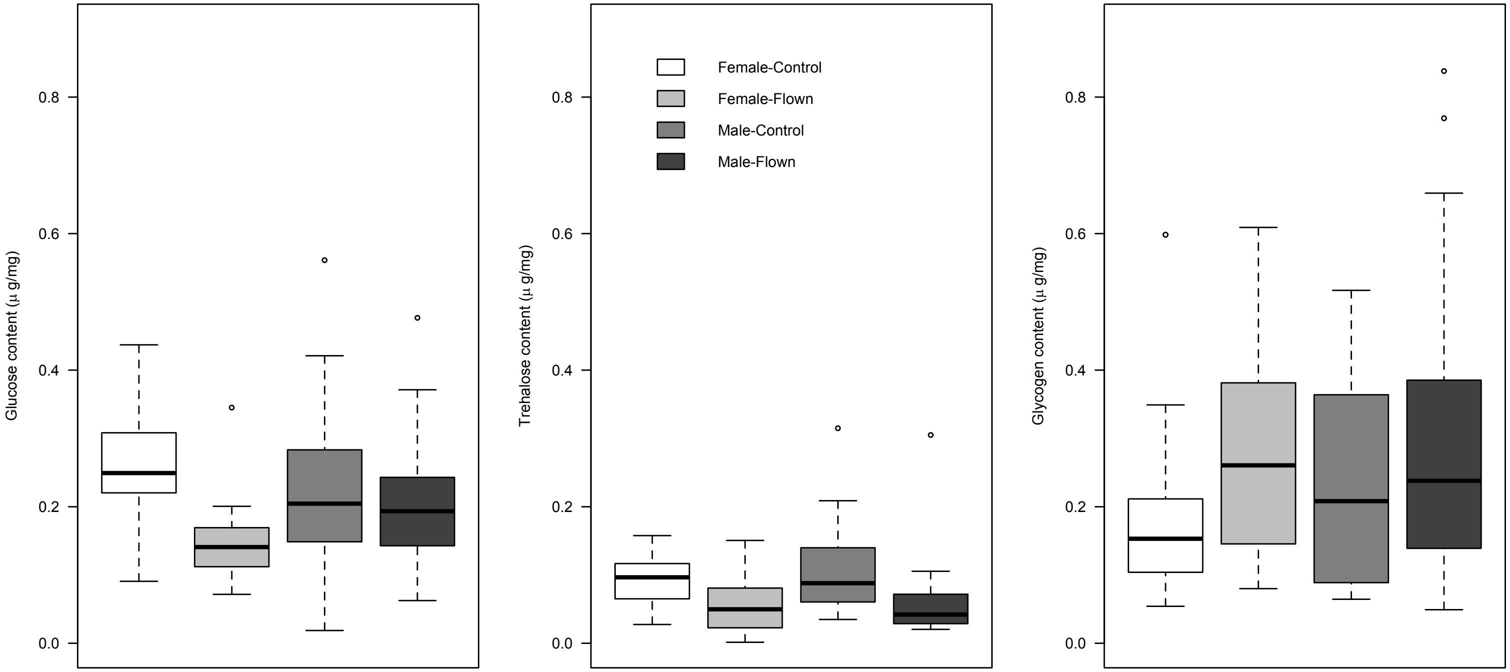

Figure 2.5: Carbohydrates as a proportion of individual body weight, determined on female and male mountain pine beetle (n=20-32) in accordance with flown and un-flown (control) flight conditions. Mountain pine beetles were flown on flight mills for 23 h. The glucose, trehalose and glycogen were extracted from flown and un-flown beetles using a Hot anthrone reaction. Glucose and glycogen contents of beetles were compared with the known contents of +D-glucose as the standard. Trehalose content of beetles was compared with the known contents of +D-trehalose as the standard. Data were analyzed using generalized mixed effects models for each carbohydrate. Raw data are plotted.

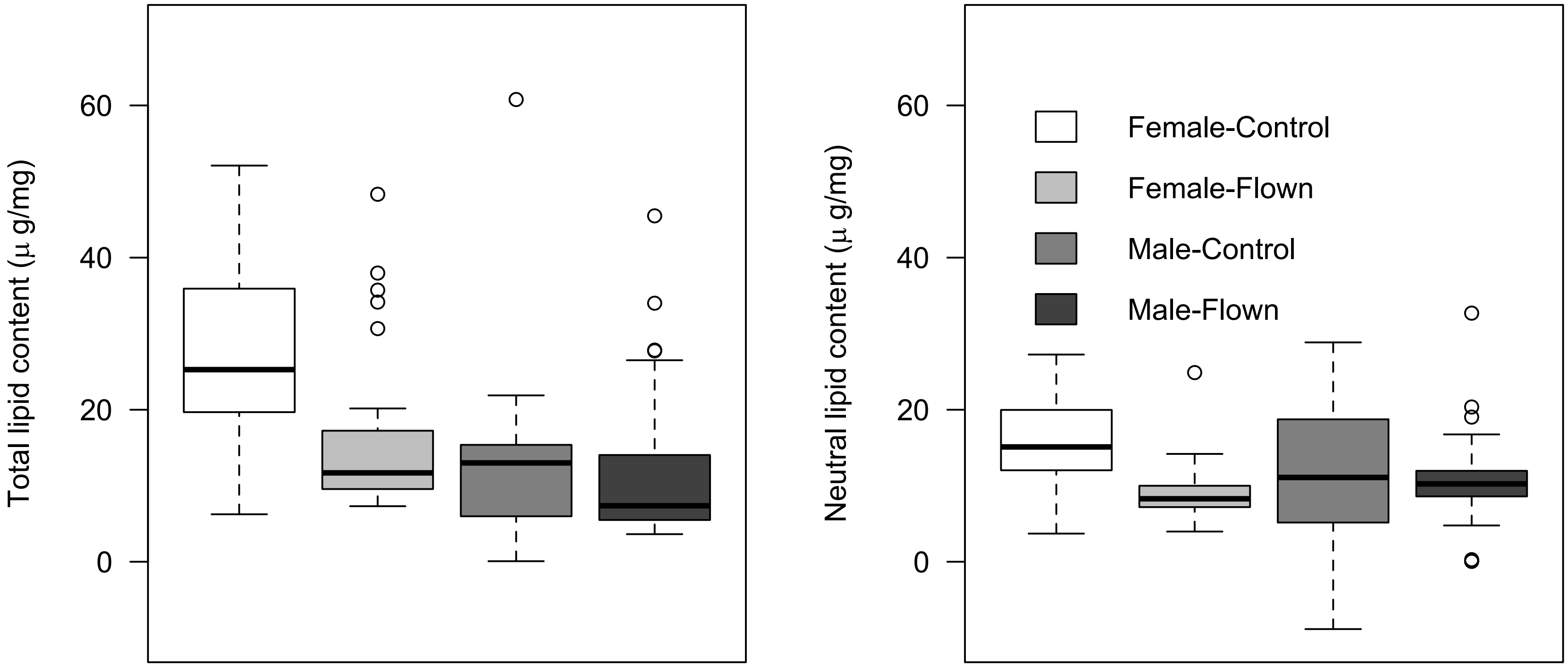

Figure 2.6: Total lipid and neutral lipid contents as a proportion of individual body weight, determined on female and male mountain pine beetle (n=20-32) in accordance with flown and control flight conditions. Mountain pine beetles were flown on flight mills for 23 h. The lipids were extracted from flown and un-flown (control) beetles using a Vanillin assay. Lipids of beetles was compared with the known contents of triolein as the standard. Data were analyzed using generalized mixed effects models for total lipids and neutral lipids. Raw data are plotted.

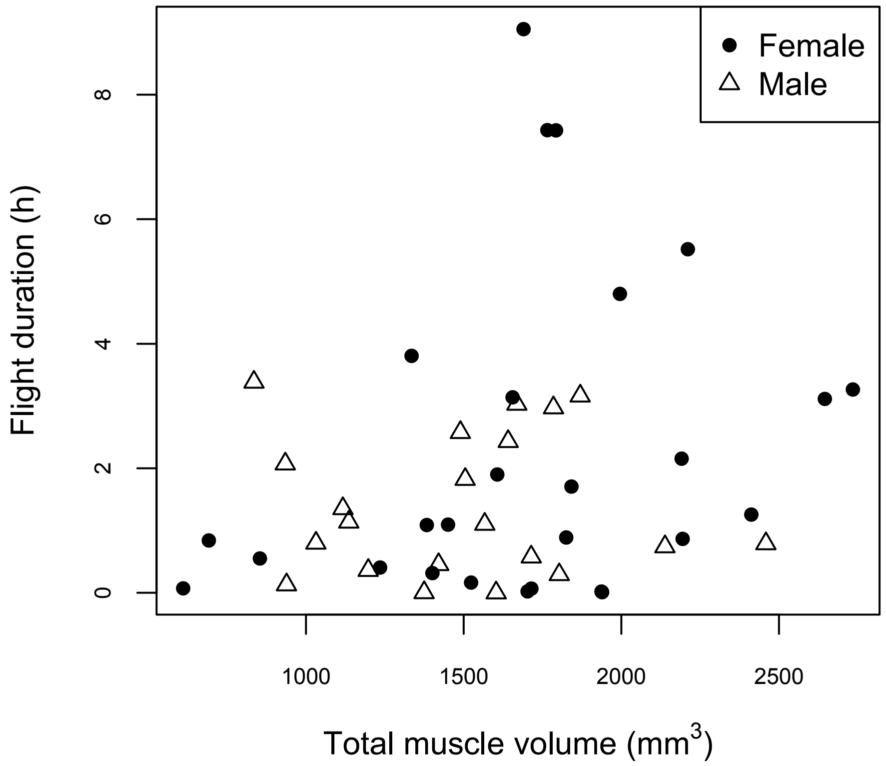

Figure 2.6: Mountain pine beetle flight duration in relation to flight muscle volume. Beetles were flown on flight mills for 23 h and the beetles were dissected to measure the flight muscle volume. Data were analyzed using a general mixed effects model.

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Chapter 3 : Variation of flight capacity in mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) with environmental conditions.

3.1 Introduction

Dispersal is a basic element in the population dynamics of irruptive insect species (Bjornstad 2002, Aukema et al. 2006, Sturtevant et al. 2013) because low resource availability at higher population densities affects insect flight (Elliott and Evenden 2009, 2012, Evenden et al. 2015). Insect dispersal consists of long- or short-distance movement of individuals from natal habitats (Nathan et al. 2003) to feeding or breeding habitats (Loxdale and Lushai 1999, Bowler and Benton 2005). Dispersal and the movement of insects is influenced by many factors that can act at individual to ecosystem levels (Loxdale and Lushai 1999). Many insect species show long-distance aerial movements in response to seasonal habitat changes, internal physiological cues and climatic factors (Drake et al. 1995).

Mountain pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins Coleoptera: Curculionidae: Scolytinae) is the most important pest of pine in western North America. It is an economic and ecologically important native insect species that has caused several million hectares of pine forest loss after the most recent outbreak that occurred in the early 2000 (Bentz et al. 2010, Safranyik et al. 2010). Mountain pine beetle movement between infested patches and from infested patches to new un-infested patches occurs through stand-level movement (Safranyik et al. 1992, Robertson et al. 2007) and long-distance movement aided by wind (Jackson et al. 2008, de la Giroday et al. 2011, 2012). High population densities of MPB in the most recent outbreak generated long-distance dispersal events that resulted in range expansion into pine forests east and north of the Rocky Mountains in Alberta (Safranyik et al. 2010). The historic host range of MPB includes lodgepole pine (*Pinus contorta* Douglas ex. Loud. var. *latifolia*), ponderosa pine (*P. ponderosa*), whitebark pine (*P. albicaulis*), limber pine (*P. flexilis*), and sugar pine (*P. lambertiana*) (Wood 1982). In its expanded range, MPB has encountered the novel host, jack pine (*P. banksiana* Lamb) in the boreal forest of Canada (Cullingham et al. 2011). Warming temperatures have allowed MPB to invade previously unsuitable forests of northern British Columbia and Alberta (Cudmore et al. 2010, Safranyik et al. 2010) and drought conditions are linked to irruptive population dynamics in MPB (Alfaro et al. 2010). An understanding of MPB dispersal is important to predict the range expansion of the beetle. In the new host range, MPB will encounter climatic conditions that differ from its historical habitats such as drought, which may influence dispersal in the new host range. Drought conditions benefit MPB by increasing the fat content of emerging beetles (Lusebrink et al. 2013) and fat content is positively related to MPB dispersal capacity (Evenden et al. 2014). Jack pine facilitates the reproduction of MPB-associated fungi and bacteria (Therrien et al. 2015) and microbial growth varies with temperature (Addison et al. 2013). This may create favourable subcortical environments for MPB reproduction in the novel jack pine host, which can influence MPB dispersal in new habitats.

Mountain pine beetle has an obligatory dispersal phase (Rudinsky 1962) which largely depends on short-distance or stand-level (Safranyik 1989) flight. This dispersal can be influenced by weather (Safranyik et al. 1992), host tree availability (Robertson et al. 2007) and beetle physiology (Evenden et al. 2014). Pioneer females release an aggregation pheromone after reaching a suitable host (Pureswaran and Borden 2005) that attracts both sexes of MPB to initiate the mass attack on the host tree (Pitman 1968). Arriving males produce different aggregation pheromones that mainly attract females until the attack density reaches the mass-attack level. Both sexes produce anti-aggregation pheromones to discourage further colonization of the host tree (Rudinsky et al. 1974). Despite our vast knowledge of MPB mass attack biology, it is unclear how abiotic factors influence the flight capacities of beetles during this obligatory dispersal phase.

Weather factors are frequently correlated and have a combined effect on insect flight. Temperature, light and wind may affect dispersal by flight of MPB. Inner bark temperature may be important for bark beetle flight in nature, as higher ambient temperatures are required for flight take-off than for sustained flight in most insects (Johnson 1969). The Douglas-fir beetle, *Dendroctonus pseudotsugae* Hopk., is not able to take off below 18-20°C but is able to carry out sustained flight at 13°C (Rudinsky and Vite 1956). The total flight duration of *D. pseudotsugae* does not vary between temperatures of 17-32°C. This is probably because their wing beat frequency increases with temperature only until it reaches the take-off threshold, after which it does not increase (Atkins 1960). The minimum temperature limits for the flight of many *Dendroctonus* species lie between 16 to 20°C (Miller and Keen 1960, McMullen and Atkins 1962, Atkins 1966, Shepherd 1966, Rasmussen 1974). Another bark beetle species, *Conophthorus coniperdus* (Schwarz) maintains a constant flight speed between 25°C and 32°C on flight mills in the laboratory, but conducts shorter and more frequent flights at higher temperatures (Henson 1962). Temperature and light influence dispersal by flight of the bark beetle *D. brevicomis* Lec. and *Ips confusus* Lec. by changing the orientation towards olfactory stimuli (Gara and Vite 1962). Flight by MPB is severely restricted at temperatures above 38°C and they are not capable of flying above 41°C (McCambridge 1971). The lower limit for flight initiation of MPB is 19°C (McCambridge 1971). Temperature might also affect bark beetle flight through an indirect effect on the energy reserves used in flight. High bark temperatures can lead to low quality MPB individuals (Chubaty et al. 2014) and this might reduce energy availability for flight. Mountain pine beetles with larger fat reserves fly further and for longer durations compared to those with lower fat reserves (Evenden et al. 2014). Individual energy level can influence host selection decisions (Atkins 1966) of MPB as beetles with higher energy reserves are more host selective than beetles with low energy reserves that accept poor quality trees (Chubaty et al. 2009, Latty and Reid 2010).

Light also influences insect flight after take-off, and the effects of light and temperature are generally confounded in nature (Johnson 1969). Mountain pine beetles are photopositive at emergence under room temperature and higher number of females orient toward light than do males (Atkins 1966). Mountain pine beetles become negatively phototactic when temperature exceeds 35°C (Shepherd 1966). *Dendroctonus armandi* Tsai and Li displays a phototactic flight behaviour in which total flight distance is greater under artificial illumination than in natural light and dark conditions in a flight mill study. Flight occurs most in the morning and afternoon and declines under dark conditions (Chen et al. 2010). The current MPB range expansion toward more northerly habitats will expose beetles to a longer day length, which may change the distance and duration that beetles can fly during a day.

Insect orientation during dispersal is linked to the direction and speed of air movement. Emerging MPB normally fly downwind until they encounter an odor plume at which point beetles turn to orient upwind in response to the odour source (Safranyik et al. 1992). Beetle response to attractive semiochemicals decreases with increasing wind speed (Gray et al. 1972). Larger scolytids are able to navigate at wind speeds up to about 2 m/s (Rudinsky 1962, Coster and Gara 1968). Flight of *D. frontalis* ceases at wind speeds greater than ~ 2 m/s (Coster and Gara 1968). Hence, changes in wind speed might alter beetle flight patterns and response toward pheromones and host volatiles.

The purpose of this study is to understand MPB flight capacity under different abiotic conditions in an effort to predict how environmental factors will affect dispersal in its expanded range. First, we hypothesize that MPB will decrease flight capacity with an increase of pre-flight and flight temperatures due to energy metabolism. Our second hypothesis tests whether MPB flight capacity will increase with longer day length. We use computer-linked flight mills to determine the effects of variation in day length and temperature before and during flight. We measure flight distance, duration, speed, and propensity of beetles under variable conditions. We predict that MPB reared and flown at low temperatures and flown under a longer photophase will have greater flight capacity compared to those reared and flown at higher temperatures under shorter photophases, respectively. Mountain pine beetle flight capacity will decrease with increasing wind speeds. We use a wind tunnel bioassay to examine the effects of wind speed on MPB flight propensity and duration. Assessment of flight capacity in a controlled environment can indicate factors that may be driving variation in dispersal of MPB under natural conditions.

3.2 Methods

3.2.1 Beetles

Mountain pine beetle-infested lodgepole pine bolts were obtained from five different sites near Grande Prairie, AB in October 2014 (Appendix I). One 50-cm bolt from 1 m above the soil surface was cut from each tree. Bolts were transported to the laboratory at the University of Alberta where the ends were sealed with paraffin wax before storage at 5°C. Those were kept in cold storage for 4 to 6 months to mimic the winter conditions required for MPB development (Lusebrink et al. 2013). After removal from cold storage, bolts were placed in separate 121-L bins made of opaque plastic and fitted with glass emergence jars and held at room temperature. The emergent adult beetles were separated by sex (Lyon 1958) and beetles were stored at 5°C in microcentrifuge tubes (2.0 ml) with a piece of paper to provide to provide a surface for beetles to cling to.

3.2.2 Beetle Flight

Two types of flight bioassays were used to test the effects of environmental conditions on MPB flight. To test the hypothesis that temperature and light affect beetle flight capacity, bioassays were conducted on computer-linked flight mills. Beetles (3-5 days post emergence) were prepared for flight by attaching a 0.14-mm-diameter loop of a tether made from 0.03-mm-diameter aluminum wire to the beetle pronotum with Press-Tite Contact Cement (LePage, Mississauga, ON, Canada). Tethered beetles were attached to the distal end of each flight mill arm by inserting a straight 2-cm portion of the aluminum tether at an approximately 100° angle with the mill arm. The flight assay was initiated 4 h after the beginning of the photophase and lasted 23 h. Males and females were flown on alternate days (n=2-22 per day) in order to avoid sensory cues from the opposite sex affecting flight. As beetles propelled the mill arms, a magnetic sensor on each flight mill indicated the arm rotation of each mill to the computer. One revolution of the mill arm equaled 94.2 cm. The software (LabView, National Instruments Corporation, Austin, TX) output included total distance flown, longest single flight, flight duration and flight speed. A random sample of beetles was selected to serve as controls. Control beetles were tethered in the same manner, but the tether was then removed from the beetles, which were kept in a perforated microcentrifuge tube (2.0 ml) during the flight period in the environmental chamber that housed the flight mills.

To test the hypothesis that beetle flight is affected by wind speed, a second set of flights was conducted in a small wind tunnel (Fig. 3.1). The small wind tunnel (0.1m X 0.05m X 0.05m) was positioned within a large wind tunnel (1.7m X 0.85m X 0.9). The small wind tunnel was connected to a variable-speed fan at the upwind end. The top of the wind tunnel was covered with a transparent sheet (80cm X 40cm) to allow light penetration. A small window (30cm X 20 cm) covered with a plastic transparent sheet on the side of the small tunnel allowed the experimenter to observe the flight trials. Wind speed was measured at the beginning of each flight bioassay using a hand-held anemometer (Model 9870, Sunshine Instruments) inserted into the wind tunnel from above 40 cm downwind from the fan (Fig 3.1).

3.2.3 Temperature-flight bioassay

Flight mill experiment was conducted to test the hypothesis that MPB will decrease the flight capacity with the increase of pre- and post- flight temperatures due to the low energy availability for flight, because energy metabolism is higher at higher temperatures. The effects of different holding and flight temperatures on MPB flight were tested. (Fig 3.1) After emergence from bolts, adult beetles were held individually in perforated microcentrifuge tubes (2 ml) with a piece of paper at 5°C until the flight experiment. Beetles (2-4 days post-emergence) were separated into different holding temperatures (5 and 24°C) 23 hours before the flight bioassay. After 23 h at the assigned holding temperature, beetles were weighed to the nearest 0.0001 g (Mettler Toledo, XS105, Columbus, OH) and the pronotum width and body length (0.01mm) were measured using an ocular micrometer on a dissecting microscope (6.3 X magnification). Tethered beetles from both holding temperature treatment groups were flown at one of two flying temperatures (20 and 24°C). Beetles from both holding temperature treatment groups were flown on a single day under each flying temperature, but males and females were flown on alternate days (n=2-22 per day) in order to avoid sensory cues from the opposite sex affecting flight. A minimum of 50 male and 50 female beetles from each holding temperature group were flown at each flight temperature (n_{total}=401). Beetles were flown for 23 hours under 16L: 8D at 621 Lux during the light cycle. A separate set of control beetles was held at the same holding temperature for 23 hr before the flight and treated in the same manner as the flown beetles. Control beetles had their tether removed and were kept separately inside perforated micro centrifuge tubes (2 ml) with a piece of paper under the same conditions as the flown beetles during the flight period. Beetles were weighed to the nearest 0.0001 g (Mettler Toledo, XS105, Columbus, OH) after the 23 h flight.

3.2.4 Light-flight bioassay

Another flight mill experiment was conducted to test the hypothesis that beetle flight capacity will increase with increasing photophase duration in the photoregime (Fig 3.2). Beetles 3-5 days post-emergence were tethered as described above. Beetle flights were conducted under two different photoregimes with different photophase lengths (short [16L: 8D] and long [18L: 6D]). Two to 22 beetles were flown per day for 23 hours at 24°C under 621 Lux during the photophase. Males and females were flown on alternate days in order to avoid sensory cues from the opposite sex affecting flight. A separate set of control beetles was housed individually inside perforated microcentrifuge tubes (2 ml) with a piece of paper with tethers removed under the same conditions during the flight bioassay. Ninety-five females and 95 males were flown on the short day length cycle and 52 males and 38 females were flown on the long day length cycle (n_{total}=280). The pronotum width and body length (0.01mm) (6.3X mag) of each beetle were recorded before the flight. Pre-flight and post-flight weights were measured to the nearest 0.0001 g (Mettler Toledo, XS105, Columbus, OH).

3.2.5 Wind speed-flight bioassay

To test the hypothesis that wind speed influences beetle flight propensity and duration, beetles were tethered following the same procedure explained above, beetles were tethered following the same procedure explained above. Tethered beetles were suspended 15 cm from the roof of a wind tunnel, 40 cm from the upwind end (Fig 3.4). Five tethered beetles of the same sex were positioned 5 cm apart in each trial. Beetles were introduced facing both upwind and downwind to the flight chamber at one of four different wind speeds (0, 0.5. 1, 2 m/s). Beetles did not fly when facing downwind (Fig 3.3). Therefore, beetles were flown facing upwind during the flight bioassay. Beetles were given 10 minutes to acclimatize before the experiment. Five beetles were observed for 50 minutes on each flight day at each wind speed. The number and duration of flights were recorded at each tested wind speed within the observation period. Wind speed treatments were alternated during the day to prevent confounding effects of time of day on beetle flight. Beetle pronotum width and body length (0.01mm) were measured before the flight. Pre-flight and post-flight weights were measured to the nearest 0.0001 g (Mettler Toledo, XS105, Columbus, OH). A total of 223 beetles were flown during the wind speed bioassay. Conditions within the wind tunnel were maintained at light~650 lux and temperature=24±2°C.

3.2.6 Data Analysis

Data were analyzed using R v. 3.1.1 2014.07.10 (R Core Development Team 2014). Data were analyzed separately for the three experiments (temperature, light and wind speed bioassays) using mixed effects models. Initial models contained all explanatory variables and interactions between all explanatory variables. In all analyses, model simplification was achieved by removal of nonsignificant factors based on ANOVA hypothesis testing ($p < 0.05$) for full and reduced models, until the most parsimonious model remained using backward model selection (Table 3.1).

The flight propensity of beetles in the temperature bioassay was analyzed using a logistic regression model. The fixed factors were holding and flying temperatures, beetle sex and pre-flight weight. Pine bolt was treated as a blocking factor. We present the results of the minimal model (Table 3.1); results are presented with chi-squared values and *p* values (Table 3.2). For the temperature and light flight mill bioassays, flight duration, flight distance and flight speed were the dependent variables in separate general mixed effects models. These dependent variables were transformed to fourth root to meet the assumption of normality. A Shapiro test was conducted to test the normality of the data. In the temperature flight mill bioassay, hoding temperature, flying temperature, sex and pre-flight weight were treated as fixed factors in each model. The bolt that beetles were reared in was treated as a random factor. We report the results of minimal models (Table 3.1); results are presented with F values and *p* values (Table 3.2).

Dependent variables tested in general mixed effects models in the light-flight experiment were flight distance, duration and speed. The light cycle (short and long), light condition (photophase or scotophase), sex and pre-flight weight were used as independent variables in each separate model in the light experiment. Tree bolt was treated as a random factor. Results are presented for minimal models (Table 3.1) with F values and *p* values (Table 3.2).

Flight propensity of beetles in the wind speed bioassay was analyzed using a logistic regression model. The fixed factors were wind speed, sex and pre-flight weight. Bolt was treated as a random blocking factor. Results of the minimal model are presented in Table 3.1, with chi-squared values and *p* values (Table 3.2). The total flight duration was treated as dependent variables in a general mixed effects model. Fixed effects were wind speed, sex and pre-flight weight. The bolt that beetles were reared in was treated as a random effect. Again, results of the minimal model are presented with F values and *p* values. The number of flight and beetle flight patterns were analyzed using generalized mixed effects models. In order to analyze beetle flight patterns, flights were categorized depending on the duration for each flight: 1) no flight; 2) burst flight <15 s; 3) intermediate flight between 15 s-25 min; and 4) sustained flight for >25 min. The percentage of each flight type conducted in the 50 min bioassay was calculated and used as dependent variables in generalized linear model. The wind speed, sex and pre-flight weight were independent variables of each model. We present the results of the minimal model (Table 3.1); results are presented with chi-squared values and *p* values (Table 3.2).

3.3 Results

3.3.1 Temperature flight bioassay

3.3.1.1 Beetle flight propensity

Between 52 and 83% of beetles flew in the different holding temperature and flight temperature combinations (Table 3.3). The flying temperatures had a significant effect on beetle flight propensity (χ^2=6.2187, p=0.01264) (Table 3.2). Beetles flown at 20°C were more likely to fly than beetles flown at 24°C. Flight propensity was similar between at both holding temperatures, 5 and 24°C. Flight propensity was significantly influenced by the pre-flight weight of beetles (χ^2= 4.3211, p=0.03764), as heavier beetles were more likely to fly than lighter beetles. Flight propensity was not different between the two sexes.

3.3.1.2 Beetle flight capacity

Beetle pre-flight weight significantly influenced the total distance (χ^2=9.5665, p=0.001982) of flight with heavy beetles flying more than light beetles (Table 3.2). Flight capacity was similar under both flying temperatures. Holding temperature significantly affected MPB flight distance in which, beetles held at the cold temperature (5°C) flew for longer distances than beetles held at the warm (24°C) temperature (χ^2=5.3019, p=0.0213) (Fig 3.6). Neither holding nor flying temperatures affected flight duration (Fig 3.5) or flight speed (Fig 3.7). Beetle flight duration was not different between two holding temperature, however, beetles held at cold temperatures showed trends of longer flight durations than the beetles held at warmer temperatures for one day before flight (Figs 3.5). Beetle weight loss as a result of flight was significantly affected by flying temperature in which, weight loss is higher in beetles that flew at 24°C than beetles flew at 20°C (χ^2=74.975, p<0.0001). Beetle weight loss was similar between both holding temperatures. Females loss more weight than male beetles (χ^2=62.836, p<0.0001).

3.3.2 Light-flight bioassay

3.3.2.1 Beetle flight capacity

There is an interaction effect between light cycle and light condition on MPB flight duration (F=7.4441, df= 407, P= 0.0066) (Table 3.2). As there is an interaction effect, we tested for simple effects that showed beetles flew for a longer duration in the photophase of the longer light cycle (18L: 6D) than in the photophase of the shorter light cycle (16L: 8D) (F=7.642, df= 277, P=0.0177) (Fig 3.8). Beetle flight duration was similar, however, between the scotophases of both light cycles tested (F=1.3344, df= 125, P= 0.2859). Beetles flew for longer distances during the longer light cycle than the shorter light cycle (F= 23.4937, df=407, P=0.0013) (Fig 3.9). Also, beetles flew for longer distances in the photophase compared to the scotophase (F= 230.4626, df= 407, P<0.0001). Females flew for a longer duration (F=11.6643, df= 407, p=0.007) and distance (F= 9.6557, df= 407, P= 0.0001) compared to males. Beetles with higher pre-flight weight flew longer (F= 2.1688, df= 404, P=0.0307) and further (F= 33.309, df= 407, P<0.0001) compared to beetles with a lower pre-flight weight. There is an interaction effect between the light cycle and light condition (F= 33.309, df= 407, P<0.0001) that affects beetle flight speed. Therefore, we tested for simple effects. Flight speed was higher in both male and female beetles in the scotophase of the long light cycle (F= 42.672, df=131, p<0.0001) (Fig 3.10) than during the photophases of both light cycles and the scotophase of the short light cycle.

Table 3.1: Statistical tests and statistical models used in temperature, light and wind speed flight bioassays.

Flight Bioassay	**Independent variable**	**Statistical analysis and statistical model**
Temperature		*Generalized mixed effects models*
	Flight duration	M1= (Flight duration~ HT+FT+SEX+PFW, random= bolt, family=Gamma)
	Flight distance	M2= (Flight distance~ HT+FT+SEX+PFW, random= bolt, family= Gamma)
	Flight speed	M3= (Flight speed ~ HT+FT+SEX+PFW, random= bolt, family=Gamma)
		Logistic regression model
	Flight propensity	M4= (Flight propensity~ HT+FT+SEX+PFW, random= bolt, family=binomial)
	Weight loss	M5=(Weight loss~ HT+FT+SEX, random= bolt, family=Gamma)
Light		*General mixed effects models*
	Flight duration	M5= (Flight duration~ LC*LCO+SEX+PFW, random= bolt, family= gaussian)
	Flight distance	M6= (Flight distance~ LC+LCO+SEX+PFW, random= bolt, family= gaussian)
	Flight speed	M7= (Flight speed ~ LC+LCO+SEX+PFW, random= bolt, family= gaussian)
Wind		*Logistic regression model*
	Flight propensity	M8= (Flight propensity~ WS+SEX+PFW, random= bolt, family=poisson)
		General mixed effects models
	Total flight duration	M9= (Total flight duration~ WS+SEX+PFW, random= bolt,

	family= gaussian)
	Generalized mixed effects model
Number of flights	M10= (Number of flights~ WS+SEX+PFW, random= bolt, family=poisson)
	Generalized linear model
Flight pattern	M11= (Percentages of each flight pattern~ WS+SEX+PFW, family=poisson)

*Each model includes: dependent variable~ fixed effects and random effects. Symbol * indicates interactions between fixed factors and symbol + indicates no interactions between fixed factors. HT: Holding temperature, FT: Flying temperature, PFW: Pre Flight Weight, LC: Light Cycle, LCO: Light Condition, WS: Wind Speed.*

3.3.3 Wind speed-flight bioassay

3.3.3.1 Beetle flight propensity

When beetles were positioned facing upwind in the wind tunnel, flight propensity was not affected by wind speed (χ^2= 1.6365, p= 0.6512) and was not influenced by beetle sex (χ^2= 0.1712, p= 0.679) (Table 3.2). Heavier beetles were more likely to fly than beetles with lighter body weight (χ^2= 5.4119, p= 0.02) in the wind tunnel assay.

3.3.3.2 Beetle flight capacity

The flight duration of beetles within the 50-minute observation period was not different among the wind speeds tested (Fig 3.11). The total flight time during the 50 minute-

Table 3.2: Statistical results of temperature, light and wind speed bioassays.

Flight Bioassay	**Independent variable**	**Fixed effects (*=interactions)**	**Statistical results**
Temperature			
	Flight propensity	Flying temperature	χ^2=6.2187, p=0.01264
		Pre-flight weight	χ^2= 4.3211, p=0.03764
	Flight duration	Pre-flight weight	χ^2=9.5665, p=0.001982
	Flight distance	Holding temperature	χ^2=5.3019, p=0.0213
	Weight loss	Flying temperature	χ^2=74.975, p<0.0001
		Sex	χ^2=62.836, p<0.0001

Light			
	Flight duration	Light cycle * light condition	F=7.4441, df=407, P=0.0066
		Photophase (light condition)	F=7.642, df=277, P=0.0177
		Scotophase (light condition)	F=1.3344, df=125, P=0.2859
		Pre flight weight	F= 0.0021, df=407, p=0.0021
		Sex	F=11.6643, df=407, p=0.007
		Pre-flight weight	F= 2.1688, df=404, P=0.0307
	Flight distance	Light cycle	F= 23.4937, df=407, P=0.0013
		Light condition	F= 230.4626, df=407, P<0.0001
		Sex	F= 9.6557, df=407, P=0.0001
		Pre-flight weight	F=16.4078, df=407, p=0.0001
	Flight speed	Light cycle* light condition	F=33.309, df=407, P<0.0001
		Scotophase (light condition)	F=42.672, df=131, p<0.0001
Wind			
	Flight propensity	Wind speed	χ^2=1.6365, p=0.6512
		Pre-flight weight	χ^2=5.4119, p=0.02
		Sex	χ^2=0.1712, p=0.679
	Total flight duration	Pre-flight weight	F=18.32256, df=205, P<0.0001
		Sex	F=12.31761, df=205, P=0.0006
	Flight pattern	Wind speed	χ^2=26.402, p<0.0001

flight period varied with beetle pre-flight weight. Beetles with a higher pre-flight weight flew for a longer duration (F= 18.32256, df= 205, P<0.0001) compared to lighter beetles (Table 3.2). Beetle sex affected total duration of flight (F=12.31761, df= 205, P= 0.0006) at different wind speeds: females always flew for a longer duration than males.

Number of flights and flight patterns. The number of flights was not affected by the wind speed, beetle sex or pre-flight weight over the 50- minute flight period (Fig 3.12). The percentage of each flight type exhibited by beetles was different between wind speeds (χ^2= 26.402, p< 0.0001) but, not with beetle sex (Fig 3.13) (Table 3.2). Intermediate and burst flights were the prominent flight type in both males and females at most of the wind speeds (0.5, 1 and 2 m/s). Females were capable of more sustained flights with the absence of wind. In contrast, a large percentage of males did not fly in the absence of wind. Males conducted more intermediate flights at 2 m/s wind speed than females.

3.4 Discussion

This study explores the impact of abiotic conditions on flight propensity and capacity in MPB. As the range of MPB expands northward and eastward, the beetle will encounter different environmental conditions including temperature and hours of daylight. Wind speed and direction is also known to influence bark beetle flight capacity (Rudinsky 1962, Coster and Gara 1968), but this has not been tested on MPB. Beetle flight propensity is higher at the lower flying temperature tested in the current study and flight propensity is not affected by pre-flight holding temperature. Beetles are ectothermic, and expend energy more quickly at higher temperatures than at cooler temperatures. Therefore, when beetles are flown at high temperatures, the quality of individuals in terms of energetic condition will be lower than for individuals flown at lower temperatures (Chubaty et al. 2014). Bark beetles with high lipid content are expected to have a long adult life span (Safranyik 1976) and have higher flight capacity (Williams and Robertson 2008). Low flight propensity of beetles flew at the higher temperature could indicate lower quality of these individuals with reduced resources to dedicate to flight.

Table 3.3: The effects of sex and holding-flying temperatures on flight performance of *D. ponderosae*.

Beetle sex and holding-flying temperature regimes(°C)	**Proportion that flew**	**Average distance flown (km)**	**Average duration (h)**	**Fight velocity (km/h)**
Female				
5-20	0.70	5.43±5.06 (47)	3.67±3.20 (47)	2.16±4.98 (47)
5-24	0.70	3.98± 3.86(49)	2.88±2.46 (49)	1.41±0.64 (49)
24-20	0.79	4.21± 4.61(57)	3.06±3.29 (57)	1.47±0.56 (57)
24-24	0.80	4.6 ± 5.83(49)	3.02±3.15 (49)	1.62±1.23 (49)
Male				
5-20	0.72	3.65± 4.51(49)	2.37±2.77 (49)	1.82±1.47 (49)
5-24	0.69	3.35±3.20 (49)	2.74 ±2.61 (49)	1.34±0.90 (49)
24-20	0.77	3.98± 4.95(52)	2.54± 3.08 (52)	2.09±2.67 (52)
24-24	0.52	12.95± 4.67(49)	2.27± 3.06(49)	2.79±4.76 (49)

Values are mean± SE, and sample size is stated in brackets.

The average flight distance of MPB during the temperature-flight bioassay ranged between 3.35 km to 5.43 km (Table 3.2) during the 23 h temperature flight bioassay. These results are comparable to previous findings for MPB flight distances that varied between 2.12 and 5.95 km on a 23-h flight mill bioassay (Evenden et al. 2014). The longest individual beetle flight was 52 km during this bioassay. *Dendroctonus amandi* flight distance ranges between 0.0003 km and 48.9 km on laboratory flight mills (Chen et al. 2010) while *D. pseudotsugae* is capable of flights between 24-32 km in a single day (Atkins 1961). *D. frontalis* flight distance ranges between 2.7 km to 32 km in a flight mill study (Kinn et al. 1994). Flight distance and duration did not vary significantly with flying temperatures in the current study, but beetles held at the cold holding temperature show a trend to fly further and longer than those held at the warm temperature before flight. Energy utilization is low at low temperatures (Chubaty et al. 2014) which allows beetles to fly longer distances, and their dispersal is positively correlated with remaining energy reserves for flight (Safranyik, 1976; Evenden et al. 2014). Similar to our findings, total flight duration of *D. pseudotsugae* remains constant over the temperature range 17°-32°C (Atkins 1960).

The wing beat frequency of *D. pseudotsugae* increases with temperature until temperature reaches the take-off threshold, after which further increase in temperature does not change the wing beat frequency (Atkins 1960). *Conophthorus coniperdus*, maintains a similar average air speed between 25 and 32°C in a flight mill study, but does shorter and more frequent flights at higher temperatures (Henson 1962). The flight velocity of MPB in this study is relatively constant at all of the temperature regimes tested. Flight velocity ranged from 1.4 and 2.7 km/h, and did not differ between male and female beetles. Mountain pine beetles in a previous study held before flight at 5°C and flown at 24°C have a flight velocity between 1.55 and 1.93 km/h (Evenden et al. 2014). Flight velocity of beetles in the current study is comparable with these results, in which beetles held at 5°C and flown at 24°C had flight velocities of 1.4-1.5 km/h. In the current study, flight reaches a maximum of 2.7 km/h speed at the high holding and flight temperatures. A field study estimate for MPB flight velocity is approximately 2 m/s (7.2 km/h) (Safranyik 1989) which is much higher than the flight velocity measured in the current study and could suggest that the tether on the beetle significantly slowed their flight. It is not clear, however, under what temperature, light and wind speed conditions the velocity was estimated in the field study.

Mountain pine beetles fly for a longer distance and duration with increased day length under a constant temperature. This finding suggests that the distance beetles can fly in a day will increase in the most northern parts of its expanded range because of longer summer days. Temperature and light influence navigation during the flight phase of *D. brevicomis* and *Ips confusus* (Gara and Vite 1962). Flight activity of the bark beetle *Xylosandrus germanus* Blanford is influenced by photoperiod in the field in which beetles prefer low light intensities for flight, but do not fly in the dark (Weber 1982). Photoperiod is the major factor determining flight activity of *Ips typographus* while other environmental factors play a secondary role. Swarming behaviour of *I. typographus* depends on sunshine in which more beetles take flights during periods of sunshine than without sunshine (Wermelinger 2004). Beetles flew more in photophase compared to the scotophase during the assay in the current study. The time available for beetle flight in the scotophase is lower than that of photophase during the current study and this difference in time was not considered for data analysis.

Beetle flight velocity is significantly higher in the scotophase of the long light cycle than in other light conditions tested. The significance of this finding is not clear, as beetles are not known to fly in the dark in nature, and very few beetles flew in the dark in the current assay. Mountain pine beetles are photopositive at emergence under room temperature and females orient toward a single light source more than males (Atkins 1966). In the current study, the length of the photophase influences the flight capacity of male and female beetles differently as females fly further and longer in the photophase of the longer light cycle compared to males. *Dendroctonus armandi* display a phototactic flight behaviour in which total flight distance and flight time is greater under artificial illumination than in natural light conditions. Flight activities in the field are highest in the morning and afternoon and decline with the onset of darkness (Chen et al. 2010). The bark beetle *Scolytus multistriatus* Marsham is photopositive during the initial flight in a wind tunnel flight assay (Choudhury and Kennedy 1980). Local flight patterns and navigation by olfactory cues by *D. brevicomis* are influenced by light conditions (Gara and Vite′ 1962). *Ips typographus* fly only during conditions of sunshine and do not fly in the dark (Lobinger and Skatulla 1996). The current study is the first to record an increased flight capacity of MPB with longer day length. This finding may be important in the modeling of beetle dispersal in novel habitats.

Mountain pine beetles fly in the presence or absence of wind and are capable of flight against all of the wind speeds tested in the current study. In our study, both sexes flew at the tested wind speeds and bigger beetles were more likely to fly. Although beetles are known to fly upwind against a wind speed of 2 m/s in the field (Safranyik et al. 1992), beetles showed only non-significant trends for reduced flight at 2 m/s wind speed compared to 0, 0.5 and 1 m/s wind speeds during this study. Some scolytid beetles cease flight at wind speeds greater than their maximum flight speed (Seybert and Gara 1970, Meyer and Norris 1973). Such conditions may similarly affect flight activity of MPB. Intermediate and burst flights were more prominent in both sexes in the wind tunnel as compared to sustained flight. During the current study, beetles flew against the wind without the presence of any semiochemical attractants. Mountain pine beetles are known to fly downwind at emergence even in the presence of aggregation pheromones (Safranyik et al. 1992) before flying upwind after encountering an odour source (Gray et al. 1972). In the absence of an odour source, MPB are capable of flight without wind or against all the different wind speeds tested in the current study. The orientation of beetles may vary, however, with the presence of semiochemicals. Mountain pine beetles fly with wind in the absence of odour plumes and fly against the wind in the presence of aggregation pheromones (Gray et al. 1972).

In conclusion, the tested holding temperature did not influence MPB flight propensity, but flight propensity was higher at the lower flying temperature than at the higher flying temperature. Beetles held at colder temperatures before flight fly longer distances and showed a trend to fly for longer durations at both flight temperatures. Flight temperature did not affect the beetle flight capacity. The flight temperatures tested during the current study are above the lower limits for MPB flight and in the range of beetle spontaneous flight activity. Longer artificial day lengths increased the flight capacity of beetles and beetles showed very low flight capacity in the scotophase. Mountain pine beetles flew, with and without wind, in the absence of an odour source at each tested wind speed. These findings will be important in modeling MPB dispersal in natural habitats and may help model beetle epidemics during the current range expansion.

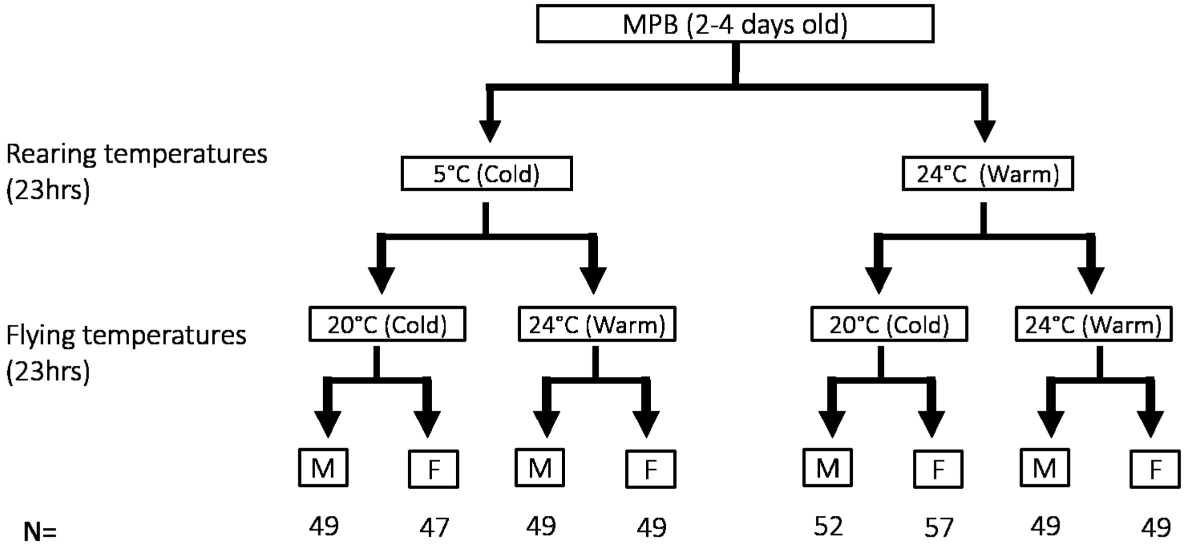

Figure 3.1: Experimental design of temperature flight bioassay. Mountain pine beetles flight capacities were tested at four different holding-flying temperature regimes.

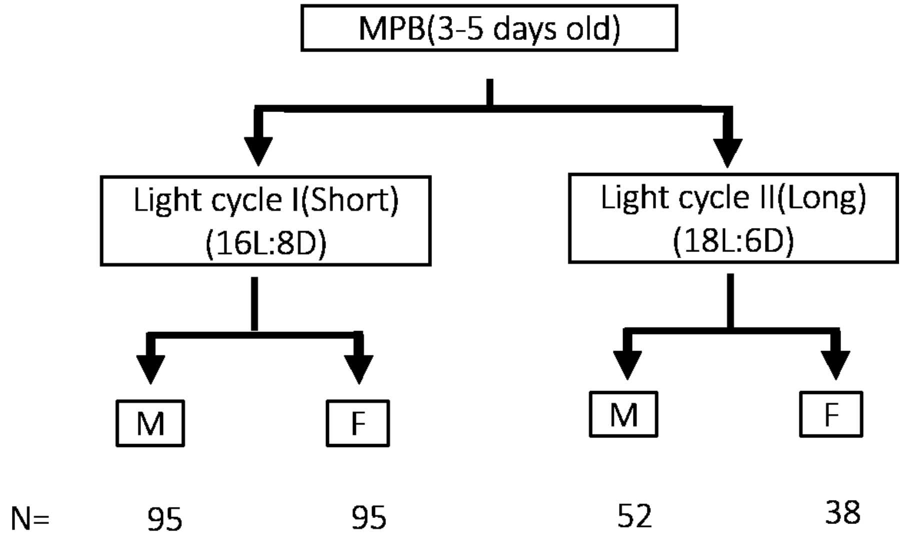

Figure 3.2: Experimental design of light flight bioassay. Mountain pine beetles flight capacities were tested at two different light cycles (16L:8D and 18L:6D) with different day lengths.

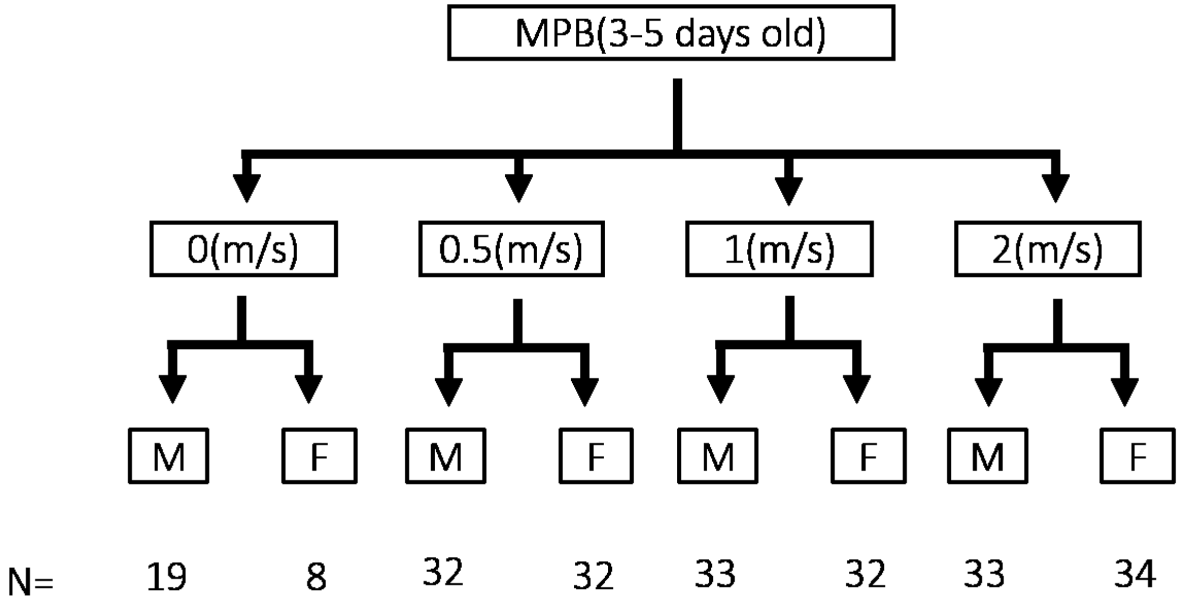

Figure 3.3: Experimental design of wind speed flight bioassay. Mountain pine beetles flight capacities were tested at four different wind speeds (0, 0.5, 1, 2 m /s).

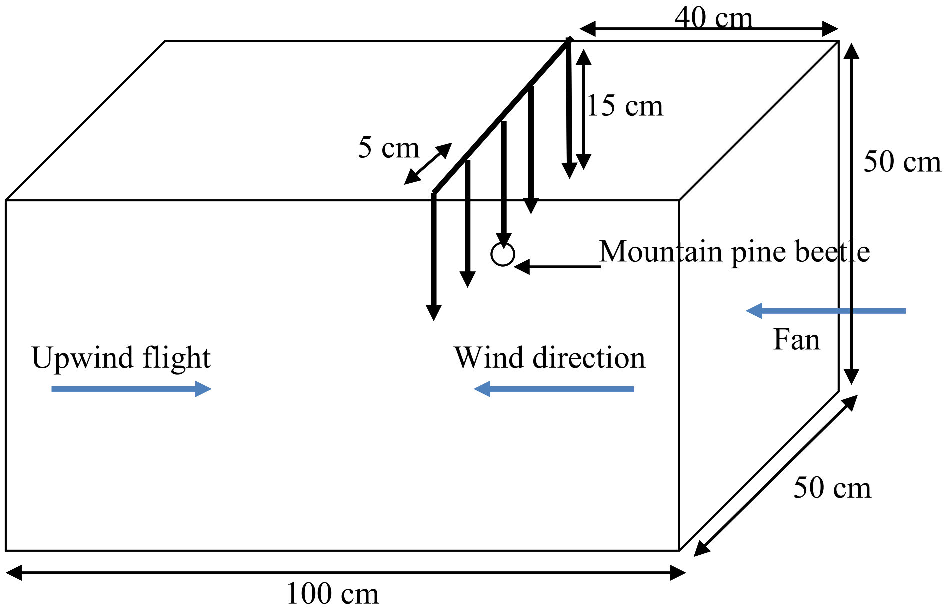

Figure 3.4: Diagrammatic view of the wind tunnel (0.1m X 0.05m X 0.05m) used for the wind speed bioassay. Beetles were tethered by attaching a beading wire to the pronotum. Tethers were attached to the roof of the wind tunnel so that beetles were separated by 5 cm and suspended 15 cm from the roof and 40 cm from the upwind end of the tunnel. Five beetles were tested at each trial and were placed facing upwind.

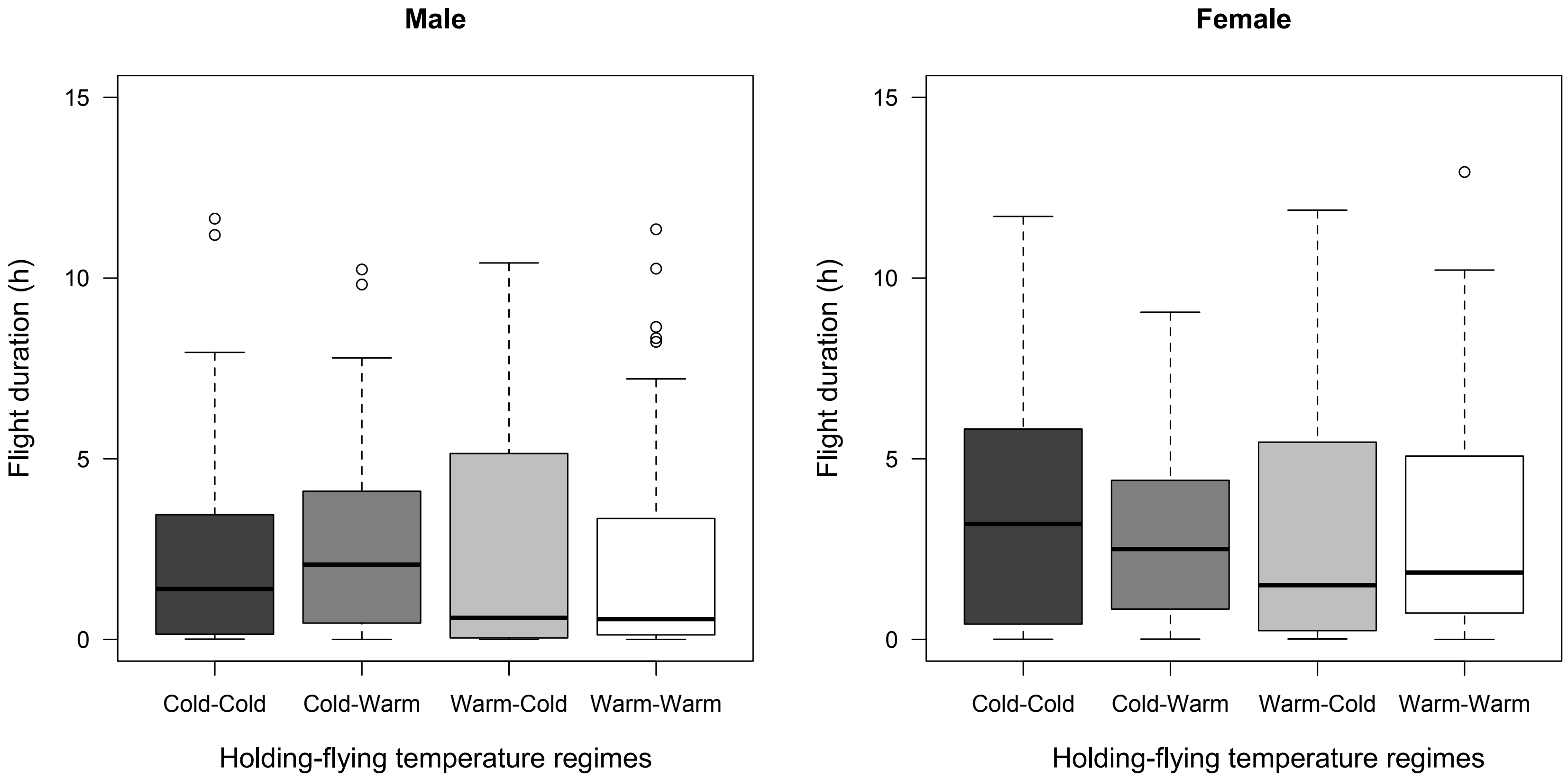

Figure 3.5: Mountain pine beetle flight duration (h) under the tested holding and flight temperature regimes. Male and female beetles were held separately at two holding temperatures: Cold (5°C) and Warm (24°C) for 23 hours before the initiation of the flight bioassay which was conducted under cold (20°C) or warm (24°C) conditions for 23 hours. Beetles from both holding temperatures were flown at the same time under a selected flying temperature. Females and males were flown separately on alternative days. Data were analyzed using a general mixed effects model. Raw data are plotted.

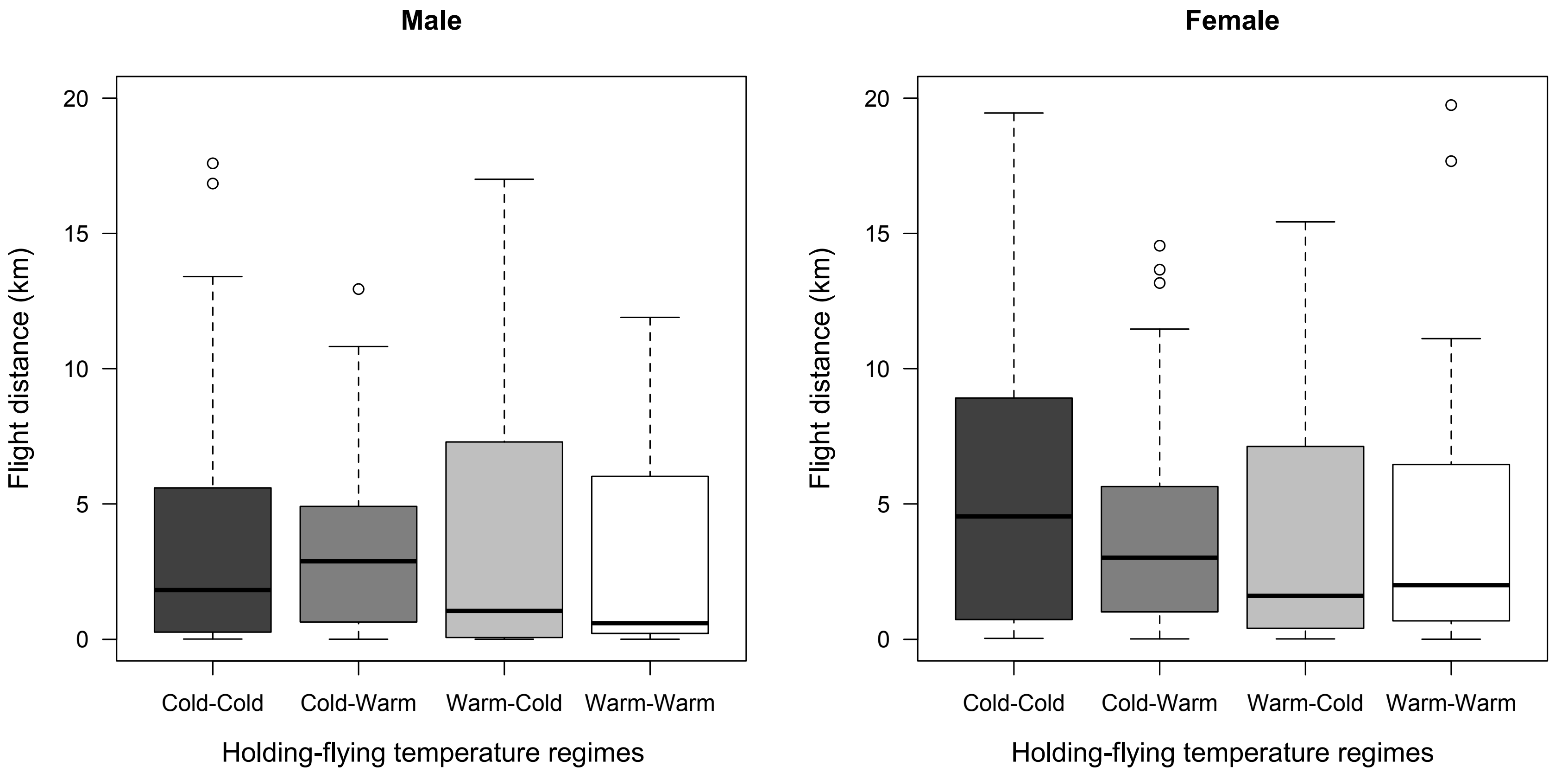

Figure 3.6: Mountain pine beetle flight distance (km) under the tested holding and flight temperature regimes. Male and female beetles were held separately at two holding temperatures: cold (5°C) and warm (24°C) for 23 hours before the initiation of the flight bioassay which was conducted under cold (20˚C) or warm (24˚C) conditions for 23 hours. Beetles from both holding temperatures were flown at the same time under a selected flying temperature. Females and males were flown separately on alternative days. Data were analyzed using a general mixed effects model. Raw data are plotted.

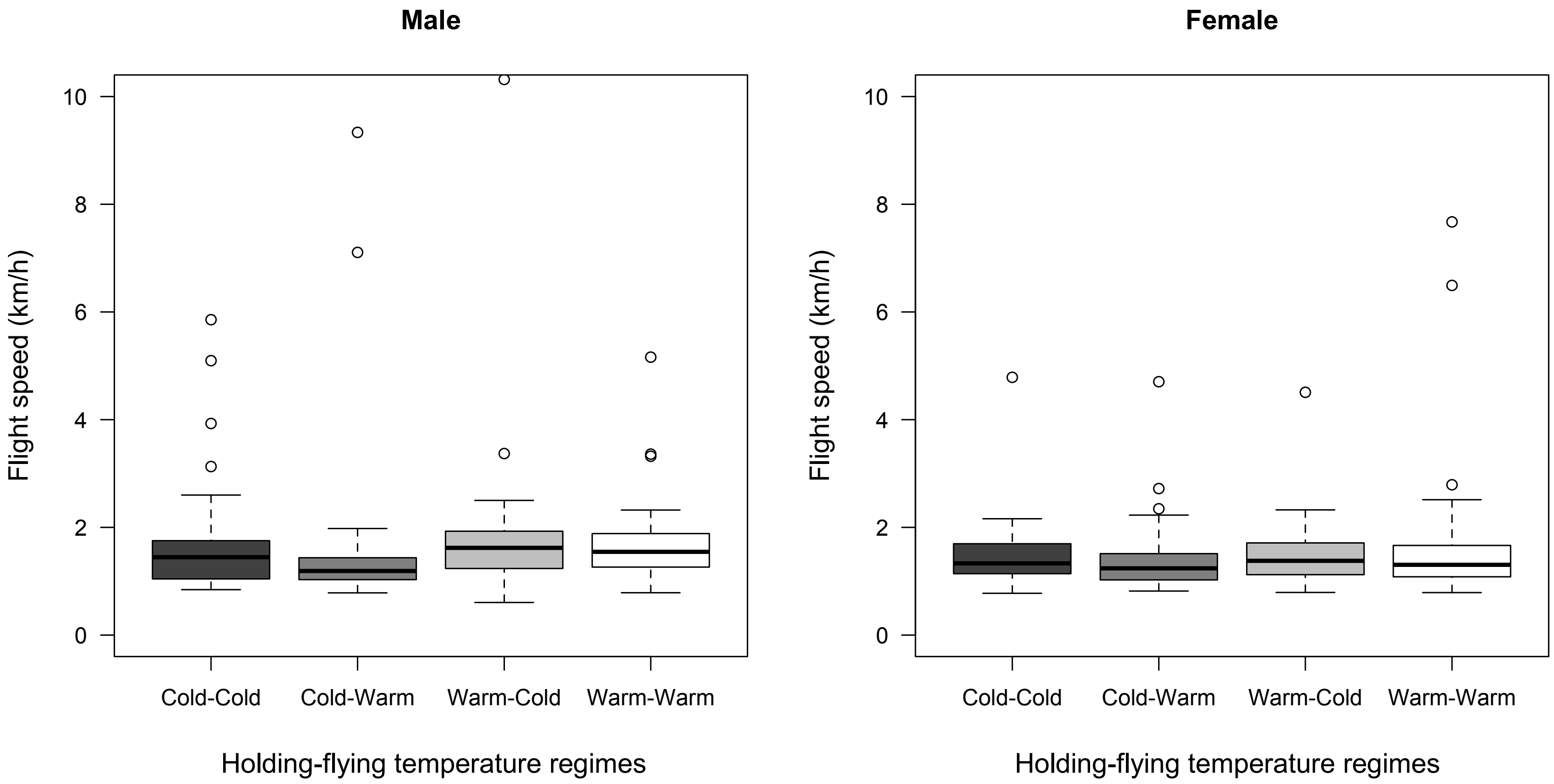

Figure 3.7: Mountain pine beetle flight speed (km/h) under the tested holding and flight temperature regimes. Male and female beetles were held separately at two holding temperatures: cold (5°C) and warm (24°C) for 23 hours before the initiation of the flight bioassay which was conducted under cold (20˚C) or warm (24˚C) conditions for 23 hours. Beetles from both holding temperatures were flown at the same time under a selected flying temperature. Females and males were flown separately on alternative days. Data were analyzed using a general mixed effects model. Raw data are plotted.

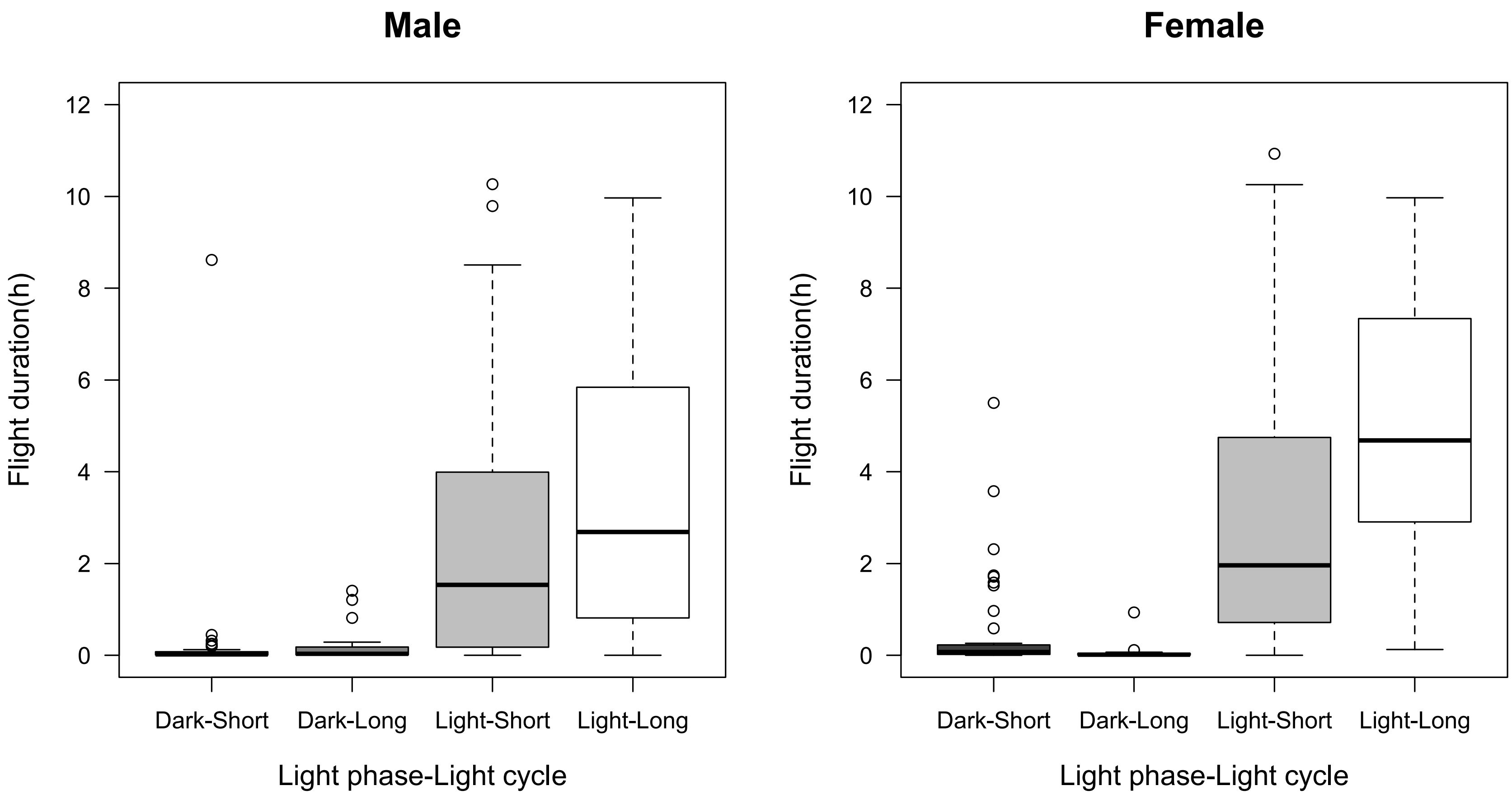

Figure 3.8: Mountain pine beetle flight duration (h) in the scotophase and photophase of the two tested light cycles: short (16L: 8D) and long (18L: 6D). Females and males were flown separately on alternate days. Data were analyzed using a general mixed effects model. Raw data are plotted.

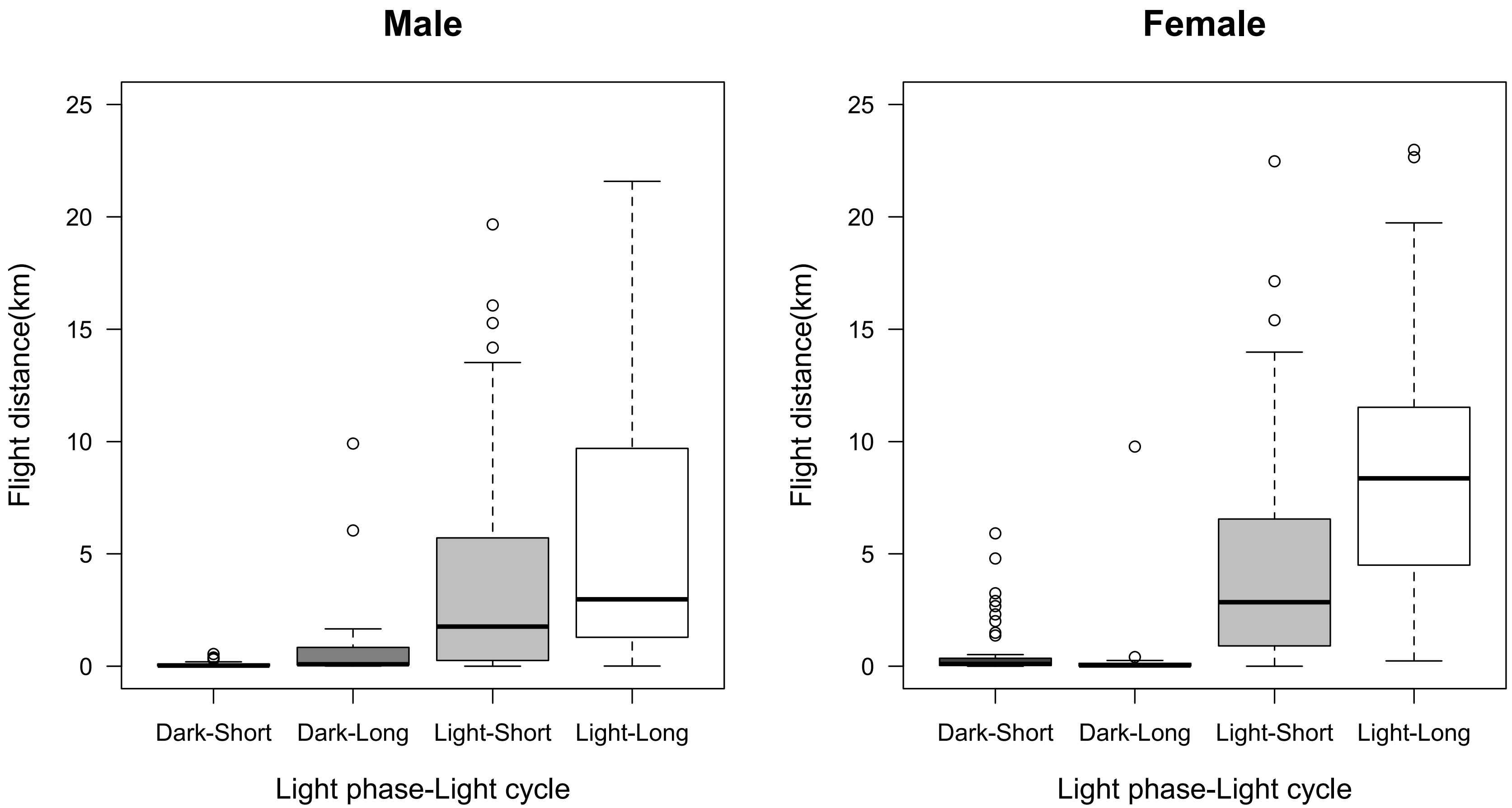

Figure 3.9: Mountain pine beetle flight distance (km) in the photophase and scotophase of the two tested light cycles: short (16L: 8D) and long (18L: 6D). Females and males were flown separately on alternate days. Data were analyzed using a general mixed effects model. Raw data are plotted.

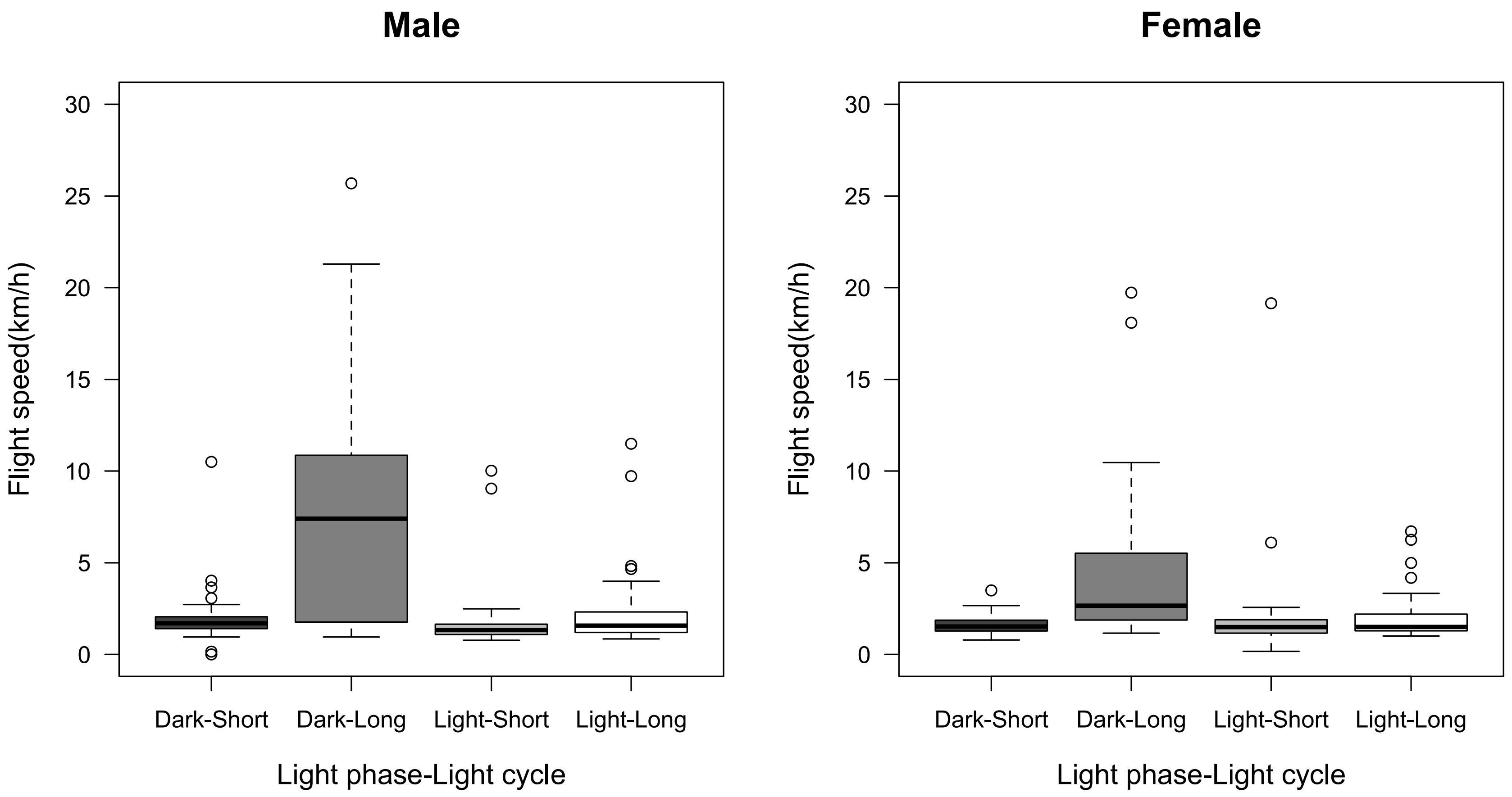

Figure 3.10: Mountain pine beetle flight velocity (km/h) in the photophase and scotophase of the two tested light cycles: short (16L: 8D) and long (18L: 6D). Females and males were flown separately on alternate days. Data were analyzed using a general mixed effects model. Raw data are plotted.

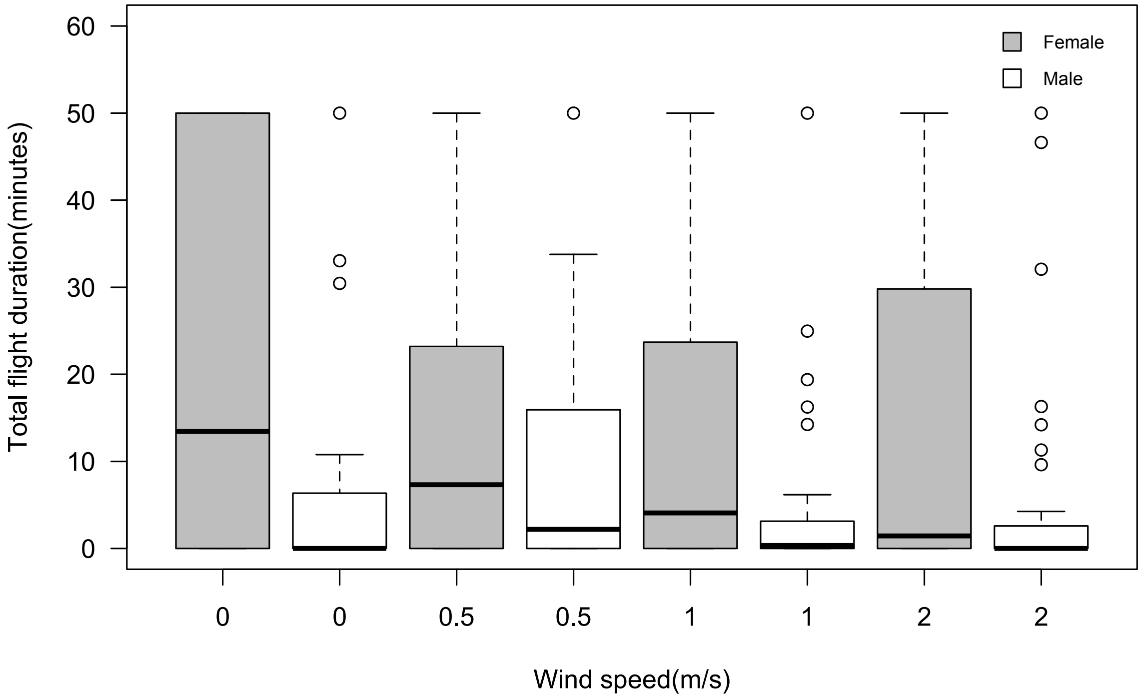

Figure 3.11: Mountain pine beetle total flight duration (min) at the tested wind speeds during the 50 min wind tunnel bioassay. Male and female beetles, 3-5 days post emergence were held separately at 5°C prior to flight for 23 h. Beetles were suspended within the wind tunnel for 50 minutes. Beetles were attached by tethers to the roof of the wind tunnel facing upwind. Tested wind speeds were: 0 m/s, 0.5m/s, 1m/s, 2 m/s. Females and males were flown separately on alternate days. The wind treatments were alternated during the day. Data were analyzed using a general mixed effects model. Raw data are plotted.

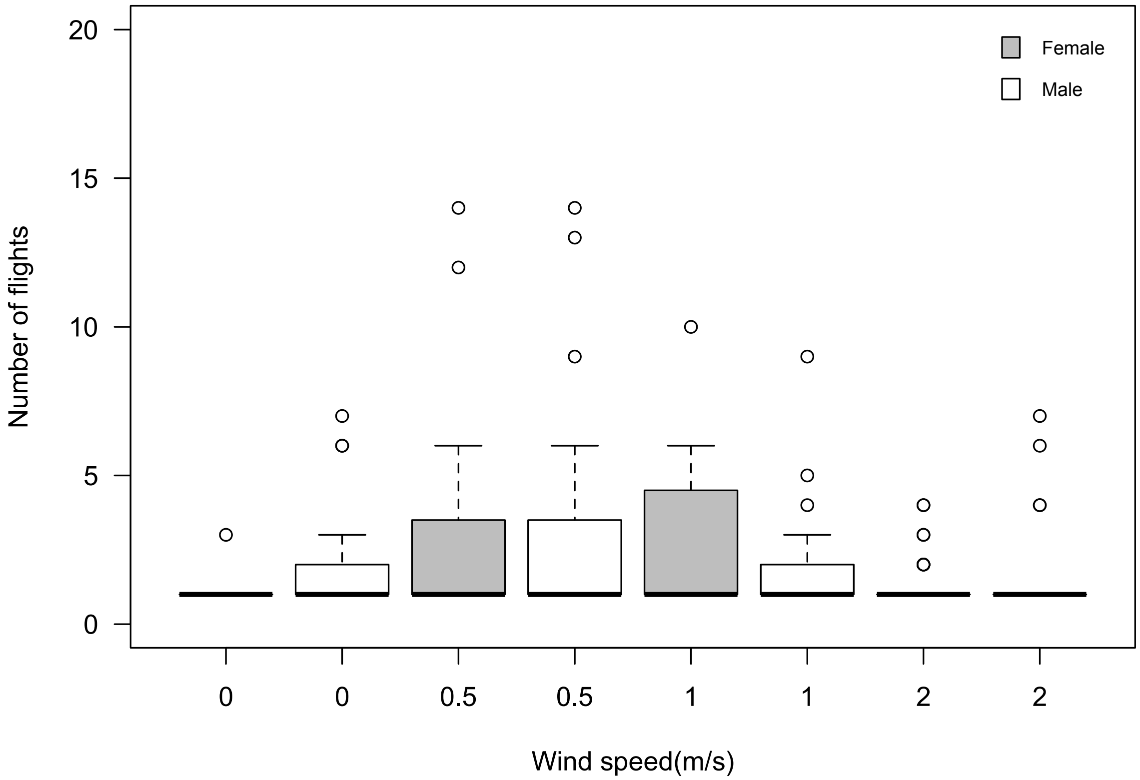

Figure 3.12: Number of flights conducted by MPB at the tested wind speeds 50 min wind tunnel bioassay. Male and female beetles, 3-5 days post emergence were held separately at 5˚C prior to flight for 23 h. Beetles were suspended within the wind tunnel for 50 minutes. Beetles were attached by tethers to the roof of the wind tunnel facing upwind. Tested wind speeds were: 0 m/s, 0.5m/s, 1m/s, 2 m/s. Females and males were flown separately on alternate days. The wind treatments were alternated during the day. Data were analyzed using a generalized mixed effects model. Raw data are plotted.

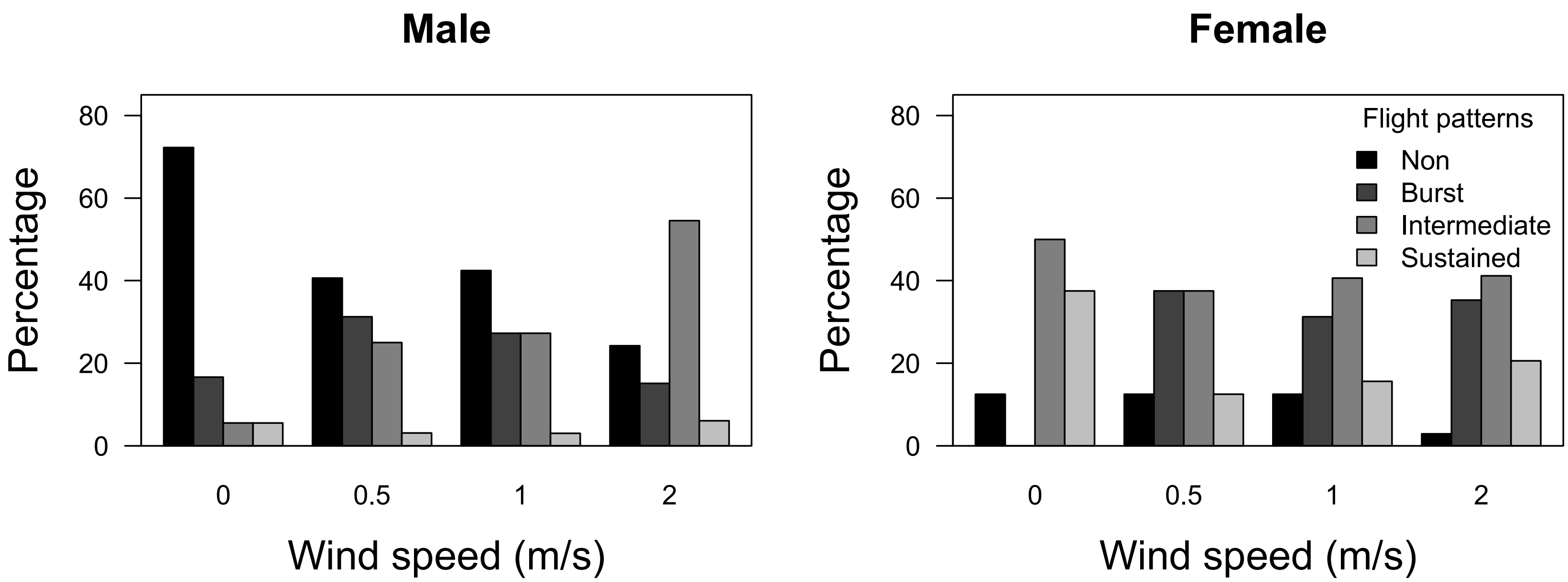

Figure 3.13: Mountain pine beetle flight patterns at the tested wind speeds 50 min wind tunnel bioassay. Male and female beetles, 3-5 days post emergence were held separately at 5˚C prior to flight for 23 h. Beetles were suspended within the wind tunnel for 50 minutes. Beetles were attached by tethers to the roof of the wind tunnel facing upwind. Tested wind speeds were: 0 m/s, 0.5m/s, 1m/s, 2 m/s. Females and males were flown separately on alternate days. The wind treatments were alternated during the day. Data were analyzed using a generalized linear model. Raw data are plotted.

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Chapter 4 : The potential for a trade-off between flight and reproduction in the mountain pine beetle (Coleoptera: Curculionidae: Scolytinae) on two pine hosts.

4.1 Introduction

Dispersal is important for organisms as it has the potential to increase individual fitness through movement to a more suitable habitat (Bowler and Benton 2005), but it remains a poorly understood phenomenon in most biological systems (Turchin et al. 1998, Cronin and Reeve 2005). Although dispersal is advantageous for the colonization of new habitats, it may be energetically costly (Zera and Harshman 2001, Harshman and Zera 2007, Zera 2009). Insects disperse in search of food (Loxdale and Lushai 1999, Dingle 2001), mates (Rudinsky 1962, Dingle and Drake 2007) or to escape from deteriorating habitats (Dingle 2001). Energy investment in dispersal can limit female reproductive investment (Hanski et al. 2006) and can result in trade-offs with other life history traits (Johnson 1969, Stearns 1992).

Flight is one of the most energy demanding activities conducted by insects (Candy et al. 1997) and high investment in flight (Shirai 1995, Zhao and Zera 2002) and flight muscle development and maintenance (Marden 2000) is required for dispersal between suitable habitats. Energy reserves for reproduction by insects can be inherited (Honěk 1993, Wedell and Karlsson 2003) or acquired through larval (Rivero et al. 2001, Awmack and Leather 2002, Nealis and Régnière 2004) and adult (Wheeler 1996, Oberhauser 1997) feeding. Trade-offs between flight capacity and reproduction are common in many insects in which flight is the main mode of dispersal. Trade–offs between flight and reproduction occur in wing-polymorphic insects in which the flying female morphs have reduced reproductive capacity (Guerra 2011) and short-winged females with low flight capacity can produce larger eggs than fully winged females (Steenman et al. 2013). In wing monomorphic species, energy use during flight can decrease subsequent reproductive output (Isaacs and Byrne 1998, Zhang et al. 2009, Gibbs and Dyck 2010, Elliott and Evenden 2012, Duthie et al. 2014).

Trade-offs between flight and reproduction are common in beetles. Across carabid species (Carabidae: Coleoptera), females of larger species have limited flight compared to smaller species due to greater investment in egg production and the cost of wing muscle production and maintenance (Matalin 2003). Female carabid species with underdeveloped ovaries at adult emergence have larger functional flight muscles than females with fully-developed ovaries (Desender 2000). Flight of alpine leaf beetles, *Oreina cacaliae* Schrank (Coleoptera:

Chrysomellidae), reduces subsequent reproduction and survival if there is no access to food after the flight (Kalberer and Martine 2003). In contrast, the long-lived pine beetle, *Monochamus galloprovincialis* Olivier (Coleoptera: Cerambycidae), shows no trade-off between flight ability and reproduction (David et al. 2015).

Bark and ambrosia beetles (Coleoptera: Curculionidae) are good models to study the trade-offs between reproduction and flight, as adults undergo an obligatory dispersal phase by flight to locate suitable hosts for brood production (Wood 1982). Dispersal can occur over long distances with the assistance of the wind (Jackson et al. 2008) or short distances by active flight (Robertson et al. 2007). Bark beetle dispersal ability is linked to both beetle physiology (Atkins 1966, Atkins 1969, Thompson and Bennett 1971, Jactel 1993, Williams and Robertson 2008, Chen et al. 2011, Evenden et al. 2014) and the number and distribution of suitable host (Robertson et al. 2007). Adult bark beetles utilize energy obtained from the natal habitat to power flight and tend to feed on the breeding host after the flight (Atkins 1969, Wood 1972).

Mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae), is a major native pest of pine (*Pinus* Linnaeus (Pinaceae)) in western North America where its principal host is lodgepole pine (*Pinus contorta* Douglas. ex. Loud. var. *latifolia*). Over 18 million hectares of forest have been killed by MPB during the most recent outbreak which occurred in early 2000 in western North America (www.nrcan.gc.ca). Host selection by MPB is associated with an obligatory dispersal phase during which beetles fly in search of a suitable host. Females are the pioneers in host location and colonization. Upon reaching a suitable host, females release the aggregation pheromone, *trans*-verbenol, which attracts both sexes of beetle to initiate mass attack on the tree (Pitman 1968). Arriving males produce another aggregation pheromone, *exo*-brevicomin, which attracts mostly females. As tree colonization progresses, both sexes produce the anti-aggregation pheromone, verbenone, and males produce frontalin to prevent further aggregation (Pureswaran et al. 2000). Mountain pine beetle utilize stored energy obtained from feeding in the natal habitat during this obligatory flight period before brood production (Bentz 2006). Although, MPBs tend to feed on the breeding host, the energy loss during flight may affect reproduction because host colonization after flight is, also energetically costly. Low energy reserves may adversely affect the reproductive capacity of MPB as male beetles synthesize *exo*-brevicomin in the fat body (Song et al. 2014) and starved female MPB produce smaller eggs compared to fed females (Elkin and Reid 2005).

The range of MPB has recently expanded eastward across the Rocky Mountains due to long-distance dispersal aided by the wind (Jackson et al. 2008, de la Giroday et al. 2011, 2012). In its expanded range, MPB has encountered different environmental conditions and different pine hosts and has successfully colonized a novel host, jack pine, *P. banksiana* Lamb. on the western edge of the boreal forest (Cullingham et al. 2011). The chemical profile emitted by jack pine is different from that of the historic host, lodgepole pine (Lusebrink et al. 2016), which may alter the host colonization process in the expanded range. Production of the aggregation pheromone, *trans*-verbenol is altered when beetles feed on jack pine vs. lodgepole pine (Erbilgin et al. 2014). These variable host conditions provided by the two pine species may change the reproductive success of MPB in its expanded range. Direct measurements of brood production in bolts of various pine species have shown that reproductive success of MPB is similar in lodgepole, jack and red pine (*P. resinosa* Aiton.) (Cale et al. 2015). Laboratory studies on naturally infested pine bolts also show that jack pine and whitebark pine (*P. albicaulis* Engelmann.) are equally suitable hosts for MPB brood production when compared to lodgepole pine (Cerezke 1995, Esch et al. 2016). Pine hosts that have not evolved under constant beetle pressure may not have as strong a chemical defense system as lodgepole pine, as reproductive success of MPB in lodgepole pine can be lower than in other pine species (Amman 1982, Langor 1989, Lusebrink et al. 2016). It is unknown if the effect of flight on subsequent reproduction will vary when MPB colonizes host species that vary in levels of chemical protection.

Here we hypothesize that energy use during dispersal may reduce the reproductive capacity of MPB resulting a trade-off between dispersal and reproduction. The magnitude of the trade-off may vary between the pine host species, because of the different levels of chemical defense and rearing conditions provided by each host. The present study examines the effects of the principal host, lodgepole pine, and an evolutionarily naïve host, jack pine, on reproduction of flown MPB compared to those that were not given the opportunity to fly before host colonization. We examine the effects of parental flight on MPB offspring number and the condition of offspring reared in two pine hosts. Beetles flown on flight mills will produce low number of offspring and offspring quality will be lower compared to un-flown beetles, because flown MPB utilize stored energy during the flight which reduce the energy availability for reproduction.

4.2 Methods

4.2 1 Beetles

Mountain pine beetle-infested lodgepole pine bolts (n=5/site) were obtained from three different sites near Grande Prairie, AB on September 2012 (Appendix II). The cut ends of the bolts were sealed with paraffin wax and housed at 5°C for 4 to 6 months to emulate winter conditions for MPB development. Un-infested lodgepole and jack pine bolts were obtained from Edson and Lac La Biche, AB, respectively, in May 2012. One 50 cm bolt from 1 m above the soil surface was cut from each tree. Bolts were transported to the laboratory at the University of Alberta where the ends were sealed with paraffin wax before storage at 5°C until use.

4.2.2 Parental Beetle Flight Treatment

After removal from cold storage, the infested bolts were placed at 24°C in separate 121 L bins made of opaque plastic and fitted with glass emergence jars. The emergent adult beetles were separated by sex (Lyon 1958) and stored at 4°C in microcentrifuge tubes (2.0 ml) with a piece of paper to provide a surface for beetles to cling to before flight (Evenden et al. 2014). Beetles were weighed to the nearest 0.0001 g (Mettler Toledo, XS105, Columbus, OH) before flight. Beetles (5-7 days post emergence) were prepared for flight by attaching a tether of 0.03-mm-diameter aluminum wire with a 0.14-mm-diameter loop and a 2-cm straight portion of wire perpendicular to the loop to the beetle pronotum with Press-Tite Contact Cement (LePage, Mississauga, ON, Canada). Flight experiments were conducted in a controlled environmental chamber maintained at 24°C and 16L:8D photoperiod (621 lux during the photophase). Tethered beetles were attached to the distal end of each flight mill arm by inserting the 2 cm portion of the aluminum tether at approximately 100° angle with the mill arm. The beetles were attached to the mill for 23 h. The flight assay was initiated 4 h after the beginning of the light period. Males and females were flown on alternate days (n=3-15 per day) in order to avoid the effect of sensory cues from the opposite sex. As beetles propelled the mill arms, a magnetic sensor on each flight mill transmitted the arm rotation of each mill to the computer. One revolution of the mill arm was equivalent to 94.2 cm of flight by a beetle. The software (LabView, National Instruments Corporation, Austin, TX) output included number of revolutions, longest single flight and flight duration. The flight distance was calculated by multiplying number of revolutions by 94.2 cm.

A random sample of beetles was selected to serve as control beetles. Control beetles were initially tethered in the same manner, but the tether was then removed from the beetle and beetles were kept in a perforated microcentrifuge tube (2.0 ml) during the flight period in the environmental chamber that housed the flight mills.

4.2.3 Bolt Inoculation and Offspring Rearing

Adult beetles that flew were removed from the tether after each flight period. Both flown and control adults were weighed and stored at 5°C for a day. Control beetles and beetles that flew >3 m were introduced into separate un-infested lodgepole pine and jack pine bolts. Phloem width of the pine bolts was measured with digital calipers before introduction of beetles. Four pairs of male and female beetles were introduced in perforated microfuge tubes to each of four bolts per pine species. A female beetle was introduced in each tube followed by a male beetle after successful excavation by the female into the bolt. Dead beetles were replaced with beetles from the same experimental treatment until pair establishment was successful. The bolts were kept for one month at 24°C to allow for beetle mating and initial larval development of offspring beetles. Bolts were then transferred to cold storage (5°C) for seven months to emulate overwintering conditions. Following the cold period, offspring beetles were reared using the same method as the parental generation described above. Adult offspring from the four pairs of beetles introduced to each bolt were counted and separated according to sex. Pronotum width and the body length of the offspring were measured using an ocular micrometer on a dissecting microscope (6.3 X magnification) to the nearest 0.01 mm. Body size of the individual beetles was estimated by calculating the area of an ellipsoid (Knud Thompson Formula $S \approx 4\pi \, [a^p b^p + a^p c^p + b^p c^p]^{1/p}$) in which a=b=half the pronotum width, c=half the length of the beetle and p=1.6075 (Mori et al. 2011). Beetles were weighed and stored at -20°C for subsequent fat extraction.

4.2.4 Fat Extraction and Offspring Condition

The adult offspring of flown and control beetle parents reared from the two pine hosts were dried for 24 h at 60°C in an oven and weighed to the nearest 0.0001 g. Beetles were placed in perforated microcentrifuge tubes (0.2 ml) and submerged in petroleum ether (Fisher Chemical, Fair Lawn, NJ) in a Soxhlet apparatus (45/50 Pyrex; Fisher, Canada). After 8 h of fat extraction, the beetles were dried again at 60°C for 24 h and re-weighed. Individual fat content was determined by subtracting the final dry weight after fat extraction from the initial dry weight.

Offspring condition was calculated using a body condition residual index that controls for body size (Elkin and Reid 2005) by regressing offspring fat content against the beetle body size. The residuals of the regression were used to create the residual index.

4.2.4 Data Analyses

Data were analyzed using R v. 3.1.1 2014.07.10 (R Core Development Team 2014). Mean phloem width of the two pine species was compared using a two-sample t-test. The flight distance and duration of flown parent beetles subsequently introduced into the two pine hosts were compared using two different two-sample t-tests. Data were separately analysed using mixed effects models. Initial models contained all explanatory variables and interactions between all explanatory variables. In all analyses, model simplification was achieved by removal of nonsignificant factors based on ANOVA hypothesis testing ($p < 0.05$) for full and reduced models, until the most parsimonious model remained using backward model selection.

Generalized mixed effects models were used to determine if parental flight treatment or the pine species that offspring were reared in had an effect on the number of offspring produced or offspring condition (Table 4.1). For offspring-number models, the dependent variables were total number of offspring, number of female offspring and number of male offspring in separate models. Parental flight treatment and tree host were specified as fixed factors in each model and the bolt beetles emerged from was treated as a random factor. We report the results of minimal models; results are presented with chi-squared values and p values.

Offspring condition was assessed by measures of offspring fat content and offspring body condition residual indices. Fat content was analyzed with three general mixed effects models and the fat content of female, male, and all offspring were specified as the dependent variables in separate models. Fixed effects were parent beetle flight treatment and pine species that offspring were reared in. The bolt that offspring emerged from was treated as a random factor.

Two general mixed effects models were used to compare the body-condition residual indices of female and male offspring among treatments in two separate models. Parent flight treatment and pine species were specified as fixed effects while bolt was treated as a random factor.

Offspring sex ratio of flown and control beetles from the two pine hosts was compared with the expected sex ratio of 1:1 using separate χ^2 tests. The sex ratios of flown beetles were compared with sex ratios of control beetles using two separate χ^2 tests in the two pine hosts.

4.3 Results

Flight capacity was similar (flight duration: t=0.8928, df= 29, p=0.3698 and flight distance: t=1.0465, df= 25, p=0.3054) for the flown parents that were subsequently introduced into bolts of the two pine species (Table 4.2). The phloem width was similar between jack and lodgepole pine bolts used for introduction of the flown and control parent beetles (t=-0.8297, df=11, p=0.424). Flight treatment of adult beetles significantly affected the number of offspring they produced (Table 4.2). Control beetles produced a higher number of male (χ^2=13.599, p=0.0002), female (χ^2=6.2517, p=0.01241) and total offspring (χ^2=13.356, p<0.0001) compared to the flown beetles (Fig 4.1). The number of offspring was also significantly affected by the pine species in which beetles were reared. Beetles produced more offspring, regardless of sex (male, χ^2=7.0156, p=0.008; female, χ^2=7.9001, p=0.005) in jack pine than in lodgepole pine bolts. The sex ratio of the offspring of flown adults significantly differed from a 1:1 ratio in lodgepole pine (χ^2=18, df=1, p=2.209e-05) and in jack pine (χ^2=5.8824, df=1, p=0.01529) (Table 4.2). Flown beetles produced more female offspring in both hosts (2 female: 1 male). Similarly, sex ratio of the offspring from control adults was significantly different compared to 1:1 ratio in both hosts. Sex ratios for control beetles were 1.3 female:1 male in lodgepole pine (χ^2=23.104, df=1, p=1.535e-06) and 1.5 female:1 male in jack pine (χ^2=22.443, df=1, p=2.165e-06) (Table 4.2). The sex ratio of offspring of flown adults was marginally different from the sex ratio of offspring of control adults in lodgepole pine (χ^2=3.443, df=1, p=0.05453) while sex ratios of offspring of flown and control adults were not significantly different in jack pine (χ^2=1.8839, df=1, p=0.1699).

Offspring fat content was not affected by parental flight status or the pine species that beetles were reared in (Fig 4.2). There was a marginal interaction effect of pine species and the parental flight treatment on body-condition residual index for male offspring (t=-0.9687991, df=12, p=0.0752) (Table 4.2). Male offspring from control adults had better body condition when reared in lodgepole compared to jack pine. Flown adults produced male offspring with lower body condition in jack pine than in lodgepole pine. The body condition residual index of both male (t=2.0259385, df=12, p=0.0656) and female (t=2.0000131, df=12, p=0.0687) offspring was marginally affected by the pine species that they were reared in. Offspring of control beetles that emerged from lodgepole pine bolts were in better condition than those that emerged from jack pine (Fig.4. 3).

Table 4.1: Mixed effects models to analyze offspring number, fat content and body condition of mountain pine beetles. Each model includes dependent variables, fixed factors, random factors and the interaction between fixed factors used in each test.

Experiment	Categories	Mixed Effects Models	
Offspring number	Total	M1=(Total number of offspring family=poisson)	~ adult flight treatment + pine species, random= Bolt,
	Male	M2=(Number of male offspring family=poisson)	~ adult flight treatment + pine species, random= Bolt,
	Female	M3=(Number of female offspring family=poisson)	~ adult flight treatment + pine species, random= Bolt,
Offspring fat content	Total	M4=(Total offspring fat content family=gaussian)	~ adult flight treatment + pine species, random= Bolt,
	Male	M5=(Male offspring fat content family=gaussian)	~ adult flight treatment + pine species, random= Bolt,
	Female	M6=(Female offspring fat content family=gaussian)	~ adult flight treatment + pine species, random= Bolt,
Offspring body condition	Male	M7=(Male offspring body condition family=gaussian)	~ adult flight treatment + pine species, random= Bolt,
	Female	M8=(Female offspring body condition family=gaussian)	~ adult flight treatment + pine species, random= Bolt,

Table 4.2: Statistical results of offspring number, sex ratio and offspring body condition of mountain pine beetles.

Experiment	Independent variables	Categories	Statistical results
Offspring number	Flight treatment	Male	χ^2=13.599, p=0.0002
		Female	χ^2=6.2517, p=0.01241
		Total	χ^2=13.356, p<0.0001
	Pine species	Male	χ^2=7.0156, p=0.008
		Female	χ^2=7.9001, p=0.005
		Total	χ^2=12.153, p<0.0001
Sex ratio	Flown	Lodgepole pine	χ^2=18, df=1, p=2.209e-05
		Jack pine	χ^2=5.8824, df=1, p=0.01529
	Control	Lodgepole pine	χ^2=23.104, df=1, p=1.535e-06
		Jack pine	χ^2=22.443, df=1, p=2.165e-06
Offspring body condition	Flight treatment * pine species	Male	t=-0.9688, df=12, p=0.0752
	Pine species	Male	t=2.0259385, df=12, p=0.0656
		Female	t=2.0000131, df=12, p=0.0687

4.4 Discussion

This study reveals a trade-off between flight and reproduction in MPB based on offspring number and condition. Adult beetles that expended energy in flight subsequently produced fewer offspring in both pine hosts compared to control beetles that were not given the opportunity to fly. The control beetles likely had more resources to allocate to reproduction as energy was not used for flight. Mountain pine beetles utilize lipids to power flight, as beetles flown on flight mills have lower fat content than beetles that do not fly (Evenden et al. 2014). The control beetles in this study may have increased their reproductive potential by resource allocation to egg production or quality. Resource allocation to reproduction does vary in MPB as beetles starved after emergence produce smaller eggs than fed individuals, but the number of eggs produced is not influenced by parental starvation treatments (Elkin and Reid 2005). Female MPB show a physiological change during dispersal and reproduction in which they produce reproductive proteins after the initial dispersal process while males consume proteins during dispersal (Pitt et al. 2014). The effect of flight on reproduction has not been studied extensively in bark and ambrosia beetles and evidence for trade-offs between flight and reproduction are equivocal. Fewer offspring are produced by the xyleborine ambrosia beetle, *Xyleborus affinis* Eichhoff when dispersal is delayed, suggesting that resource allocation to preflight activities reduces the reproductive potential of adult beetles (Biedermann et al. 2011). Despite energy loss during dispersal, males of the great European bark beetle, *Dendroctonus micans* Kug produce more offspring by searching for and mating with less-related females than sibling females that are accessible over shorter distances (Fraser et al. 2014).

Offspring condition may be affected by parental flight in MPB. There is a non-significant trend for flown MPB parents to produce offspring in poor condition. Female insects can exhibit reduced fecundity (Gu et al. 2006) and egg size (Elkin and Reid 2005) as a result of energy use. Maternal effects can influence offspring quality as reflected in egg and larval size (Fox and Czesak 2000). Mountain pine beetle utilize lipids during flight (Evenden et al. 2014) and this reduction in fat reserves may affect offspring condition through reduced investment in egg after flight. In this study, MPB have better body condition when reared in lodgepole pine compared to jack pine, suggesting that a maternal effect may render offspring better able to resist the defenses produced by lodgepole pine than the offspring of flown parents. Lodgepole pine produces higher levels of defensive monoterpenes than does jack pine (Clark et al. 2014). Nutritional quality of the two hosts may also affect offspring condition. Male MPB that emerge from jack pine bolts have lower fat stores compared to males that emerge from lodgepole pine bolts (Lusebrink et al. 2016). These interactions between flight, the rearing environment and reproduction in bark beetles highlight the need to look for trade-offs under natural conditions.

The sex ratio of the offspring from both flown and control beetles was female-biased. The offspring of control beetles emerged in 1.3:1 and 1.5:1 female:male ratios from lodgepole pine and jack pine, respectively. The sex ratio of offspring from flown beetles was 2 females:1 male in both pine species which is similar to the sex ratio of emergent MPB recorded in wild habitats (Reid 1958, Safranyik 1976, Amman 1984, Amman and Bartos 1991). One study found the adult MPB sex ratio to be female biased at the pre-dispersal stage in naturally infested lodgepole pine collected from southwest Alberta and southeast British Columbia, Canada (Amman and Bartos 1991). The female-biased sex ratio is most likely due to male mortality during the juvenile stages (Raffa and Berryman 1983). Overwintering mortality is the major reason for male-biased mortality, but an additional size-independent mechanism such as body lipid content may contribute to the sex-ratio bias in MPB (Lachowsky and Reid 2014). The different sex ratios of offspring from control and flown adult MPB in the current study may be related to body condition of the offspring as there is a trend for control beetle parents with higher fat stores than flown beetle parents to produce higher quality offspring. Cold-tolerance depends on lipid content in MPB (Lombardero et al. 2000, Bonnett et al. 2012). Both pre-flight (Reid and Purcell 2011, Graf et al. 2012) and post-flight (Evenden et al. 2014) adult males have lower absolute and relative amounts of fat compared to females and male larvae may also have less fat which could make them less tolerant to cold during overwintering. The cold conditions that MPB offspring were subjected to in the current study (5 °C), however, would not be expected to induce much mortality.

The species of pine within which beetles develop has a significant effect on beetle reproduction in terms of the offspring number. The total number of offspring produced by adults introduced to jack pine was greater than that of beetles introduced into lodgepole pine. The naïve jack pine host was more suitable for brood success than the historical host in this study. Our results differ from earlier findings that MPB reared in naïve hosts with which they have not coevolved have similar brood success compared to those reared in their historical lodgepole pine host (Cale et al. 2015). In a previous flight study (Erbilgin et al. 2014), female MPB that emerged from jack pine subsequently lost less mass during flight than females that emerged from lodgepole pine. This finding suggests that female MPB that develop in jack pine may have more resources available to allocate to reproduction when they arrive at a suitable host post-dispersal than females that develop in lodgepole pine. Bolts from the two pine hosts used in this study had similar phloem widths suggesting a similar quantity of nutritional resources was available for the parental beetles and developing larvae of the next generation however, the nutritional quality may differ between the two pine hosts. Lodgepole pine has higher phloem nitrogen content than jack pine, but phloem carbon content is similar between hosts (Lusebrink et al. 2016). Reproductive success of the ambrosia beetle, *Pityopthorus lautus* Eichhoff (Coleoptera: Platypodidae), correlates to the phloem nitrogen and carbohydrate levels (Kirkendall 1983). Host defensive chemistry may also have a major impact on brood development. Jack pine contains lower amounts of the defensive compounds 3-carene, myrcene, and terpinolene that are known to be toxic to bark beetles, than lodgepole pine. This suggests jack pine could be less defended against beetle colonization than lodgepole pine (Clark et al. 2014). Jack pine is a viable host for the MPB (Cullingham et al. 2011) and survival and progeny production in jack pine is similar to that of MPB reared in lodgepole pine (Cerezke 1995, Erbilgin et al. 2014)d. Further studies on MPB reproduction in jack pine in nature are required as jack pine monoterpene composition varies with climatic conditions and this may influence host susceptibility (Taft et al. 2015). Although parental beetles inoculated into jack pine produce more offspring than those inoculated into lodgepole pine during the current study, offspring fat content was similar among offspring reared in both pine hosts. A maximum of four pairs of beetles were allowed to reproduce in each bolt, ensuring that larval resources were not limiting. Fat content of MPB increases with beetle body size (Graf et al. 2012). The variation in individual beetle size may affect offspring fat content which could account for similar average fat reserves of offspring reared in the two pine species. The MPB body condition residual index was tested in order to account for the effect of body size on offspring condition.

Body-condition residual indices calculated for offspring in this study show a marginal difference with host species suggesting that overall body condition may be better for individuals that feed in lodgepole pine. The difference in body condition of MPB reared in the two hosts may be due to the influence of tree defenses. Variation in pine monoterpene chemistry can affect bark beetle performance metrics such as maternal gallery excavation, fecundity, survivorship, fitness and pheromone production (Robertson et al. 2007, Lusebrink et al. 2011, Reid and Purcell 2011, Davis and Hofstetter 2012, Erbilgin et al. 2014). Although lodgepole pine phloem has higher concentrations of toxic compounds such as 3-carene, myrcene, and terpinolene compared to jack pine (Clark et al. 2014), the tolerance to monoterpenes is condition-dependent in bark beetles, as beetles with lower energy reserves are less tolerant to high levels of monoterpenes (Anderbrant et al. 1985, Sallé and Raffa 2007, Reid and Purcell 2011). Although fewer offspring emerged from lodgepole than from jack pine in the current study, those that did were of higher body condition than the beetles reared in jack pine. Similarly, MPB introduced to lodgepole pine without a flight treatment produced offspring with higher fat content than those introduced to jack pine (Lusebrink et al. 2016).

Our results indicate that MPB make a trade-off between flight and reproduction in terms of offspring number and to a lesser degree offspring quality, which can be influenced by the host species. The energetic state of beetles arriving at breeding sites influences their reproductive potential (Atkins 1969, Wood 1972). Flown beetles produce fewer number of offspring than control beetles, suggesting that the prolonged dispersal phase of adult MPB can decrease beetle fitness despite the possibility of locating a higher quality host through an extended search (Chubaty et al. 2009). Pioneer MPBs that fly long-distances in search of suitable hosts produce fewer offspring than non-pioneering individuals that join the aggregation later in the dispersal period (Latty and Reid 2009). Beetles with a moderate level of energy are, however more likely to pioneer (Latty and Reid 2010) while up to 30% of emerging bark beetles do not fly (Atkins 1959, 1966, Jactel 1993, Kinn et al. 1994). This pioneering strategy may be important in balancing the trade-off between flight and reproduction in which females with greater fat reserves invest less in dispersal and more in reproduction, while females with low fat reserves conserve energy for reproduction by avoiding the pioneering behaviour. Male MPBs synthesize the aggregation pheromone, *exo*-brevicomin, in the fat body (Song et al. 2014). Reduced fat levels after flight may affect the production of *exo*-brevicomin and this may alter the subsequent host colonization of dispersing beetles. Dispersal by flight, however, did not adversely affect offspring fat content in the current study but this may be driven by the lack of larval competition for resources in our study. Adult dispersal may affect offspring fat content during the natural mass attack process because of the subsequent competition for resources by developing larvae. This and other studies (Cullingham et al. 2011, Erbilgin et al. 2014, Lusebrink et al. 2016) have shown that the novel jack pine host is a suitable host for MPB brood production, although offspring condition is better in native lodgepole pine. The degree of trade-off appears to vary with host species and is lower in the novel host jack pine compared to lodgepole pine. Jack pine grows in relatively dry habitats and is capable of producing high amounts of the attractive kairomone, myrcene under drought conditions (Lusebrink et al. 2016). Beetles infesting jack pine produce high amounts of the attractive pheromone *trans*-verbenol (Erbilgin et al. 2014) due to the high content of the pheromone precursor, α-pinene, in jack pine phloem. Future research will be needed to understand the trade-off between dispersal and reproduction that can relate to the actual host distribution and environmental factors in the expanding range of MPB.

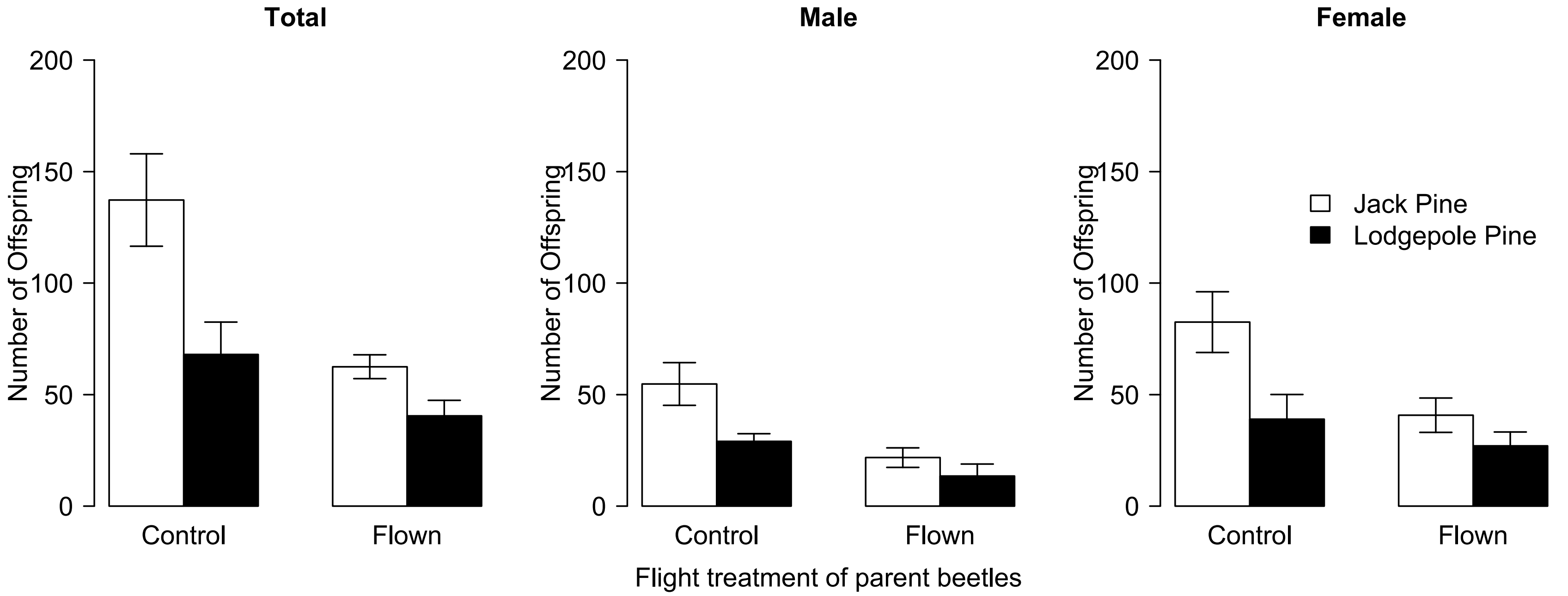

Figure 4.1: Number of offspring produced by flown and control (un-flown) parental mountain pine beetles subsequently introduced to lodgepole pine and jack pine for reproduction. Flown and control adult beetles were introduced to lodgepole pine (n=4) and jack pine (n=4) bolts and emerging male, female and total number of offspring were counted. Data were analyzed using separate generalized mixed effects models for total, female and male number of offspring. Raw data are plotted.

Figure 4.2: Proportional fat content (Offspring fat content/ offspring body weight) of the offspring that emerged from lodgepole and jack pine from flown and control (un-flown) parental mountain pine beetles. Flown and control parental beetles were introduced to lodgepole pine (n=4) and jack pine (n=4) bolts and the fat content of the emerging beetles was extracted in petroleum ether. Data were analyzed using separate generalized mixed effects models for total, female and male offspring fat content. Raw data are plotted.

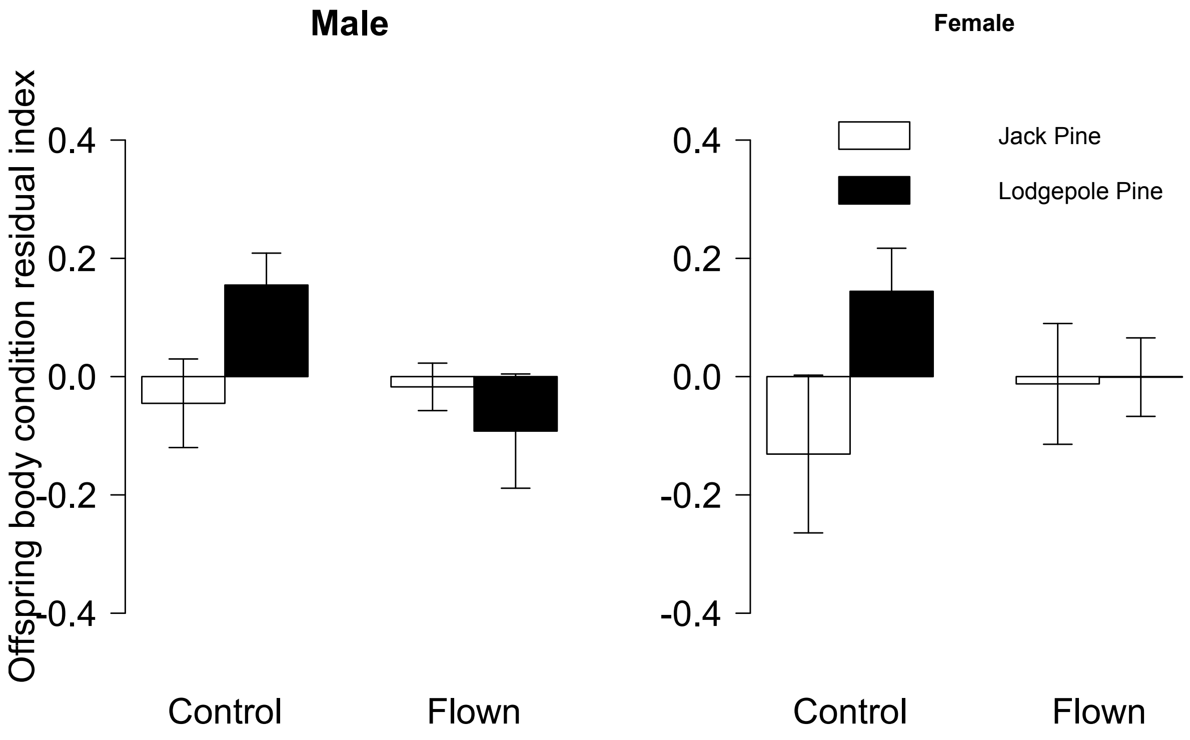

Figure 4.3: Body condition residual indices of male and female offspring mountain pine beetle produced by flown and control (un-flown) parent beetles in lodgepole and jack pine. Parental beetles were introduced to lodgepole pine (n=4) and jack pine (n=4) bolts. Offspring body condition indices were calculated by regressing offspring fat content against offspring body size. Data were analyzed using separate general mixed effects models for female and male body condition residual indices. Raw data are plotted.

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Chapter 5 : General conclusion

Analysis of factors influencing dispersal capacity of mountain pine beetle (MPB), *Dendroctonus ponderosae* Hopkins (Coleoptera: Curculionidae: Scolytinae) will help us to understand the population dynamics of this destructive forest pest and will help in modeling MPB dispersal in nature. Here, we have demonstrated that adult flight of MPB is affected by physiological and environmental factors. We further show a major trade-off between MPB dispersal and reproduction.

The mountain pine beetle energy budget is composed of higher amounts of proteins and lipids than carbohydrates, with proteins being the highest. This study is the first to reveal that MPB utilizes both lipids and carbohydrates in fueling flight. Lipids are the major fuel for MPB flight, with neutral lipids being the largest component. Mountain pine beetles use very low amounts of carbohydrates in flight compared to lipids, suggesting that MPB may utilize carbohydrates only during flight initiation and neutral lipids for sustained flight. These results agree with previous findings that MPBs utilize lipids at least in part during flight (Evenden et al. 2014). This has also been found in other *Dendroctonus* species. The majority of lipids of *Dendroctonus frontalis* Zimmermann are triglycerides (Kinn et al. 1994). Lipids are metabolized during flight in *D. frontalis* by the degradation of triglycerides into short chain fatty acids which can occur between emergence and host colonization (Hodges and Barras 1974). Females, the pioneering sex of host location, have higher lipid content compared to males in the current study and in a previous study (Evenden et al. 2014). Carbohydrates are the major fuel for flight initiation in insects (Freidman 1985) and this may be the case in MPB as flown beetles have low amounts of trehalose and glucose compared to non flown beetles. *Dendroctonus armandi* Tsai and Li use carbohydrates during the initial dispersal process (Chen et al. 2011). Female and male MPB may use different mechanisms in energy utilization in which females have higher total lipids and glucose content compared to males. These differences in energy metabolism during dispersal may affect the beetle's dispersal capacity (Williams and Robertson 2008, Evenden et al. 2014) and host finding behaviours (Chubaty et al. 2009, 2014, Latty and Reid 2010). Further studies should be done in order to characterize the energy metabolism during different stages of flight in MPB.

Insect movement is governed by both abiotic and biotic environmental factors (Loxdale and Lushai 1999). This study reveals the effects of some abiotic factors on MPB flight: pre- and post-flight temperatures, day lengths, and wind speeds. Beetles held at 5°C for one day before flight fly longer and show a trend for flying further than those held at 24°C. Energy utilization is low at low temperatures (Chubaty et al. 2014) and MPB dispersal is positively correlated with remaining energy reserves for flight (Safranyik 1976, Evenden et al. 2014) suggesting that beetles that experience high pre-flight temperatures may have lower flight capacities. Beetles flown at the higher flying temperature are less likely to fly, which may be due to higher energy expenditure at higher temperatures than at cooler temperatures. Therefore, beetles held at high temperatures may be of lower quality in terms of energetic condition compared to individuals held at lower temperatures (Chubaty et al. 2014) which might reduce the flight propensity. Therefore, changes in temperature with changing habitats might alter MPB dispersal capacity.

Mountain pine beetles fly for a longer distances and durations with increased artificial day length under a constant temperature. This finding suggests that the distance beetles can fly in a day will increase in the most northern parts of its expanded range (Cullingham et al. 2011, Erbilgin et al. 2014). The photophase influences the flight capacity of male and female beetles differently as females fly further and longer in the photophase of the longer light cycle compared to males, suggesting that day lengths might act differently on flight capacity of different sexes. Beetles flew more in the photophase compared to the scotophase during the assay in the current study. Mountain pine beetles fly in the presence or absence of wind, and are capable of flight against all of the wind speeds tested in the current study (0-2 m/s). Beetles are known to fly upwind against a wind speed of 2m/s in the field (Safranyik et al. 1992). In the current study, beetles flying at 2m/s flew shorter durations compared to those flown at lower wind speeds. Intermediate and sustained flights are more prominent in females than males. These findings will be important in modeling MPB dispersal in natural habitats. Further understanding of MPB energy metabolism under different environmental conditions may help in understanding the effects of flight on host selection and in predicting population dynamics in the habitat during an outbreak.

Energy investment in dispersal limits female reproductive investment (Hanski et al. 2006) and can result in trade-offs in other life history traits (Johnson 1969, Stearns 1992). Female MPBs show a physiological change during dispersal and reproduction, in which females produce reproductive proteins after the initial dispersal process while males consume proteins during dispersal (Pitt et al. 2014). This study revealed a trade-off between flight and reproduction in

MPB, based on offspring number and condition. Adult beetles that expended energy in flight subsequently produced fewer offspring compared to control beetles that were not given the opportunity to fly, suggesting that resource availability after flight influences MPB reproduction (Elkin and Reid 2005). There is a trend for flown MPB parents to produce offspring in poor condition. Maternal effects can influence offspring quality (Fox and Czesak 2000). Female insects can have reduced fecundity (Gu et al. 2006) and egg size (Elkin and Reid 2005) as a result of energy use. Mountain pine beetle utilize lipids during flight (Evenden et al. 2014) and this reduction in fat reserves may affect the offspring condition through investment to egg production after flight.

The pine host within which beetles develop has an effect on beetle reproduction, in terms of the offspring number. The naïve host, jack pine (*Pinus banksiana* Lamb.) was more suitable for brood success than was the historical host, lodgepole pine (*P contorta* Douglas ex. Loud. var. *latifolia*). The body condition residual indices for the offspring of individuals reared on lodgepole pine showed a trend towards better condition than those from jack pine. Host chemistry may influence MPB brood condition. Lodgepole pine produces higher levels of defensive monoterpenes than jack pine (Clark et al. 2014). Maternal effects may render offspring better able to resist the defenses produced by lodgepole pine than the offspring of flown parents. Host nutritional content also affects individual body condition. Male MPB that emerged from jack pine bolts in a previous study had lower fat stores compared to males that emerged form lodgepole pine bolts (Lusebrink et al. 2016).

This study demonstrates the importance of both endogenous and exogenous factors on dispersal of MPB, and the consequences of dispersal on subsequent reproduction. Due to the irruptive nature and the range expansion of MPB, understanding their dispersal and reproductive success is important for understanding infestation patterns and in predicting outbreaks. Understanding of MPB dispersal and the effect that beetle energy expenditure has on offspring will help in prediction of the economic impact of the pest in native and naïve hosts. Further studies are necessary to understand energy expenditure of MPB in different flight stages and to understand how environmental conditions influence the individual plasticity in energy expenditure during flight.

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Appendix I

Details of sites in Grande Prairie, AB. Mountain pine beetle-infested lodgepole pine bolts were obtained from five different sites near Grande Prairie, AB in October 2014. Five trees were harvested from each site.

Site	**Location**
1	N 54 39.041, W 118 58.332
2	N 54 32.492, W 119 06.920
3	N 54 25.320, W 118 00.677
4	N 54 36.465, W 118 13.282
5	N 54 21.376, W 118 19.112

Appendix II

Details of sites in Grande Prairie, AB. Mountain pine beetle-infested lodgepole pine bolts were obtained from three different sites near Grande Prairie, AB in September 2012. Five trees were harvested from each site.

Site	Location	
1 Central Ridge Road	N 54 30.344	W118 46.643
2 Musreau Lake Road	N 54 33.809	W118 42.501
3 Wellsite Rd @ km 7-57 on Musreau Cutacross	N 54 39.069	W118 44.287