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Chapter 2: Cold and Alone? Roost Choice and Season Affect Torpor Patterns in Lesser Short-Tailed Bats.

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Nature of contribution by PhD candidate	Sole/major contributions to: study design, field work, data analyses, and writing, submission, and revision of all drafts of the manuscript
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Extent of contribution by PhD candidate (%)	90
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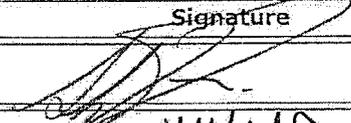
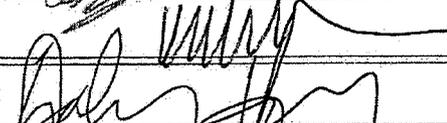
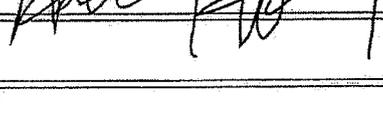
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Name	Nature of Contribution
Stuart Parsons	Assistance with: study design, field work, editing of manuscript
Mark Brigham	Assistance with: study design, field work, editing of manuscript
Tony Hickey	Assistance with: editing of manuscript

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The undersigned hereby certify that:

- ❖ the above statement correctly reflects the nature and extent of the PhD candidate's contribution to this work, and the nature of the contribution of each of the co-authors; and
- ❖ that the candidate wrote all or the majority of the text.

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Chapter 3: Stressful Summers? Torpor Expression Differs Between High and Low Latitude Populations of Bats

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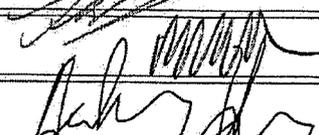
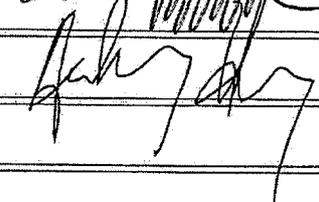
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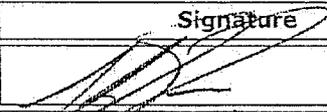
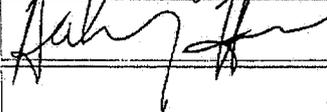
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Chapter 5: Demographics, Season, and Location Affect Dietary Preferences of New Zealand Lesser Short-Tailed Bat (*Mystacina tuberculata*)

Nature of contribution by PhD candidate	Sole/major contributions to: study design, field work, data analyses, and writing, submission, and revision of all drafts of the manuscript
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**Energy Balance in a New Zealand Bat  
(*Mystacina tuberculata*): Thermoregulation,  
Roost Choice, and Diet**

**Zenon James Czenze**

A thesis submitted in fulfillment of the requirements for the degree of Doctor  
of Philosophy in Biological Sciences.

The University of Auckland 2017.



## **Abstract**

Seasonal and latitudinal differences in ambient temperature ( $T_a$ ) differentially impact the energy budgets of endotherms, which maintain homeostasis via physiological and behavioural traits including roost choice, thermoregulation, and diet. During periods of negative energy balance, small mammals, such as bats, use torpor to reduce energy expenditure and therefore energy demands. Most thermal physiological research has focused on species from the extremely seasonal Northern hemisphere, while comparatively few have examined species living in the less seasonal Southern hemisphere. My thesis evaluated how seasonal, and latitudinal differences in energy balance relate to variation in roosting sociality, torpor expression, and diet among three populations of New Zealand lesser short-tailed bat (*Mystacina tuberculata*). I quantified  $T_a$ , roost temperature, and skin temperature responses by individuals and used molecular analysis to compare the diets of three distinct populations (Pureora, Eglinton, and Hauturu). In Pureora, individuals used communal roosts more often in summer than winter, and more often in Pureora than Eglinton during summer. During winter, individuals from Hauturu preferred to roost in uninsulated punga (*Cyathea dealbata*), which was contrary to Pureora and Eglinton individuals, who preferred insulated tree roosts. Torpor expression was greater for individuals in solitary roosts, in winter compared to summer, and in higher latitude compared to lower latitude sites. In contrast to previous morphology-based diet studies, Lepidoptera and Diptera accounted for nearly 90% of amplicons identified from faecal matter. Dietary diversity was higher in summer than winter, higher in lower latitude compared to higher latitude sites, and higher in juvenile bats compared to other demographics. I demonstrate that *M. tuberculata* have a varied roosting, thermoregulatory, and dietary strategy, likely to meet the site-specific challenges of their environment.

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# **Chapter 1: General Introduction**

## **Energy Balance**

An individual's daily and long-term energy balance (energy expenditure vs. energy intake) can be influenced by a large number of variables. These likely include: climate, weather, ambient temperature ( $T_a$ ), circannual cycles, photoperiod, reproductive state, and food/water availability (Csada & Brigham, 1994; Doucette, Brigham, Pavey, & Geiser, 2012; Körtner & Geiser, 2000; McNab, 2002; Song & Geiser, 1997). The exchange of heat through radiation, convection, conduction, and evaporation is critical and can strongly affect an individual's energy expenditure (McNab, 2002). To maintain homeostasis individuals must balance the energetic costs of environmental thermal exchange against maintenance, somatic growth, and reproduction.

Endotherms (i.e., birds and mammals) must cope with seasonal variation in ambient conditions. To avoid predictable energetic shortfalls, migratory species leave their summer habitat and travel to wintering grounds where the climate and/or food availability is more favourable (e.g., Post & Forchhammer, 2008; Visser, Holleman, & Gienapp, 2006). Conversely, hibernators accumulate an energy reserve (i.e., fat store or food cache) large enough to balance winter energy costs (e.g., Geiser, 2004; Lyman, 1982). Many hibernating species reduce energy expenditure so their energy reserve is just large enough to survive, and small increases in energy expenditure may negatively impact survival (Boyles & Brack, 2009; Speakman, Webb, & Racey, 1991).

## **Torpor**

Many clades of vertebrates employ torpor to reduce energetic expenditure during periods of food scarcity/unpredictability (Grigg, Beard, & Augee, 2004; Ruf & Geiser, 2015; Wang, 1989). Torpor bouts are characterised by drastically lowered metabolic rate (MR) and, consequently, a reduction in body temperature ( $T_b$ ) (Geiser, 2004). Torpid MR (TMR) can be reduced to <1% of resting metabolic

rate, resulting in significant energetic benefits (Geiser, 2004). Daily heterotherms typically employ torpor for <24 hours, while hibernators can display multiday torpor bouts throughout their hibernation period (see Ruf & Geiser 2015 for a review).

Torpor is not without costs and, during prolonged torpor, heart rate is depressed and breathing occurs between bouts of apnea (Heldmaier, Ortmann, & Elvert, 2004). These physiological patterns result in respiratory acidosis due to increased CO<sub>2</sub> in the blood (Elvert & Heldmaier, 2000). The inability to drink or urinate while torpid results in the buildup of metabolic wastes and osmotic stress (Thomas & Cloutier, 1992; Thomas & Geiser, 1997). The immune system of hibernating animals appears to be depressed, which can make individuals more susceptible to pathogens (Luis & Hudson, 2006; Moore et al., 2011; Prendergast, Freeman, Zucker, & Nelson, 2002). Protein synthesis, gluconeogenesis, and cell division and growth are all suppressed during torpor bouts (Frerichs et al., 1998; Staples & Hochachka, 1998; van Breukelen & Martin, 2002). Hibernators also appear to accumulate a sleep debt and may exhibit memory loss (Larkin & Heller, 1999; Millesi, Prossinger, Dittami, & Fieder, 2001; Thompson, Montiglio, & Humphries, 2013).

A fundamental characteristic of torpor displayed by most endotherms is the interruption of torpor bouts with periods of euthermia. These arousals are achieved via metabolic heat production, may counteract the harmful consequences of prolonged torpor, and most hibernators briefly and periodically arouse throughout hibernation (Ruf & Geiser, 2015). External stimuli like fire, freezing, predation, consumption of food stores, and in some cases mating may elicit "emergency arousals" (Humphries, Kramer, & Thomas, 2003; Humphries, Thomas, & Kramer, 2003; Stawski, Matthews, Körtner, & Geiser, 2015). Although

periodic arousals are brief, they account for up to 90% of the organism's overwinter energy expenditure (Geiser, 2004; Thomas, Dorais, & Bergeron, 1990; Wang, 1989). This seems counterintuitive; hibernation appears to be a strategy to maximise energy savings, yet the near ubiquitousness of arousals in hibernating mammals highlights their importance (see Klug & Brigham, 2015 for a review).

Although low  $T_a$ , along with other variables, facilitates torpor, studies on small primates and tropical bats suggest that species with limited fat-storing capacities, or those which deal with regular food shortages, also use torpor (Bartels, Law, & Geiser, 1998; Giroud, Blanc, Aujard, & Bertrand, 2008). In Australia, drought can decrease the insect biomass by >50% and during a dry year, owlet-nightjars (*Aegotheles cristatus*), a heterothermic insectivorous bird, used torpor more often, and exhibited deeper and lengthier torpor bouts compared to a wet year (Doucette et al., 2012). This highlights the importance of considering the interaction between ambient conditions and food availability when investigating questions about the thermoregulatory strategy employed by endotherms.

Torpor saves individuals considerable amounts of energy, but there are ways these individuals can further reduce energetic costs. By choosing roosts that have high daily  $T_a$  fluctuations, individuals may considerably reduce their energy expenditure during arousals thus maximising energetic savings. Some bat species use the daily fluctuations in  $T_a$  to rewarm passively and arouse when  $T_a$  is highest and rewarming costs are lowest (Stawski & Geiser, 2010). Bats with larger fat reserves select warmer microclimates, thus higher TMR, and express torpor less compared to those who are energetically stressed (Boyles, Dunbar, Storm, & Brack, 2007). All individuals have an optimal microclimate  $T_a$  dictated, in part, by the size of their energy reserve. Hibernating Indiana bats (*Myotis sodalis*) increase

TMR and energy expenditure 2°C above or below their optimal hibernacula  $T_a$  (Day & Tomasi, 2014).

The torpor optimisation hypothesis generates different predictions for species with sex-specific life history differences. Over-winter differences in sex-specific energy budgets occur in species where reproductive investment by one sex comes after spring emergence (Humphries, Thomas, et al., 2003; Jonasson & Willis, 2011; Michener & Locklear, 1990). The sex that most depends on spring fat reserves for reproduction should rely more heavily on torpor during hibernation and spend less energy mitigating the costs of torpor (Humphries, Thomas, et al., 2003; Jonasson & Willis, 2011). Male little brown bats, unlike females, do not need to conserve fat to fuel post-emergence pregnancy, and use their energy stores more rapidly during hibernation (Jonasson & Willis, 2011).

### **Hibernation in Tree-Bats**

Most of our understanding of mammalian torpor comes from studies of rodents that construct small, well-insulated burrows housing single or groups of hibernating individuals (Geiser, 2004; Karasov, 1983). Some tree-roosting bats preferentially choose tree cavities that confer energy savings through thermal insulation (Sedgeley, 2006). In areas where lingering winter weather may persist, opportunities for social thermoregulation within trees cavities is a strong factor in bats' choice of roosts, especially during pregnancy and lactation (Willis, Brigham, & Geiser, 2006; Willis, Voss, & Brigham, 2006). *Nyctophilus geoffroyi* and *N. gouldi* may further lower their thermoregulatory costs by choosing uninsulated tree roosts with exposure to solar radiation to achieve passive rewarming (Turbill & Geiser, 2008). During winter, bats may be more active in areas where winters are mild and temperatures rarely drop below freezing and arousals by tree-roosting bats can be influenced by a combination of weather patterns (Park, Jones, &

Ransome, 2000; Stawski, Turbill, & Geiser, 2009; Turbill, 2008). During winter in Australia, the highest insect activity occurred on mild nights with falling barometric pressure, and these nights also coincided with highest bat activity (Turbill, 2008). Uninsulated tree roosts may allow bats to discern environmental cues about winter prey availability.

### **Latitudinal Gradients**

Individuals may respond to daily, seasonal and climatic changes in  $T_a$  differently depending on their location (Dunbar & Brigham, 2010; Stawski & Geiser, 2011; Zervanos, Maher, Waldvogel, & Florant, 2010). Individuals respond physiologically to their environment and small changes in  $T_a$  can drastically impact the individual's energetic balance and strategy (Boyles et al., 2007). Lower latitude populations may rarely experience prolonged low  $T_a$  and individuals likely attempt to avoid the physiological costs of torpor by increasing thermogenesis. Conversely, individuals from higher latitude populations may associate cold  $T_a$  with the onset of winter and enter torpor as a response to long-term periods of cold weather.

*Nyctophilus bifax* from a higher latitude population often exposed to colder  $T_a$ , tolerate lower  $T_a$  before they begin thermogenesis compared to lower latitude individuals (Stawski & Geiser, 2011). Additionally, lower latitude *N. bifax* exhibit shorter torpor bouts and defend higher mean minimum torpid skin temperature ( $T_{sk}$ ) compared to their higher latitude counterparts (Stawski, 2012). Interestingly, the slope of the relationship between torpor bout duration and  $T_a$  was significantly steeper for the higher latitude population, suggesting that factors like  $T_a$  mediated food abundance may affect a population's thermoregulatory behaviour more severely depending on location (Stawski, 2012). It appears that individuals from different populations of the same species can exhibit variability

in their thermoregulatory patterns in response to the site-specific energetic challenges.

### **Seasonal Diet**

Certain aspects of an individual's life history (i.e. reproduction, lactation, hibernation) come with higher energetic burdens and many species alter their diet to account for these costs. During the majority of the year in Israel, greater mouse-tailed bats (*Rhinopoma microphyllum*) feed principally on Coleoptera and small numbers of Lepidoptera, Orthoptera, and Hymenoptera (Levin et al. 2009). However, during the lactation period, male and female *R. microphyllum* take advantage of the brief periodic nuptial flights of queen carpenter ants (*Camponotus felah*) and feed exclusively on these alates (Levin et al. 2009). In Canada during late fall and before hibernation, big brown bat faeces contains more Coleoptera and Trichoptera remains, which are rich in the high-energy linoleic acid (Clare et al. 2013). By switching to more energy rich prey, individuals can maximise foraging effort when under increased energetic burdens. A species with a large distributional range will likely experience different stressors to its energy expenditure (i.e., thermoregulatory costs and foraging costs) and energy intake (i.e., prey availability and dietary selection) depending on location and season. Individuals from higher latitudes will face increased thermoregulatory costs and potentially decreased food availability compared to lower latitude individuals.

### **Study Species**

The lesser short-tailed bat (*Mystacina tuberculata*) is a small forest dwelling bat endemic to New Zealand. This species has a distributional range of nearly 1400 km from Omahuta-Puketi Forest (35° 19' S; 173° 76' E) in the North Island to Whenua Hou/Codfish Island (46° 78' S; 167° 63' E) in the South (Carter & Riskin, 2006). Three subspecies of lesser short-tailed bat have been described

(Hill and Daniel 1985). All three have been assessed as requiring conservation management (O'Donnell, Christie, Hitchmough, Lloyd, & Parsons, 2010b).

Lesser short-tailed bat behaviour changes seasonally, and the species has a well-described life history (see Carter & Riskin, 2006). During the breeding season (December - February), males participate in a lek breeding system (Toth & Parsons, 2013). Males attract females to their singing roost inside a tree cavity near communal female roosts which may contain hundreds of individuals. Singing and courtship activity by males peaks during late summer (King, 1990). Although the life history is well described (Carter and Riskin, 2006), there is no information or direct comparisons between populations from the North and South Island.

Lesser short-tailed bats are an attractive study species for examining thermoregulatory variability as they are widely distributed across New Zealand and populations face different seasonal weather depending on their location. Individuals from populations in the South Island may experience  $T_a$  as low as  $-10^{\circ}\text{C}$  and populations in the North Island may never deal with  $T_a$  below  $0^{\circ}\text{C}$ . Winter behaviour is generally characterised by inactivity during cooler weather, and activity during relatively mild weather (Daniel, 1979). It is likely that, similar to other bat species with large latitudinal ranges (e.g., big brown bats; Dunbar & Brigham, 2010), populations of lesser short-tailed bats will employ varying physiological strategies in response to similar environmental conditions. Indeed, individuals from Codfish Island, at the southern limit of the species range, are more active during the winter than individuals from the milder North Island (Christie & Simpson, 2006; Daniel, 1979; Sedgeley, 2001; Sedgeley, 2003). Individual bats with the greatest energy needs increase foraging time (Audet, 1990; Kunz, 1987), and the extended winter activity in the South Island may represent increased energy demands/decreased food availability. Although some

seasonal activity of lesser short-tailed bats across New Zealand has been documented (e.g., Christie & Simpson, 2006), no research has been undertaken to examine their thermoregulatory behaviour despite its likely importance for survival and site-specific adaptation.

The lesser short-tailed bat represents a model species for examining spatial-temporal variation in diet because it is a small generalist insectivore with a wide distribution including presumably different insect communities. When available, especially in northern populations, bats will feed on nectar and fruit (Lloyd, 2001). However, lesser short-tailed bats use a combination of gleaning, aerial hawking, and terrestrial foraging to capture arthropods that make up the majority of their diet (Arkins, Winnington, Anderson, & Clout, 1999; Jones, Webb, Sedgely, & O'Donnell, 2003; Parsons, 1997; Webb, Sedgely, & O'Donnell, 1998).

Visual inspection and morphological analysis of lesser short-tailed bat faeces indicates they feed primarily on five orders of arthropods: Coleoptera, Lepidoptera, Diptera, Blattodea and Orthoptera; three of which are non-volant (Arkins et al., 1999; Lloyd, 2001). Having a diverse diet allows lesser short-tailed bats to take advantage of a variety of prey items and may allow bats to forage on evenings where  $T_a$  limits the availability of flying insects. This suggests that during nights when flying insect abundance is greatly reduced, lesser short-tailed bats may not be as energetically constrained relative to exclusively hawking bats (Anthony, Stack, & Kunz, 1981; Audet, 1990; Brigham, 1991; Sedgely, 2001). This flexibility also has implications for seasonal dietary preference as more flying insects may be available for capture by bats during summer compared to winter, particularly in the more seasonal South Island. In the warmer North Island, the highest dietary diversity occurs during summer (Arkins et al. 1999). Moreover, when  $T_a$  is highest, there is an increase in moth abundance, which is reflected in

bat fecal samples (Arkins et al. 1999). There have been no direct comparisons of diet between individuals from different populations.

### **Goals and Objectives**

The aim of my thesis was to examine how aspects of an individual's energy budget (i.e., thermoregulatory patterns, roost choice, and diet) vary with season, latitude, and demographic, and which aspects are most flexible in response to site-specific adaptations. I studied individual lesser short-tailed bats from three distinct populations (e.g., Pureora Forest Park, Eglinton Valley, and Hauturu/Little Barrier Island), over a range of latitudes, from 2014-2017. The thesis is arranged as a series of stand-alone papers, three of which have been published in international journals.

In Chapter 2 (Czenze, Brigham, Hickey, & Parsons, 2017a), my objective was to examine seasonal roosting sociality and thermoregulatory patterns of free-ranging lesser short-tailed bats. I tested the hypothesis that differences in seasonal energy budgets, due to changes in  $T_a$ , affect roost choice and thermoregulatory patterns. I predicted that individuals would choose thermally insulated roost microclimates to save energy and lower  $T_a$  would lead to more frequent, prolonged, and/or deeper torpor bouts.

In Chapter 3 (Czenze, Brigham, Hickey, & Parsons, 2017b), my objective was to examine the differences in summer thermoregulatory patterns of free-ranging lesser short-tailed bats between a North and South Island population. I tested the hypothesis that latitudinal differences in energy balance would lead to variation in roosting sociality and torpor expression between populations. I predicted that compared to the lower-latitude population, individuals from the higher-latitude population would show greater torpor expression within thermally insulated solitary tree roosts to conserve energy.

In Chapter 4 (Czenze, Brigham, Hickey, & Parsons, 2017c), my objective was to assess differences in winter thermoregulatory patterns between free-ranging lesser short-tailed bats from an inland and offshore island population. I tested the hypothesis that differences in winter  $T_a$ , and therefore energy balance, would lead to varying torpor expression and roost choice between populations. I predicted that higher  $T_a$  will cause individuals to show lower torpor expression and that individuals would prefer roosting in thermally unstable microclimates and would be more responsive to  $T_a$  to take advantage of warmer evenings for foraging.

In Chapter 5, my objective was to perform molecular analyses on faecal samples collected from bats to assess variability in lesser short-tailed bats' diet across New Zealand. First, I tested the hypothesis that spatial-temporal variation in resource use is an important form of dietary flexibility when assumed resource availability fluctuates. Specifically, I predicted that: 1) during the same season, populations from lower latitudes will eat a more diverse diet. 2) Bats will have a more diverse diet during summer compared to winter. 3) Within a season, prey abundance and diversity will be correlated with  $T_a$ . Second, I tested the hypothesis that prey consumption will differ due to demographic differences in energy demands and predicted that young-of-the-year will consume a more diverse diet.

In Chapter 6, I present general conclusions and summarise the major findings of my thesis in the context of conservation, and suggest future avenues of study.

# **Chapter 2: Cold and Alone? Roost Choice and Season Affect Torpor Patterns in Lesser Short-Tailed Bats**

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## **Abstract**

Seasonal changes in weather and food availability differentially impact energy budgets of small mammals such as bats. While most thermal physiological research has focused on species that experience extreme seasonal temperature variations, knowledge is lacking from less variable temperate to subtropical climates. We quantified ambient temperature ( $T_a$ ) and skin temperature ( $T_{sk}$ ) responses by individuals from a population of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) during summer and winter using temperature telemetry. During summer, communal roosts were more thermally stable than  $T_a$ . During winter, solitary roosts were warmer than  $T_a$  indicating significant thermal buffering. Communal roost trees were used on 83% of observation days during summer, and individuals occupying them rarely entered torpor. Solitary roosts were occupied on 93% of observation days during winter, and 100% of individuals occupying them used torpor. During summer and winter, bats employed torpor on 11 and 95% of observation days, respectively. Maximum torpor bout duration was 120.8 h and winter torpor bout duration correlated negatively with mean  $T_a$ . Torpor bout duration did not differ between sexes, although female minimum  $T_{sk}$  was significantly lower than males. The summer Heterothermy Index varied, and was also significantly affected by  $T_a$ . Mean arousal time was correlated with sunset time and arousals occurred most frequently on significantly warmer evenings, which are likely associated with an increased probability of foraging success. We provide the first evidence that torpor is used flexibly throughout the year by *M. tuberculata*, demonstrating that roost choice and season impact torpor patterns. Our results add to the growing knowledge that even small changes in seasonal climate can have large effects on the energy balance of small mammals.

## **Introduction**

All organisms must maintain a positive daily energy balance or they will perish.

Energy balance is influenced by precipitation, reproductive state, circannual cycles, photoperiod, food/water availability, and ambient temperature ( $T_a$ ) (Csada & Brigham, 1994; Doucette, Brigham, Pavey, & Geiser, 2012; Körtner & Geiser, 2000; McNab, 2002; Song & Geiser, 1997) Ambient temperature has fundamental impacts on metabolism, particularly in small endotherms (i.e. mammals and birds). Maintaining a high and stable body temperature ( $T_b$ ) requires large amounts of energy, particularly for small mammals that quickly lose heat to the environment due to their high surface area to volume ratios.

Though individuals living in highly variable temperate climates face the most drastic changes in seasonal environment, warm temperate areas also expose individuals to seasonal changes in  $T_a$  and presumably energy availability. For most temperate and subtropical endotherms, summer is a period of positive energy balance. During summer, food is usually more abundant than in winter and the costs of thermoregulation at higher  $T_a$  are lower. In cold-temperate areas, winter is challenging because endotherms must cope with energy shortages and greater heat loss to the environment. However, the influence of  $T_a$  variability in subtropical and warm temperate habitats has been less thoroughly explored.

To minimize energy loss during periods of prolonged energy scarcity, many clades of vertebrates employ daily or multiday torpor (Grigg et al., 2004; Ruf & Geiser, 2015; Wang, 1989). Torpor is a physiological state characterized by decreases in metabolic rate (MR) leading to reduced  $T_b$  (Geiser, 2004). Some hibernators accumulate a fat store or food cache large enough to balance energy costs (e.g., Geiser, 2004; Lyman, 1982). During winter, many of these species decrease energy expenditure so that their reserves are large enough to survive, as even small increases in energy expenditure may negatively impact survival (Boyles & Brack, 2009; Speakman, Webb, & Racey, 1991; although see Geiser,

2007).

Most species that hibernate periodically arouse, via metabolic heat production to a high, stable  $T_b$  (i.e. normothermia), and while periodic arousals are brief, this can account for up to 90% of overwinter energy expenditure (Geiser, 2004; Thomas et al., 1990; Wang, 1989). The “torpor optimization hypothesis” postulates that expression of torpor should reflect a balance between the benefits of reduced MR and arousal costs (Humphries, Thomas, et al., 2003; Jonasson & Willis, 2011). Furthermore, the torpor optimization hypothesis predicts differences in sex-specific energy budgets for species with differential timing of reproductive investment (Humphries, Thomas, et al., 2003; Jonasson & Willis, 2011; Michener, 1992).

Lesser short-tailed bats (*Mystacina tuberculata*) are the only extant species of family *Mystacinidae*, and this family is endemic to New Zealand. *Mystacina tuberculata* provide a good model to examine energetic flexibility in small mammals, as they live in areas with more subtle seasonal variations than most temperate mammals. Lesser short-tailed bats roost throughout the year in tree cavities, which may or may not be thermally stable (Sedgeley, 2006). Reproductive activity by males peaks during late summer and females give birth the following spring (King, 1990). Winter behaviour is generally characterized by inactivity within a tree roost; however, activity is positively correlated with  $T_a$  (Christie, 2006; Christie & Simpson, 2006; Daniel, 1979). Notably, mild winter weather (i.e. above 10°C) increases the abundance of flying insects (Paige, 1995), and although *M. tuberculata* are omnivorous, insects make up the majority of their diet (Arkins et al., 1999).

We used temperature-sensitive radio transmitters on individuals to address thermoregulatory patterns of free-ranging *M. tuberculata* in Pureora Forest Park,

in the central North Island of New Zealand. Our first hypothesis was that differences in seasonal energy budgets, due to changes in  $T_a$ , would affect roost choice, and we predicted that individuals would choose thermally buffered roost microclimates to save energy. Our second hypothesis was that seasonal variation in  $T_a$  influence the frequency, duration, and depth of torpor bouts, and we predicted that warmer  $T_a$  during summer would lead individuals to use torpor less than during winter. Furthermore, we predicted that when torpor was used, it would be more frequent, prolonged, and/or deeper during colder days when prey availability is presumably lower. Finally, we examined the hypothesis that timing differences in reproductive investment lead to intersexual differences in winter skin temperature ( $T_{sk}$ ) patterns. Specifically we predicted females, whose reproductive investment comes after winter would be more conservative and exhibit longer and/or deeper torpor bouts than males.

## **Methods**

All procedures were approved by the University of Auckland Animal Ethics Committee (AEC-R1374) and were conducted under New Zealand Department of Conservation Wildlife Act Authorization Number 39083-FAU. Our study was conducted in the Pikiariki Ecological Area of Pureora Forest Park (38°26'S, 175°39'E), central North Island, New Zealand, during the Austral "summer" (January – March) and "winter" (June – October). Pikiariki consists of approximately 450 ha of native, mature podocarp-hardwood forests and is home to approximately 700 *M. tuberculata*. Bats occupied 15 solitary roosts (housing only one individual), and four communal roosts (housing upwards of 400 individuals at a time).

We caught bats in harp traps and mist nets. Individuals were sexed, aged, weighed to the nearest 0.5 g using a spring pesola, forearm length was measured

to the nearest 1 mm, and we calculated body condition index (BCI), mass (g) forearm length (mm)<sup>-1</sup>. Young of the year were distinguished from adults by the lack of ossification of the metacarpal-phalangeal joint on the third digit (Davis & Hitchcock, 1965).

For small bats,  $T_{sk}$  provides a good approximation of  $T_b$  (Audet & Thomas, 1996; Barclay et al., 1996; Willis & Brigham, 2003). We clipped a small patch (<1 cm<sup>2</sup>) of fur between the shoulders and attached a temperature-sensitive radio-transmitter (0.9-1 g, BD-2NT; Holohil Systems Ltd, Carp, Ontario) using a latex cement (TORBOT Group, Inc., Cranston RI, USA). On average the body mass of bats affixed with transmitters was 14.5 g  $\pm$  1.5 g and transmitters represented between 5.6 and 7.5% of body mass. Individual bats were radio-tracked each morning to find day roosts using a scanning receiver (Australis 26K Scanning Receiver, Titley Electronics, Ballina, Australia) connected to a 3-element Yagi antenna. A data-logging receiver (Lotek SRX\_800; Lotek Engineering Inc. Newmarket, Ontario, Canada) was deployed at roosts and recorded individual  $T_{sk}$  at 10-min intervals whenever a radio-tagged bat was present.

Thermoregulatory stages were classified following Jonasson and Willis (2011). Warming: an abrupt increase in torpid  $T_{sk}$  (three consecutive recordings) that eventually stabilized at a normothermic level. Normothermic: the time between the end of the warming phase and the initiation of cooling. Cooling: readily obvious as a decline in  $T_{sk}$  (three consecutive recordings) preceding steady-state torpor. Torpid: stable  $T_{sk}$  (three consecutive recordings) following the cooling phase and preceding the warming phase. We excluded data from the first 24 hours after releasing the bats to avoid the influence of disturbance on our results. We recorded the date and time of each arousal from the  $T_{sk}$  trace and converted the time of arousal to radians. We then used Rayleigh's test for circular distributions

to determine if the daily distribution of arousal times was significantly different from random.

We recorded regular drops in  $T_{sk}$  during the normothermic phase which we refer to as shallow heterothermy, and did not meet our criteria for torpor (i.e. more than 30 min). To compare normothermic bouts we calculated the Heterothermy Index:  $\sqrt{\frac{\sum(\text{mean modal } T_{sk} - T_{sk})^2}{n-1}}$  (HI; Boyles, Smit, & McKechnie, 2011).

HI provides a single value for daily fluctuations in  $T_{sk}$ , and can be used to quantify, and compare highly variable  $T_{sk}$  data.

To estimate energy savings associated with drops in  $T_{sk}$  during normothermia, we estimated power (energy consumption; W) of maintaining mean modal active  $T_{sk}$  ( $E_{mm}$ ), and estimated energy consumption of recorded  $T_{sk}$  ( $E_{obsv}$ ). We assumed bat mass was 80% water, and estimated kilojoules using:  $kJ = (Mass) (specific\ heat\ capacity\ of\ water) (T_{sk} - T_a)$ . We assumed that  $T_{sk}$  and  $T_a$  remained constant during the 10 min recording interval, and then estimated energy consumption using:  $W = (kJ\ sec^{-1}) 1000$ , and daily energy consumption as the sum of these values. We subtracted daily  $E_{obsv}$  from daily  $E_{mm}$  ( $E_{diff}$ ) to estimate energy savings.

We recorded  $T_a$ , barometric pressure ( $P_{bar}$ ), and roost temperature ( $T_{roost}$ ) of one communal roost using data loggers (HOBO Micro Station Data Logger - H21-002, Onset Computer Corporation, Cape Cod, MA, USA). Ambient temperature was recorded in the shade, 2 m above the ground, and the logger inside the communal roost was placed 1 m inside the tree hole in a part of the cavity known to be used by bats.

To determine which bat-specific, and weather-specific variables best predict thermoregulatory strategy we used linear mixed effect models (LMEM). In bat-specific models, we used torpor bout duration ( $\log_{10}$ ) (to decrease the spread of

residuals) and minimum  $T_{sk}$  as response variables, sex as a fixed effect, and individual as a random effect. In weather-specific models we used torpor bout duration ( $\log_{10}$ ), minimum  $T_{sk}$ , or HI as response variables, and three variables which have been associated with bat activity in past studies: (1)  $T_a$ ; (2)  $T_{roost}$ , which may be buffered and less variable compared to  $T_a$ ; (3) Change in  $P_{bar}$ , which reflects passing weather fronts and is correlated with both insect and bat activity (Jones et al. 1995). Temperature and especially  $P_{bar}$  should rapidly equilibrate inside and outside the roost, and could be used by bats to assess relative levels of insect abundance.

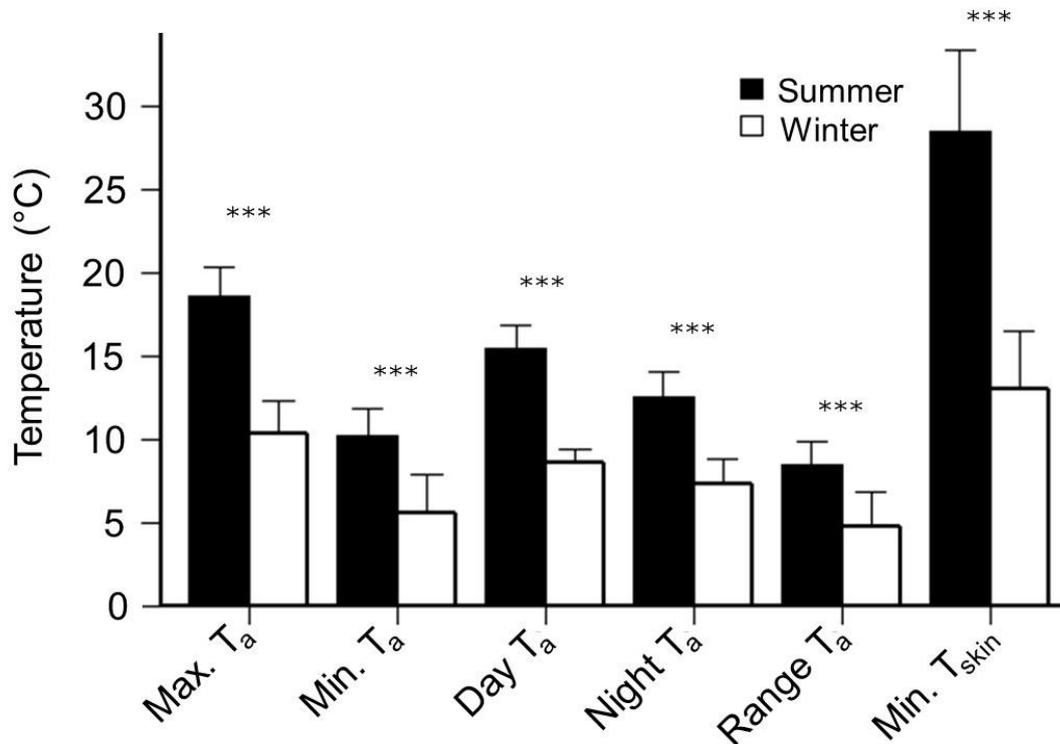
All analyses were conducted in R studio version 0.98.945 (R Development Core Team 2009) and values are reported as the mean  $\pm$  *SD* with *n* representing the number of animals and *N* the number of events/arousals. We assessed significance at the  $P < 0.05$  level.

## Results

We attached transmitters to 8 individuals (5 males, 3 females) and recorded 61 bats-days of  $T_{sk}$  data from 7 individuals (4 males, 3 females). Neither body mass ( $P = 0.65$ ,  $t = -0.47$ ,  $n = 8$  individuals) nor BCI ( $P = 0.74$ ,  $t = -0.35$ ,  $n = 8$ ) differed between sexes. Individuals caught in the summer were heavier ( $16 \pm 0.0$  g;  $n = 3$ ;  $P < 0.01$ ,  $t = 5.7$ ) than those caught during winter ( $14 \pm 0.8$  g;  $n = 5$ ), and had higher BCI ( $P < 0.01$ ,  $t = 6.1$ )

Ambient temperature during summer varied between a mean minimum of  $10.2 \pm 1.6^\circ\text{C}$  ( $N = 18$ , absolute minimum =  $2.7^\circ\text{C}$ ) and mean maximum of  $18.6 \pm 1.7^\circ\text{C}$  ( $N = 18$ , absolute maximum =  $22.3^\circ\text{C}$ ), with a mean daily range of  $8.4 \pm 1.5^\circ\text{C}$  ( $N = 18$ , absolute range =  $11.5^\circ\text{C}$ ). During winter,  $T_a$  varied between a mean minimum of  $5.6 \pm 2.3^\circ\text{C}$  ( $N = 43$ , absolute minimum =  $-2.2^\circ\text{C}$ ) and a mean

maximum of  $10.3 \pm 2.3^\circ\text{C}$  ( $N=43$ , absolute maximum= $15.9^\circ\text{C}$ ), with a mean daily range of  $4.7 \pm 2.1^\circ\text{C}$  ( $N=43$ , absolute range  $10.1^\circ\text{C}$ ; Fig. 1).



**Figure 1** Differences in temperature between summer (black bars) and winter (white bars). Max.  $T_a$  and min.  $T_a$  are the means of all respective daily maximum and minimum ambient temperature ( $T_a$ ); day  $T_a$  and night  $T_a$  are the means of all day and night  $T_a$  (sunrise to sunset, and sunset to sunrise); range  $T_a$  is the mean of all daily ranges in  $T_a$  (max.  $T_a$  - min.  $T_a$ ); min.  $T_{skin}$  is the mean of all individuals mean minimum skin temperature. Values represent mean  $\pm$  SD, asterisks represents a significant difference between seasons (Student's  $t$  test;  $P < 0.05$ ).

During summer, mean minimum communal  $T_{roost}$  ( $13.2 \pm 1.5^\circ\text{C}$ ) was higher than mean minimum  $T_a$  ( $10.2 \pm 1.6^\circ\text{C}$ ), and mean  $T_{roost}$  range ( $3.8 \pm 0.9$ ) was smaller than mean  $T_a$  range ( $8.4 \pm 1.5$ ; Table 1). Mean daily solitary  $T_{roost}$  ( $n=3$  roosts,  $N=12$  days;  $14.2 \pm 3.4^\circ\text{C}$ ) was higher than mean  $T_a$  ( $10.2 \pm 1.6^\circ\text{C}$ ), and the mean  $T_{roost}$  range ( $2.8 \pm 1.2$ ) was less than the mean  $T_a$  range ( $4.6 \pm 1.5$ ).

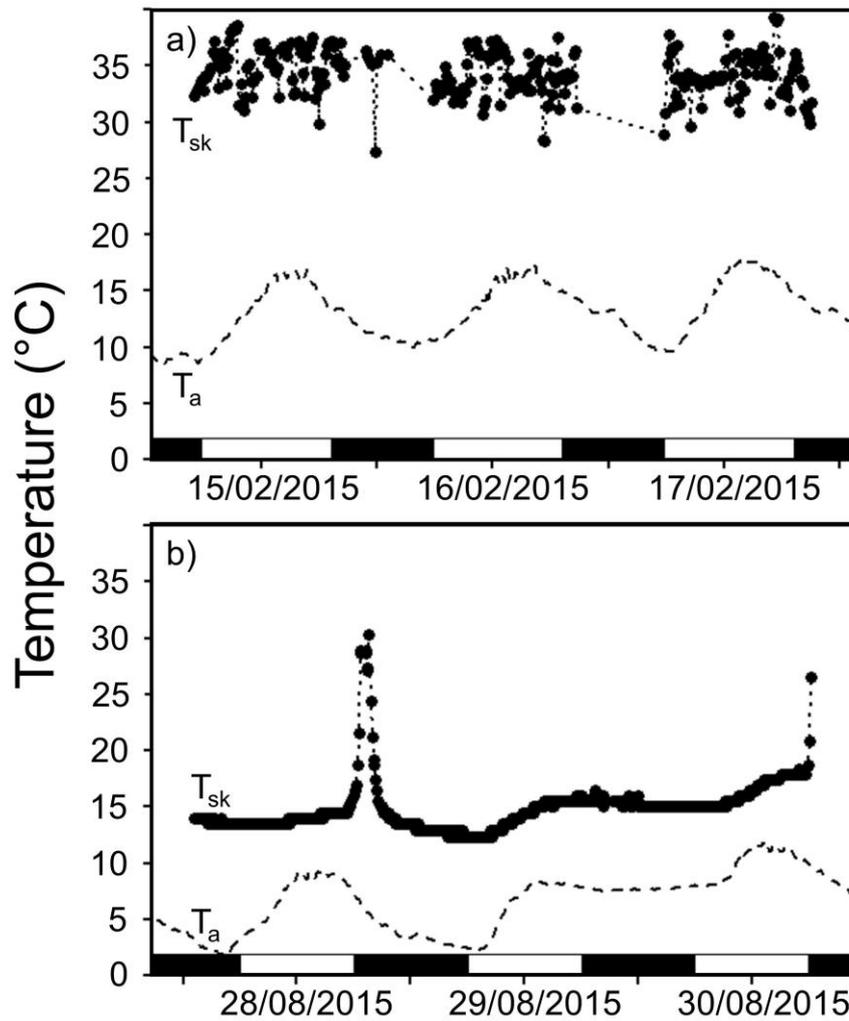
**Table 1** Communal and solitary roost thermal characteristics compared to corresponding ambient conditions in Pureora Forest Park.

	Mean $T_a$ (°C)	Max $T_a$ (°C)	Min $T_a$ (°C)	Range $T_a$ (°C)
Communal Roost	14.7±1.4	17.2±1.7	13.2±1.5	3.8±0.5
Communal Ambient	15.6±1.5 <sup>#</sup>	18.6±1.7*	10.2±1.6*	8.4±1.5*
Solitary Roost	14.2±3.4	15.4±3.0	12.8±3.5	2.8±1.1
Solitary Ambient	6.8±4.7*	8.8±4.5*	4.2±4.6*	4.6±1.5*

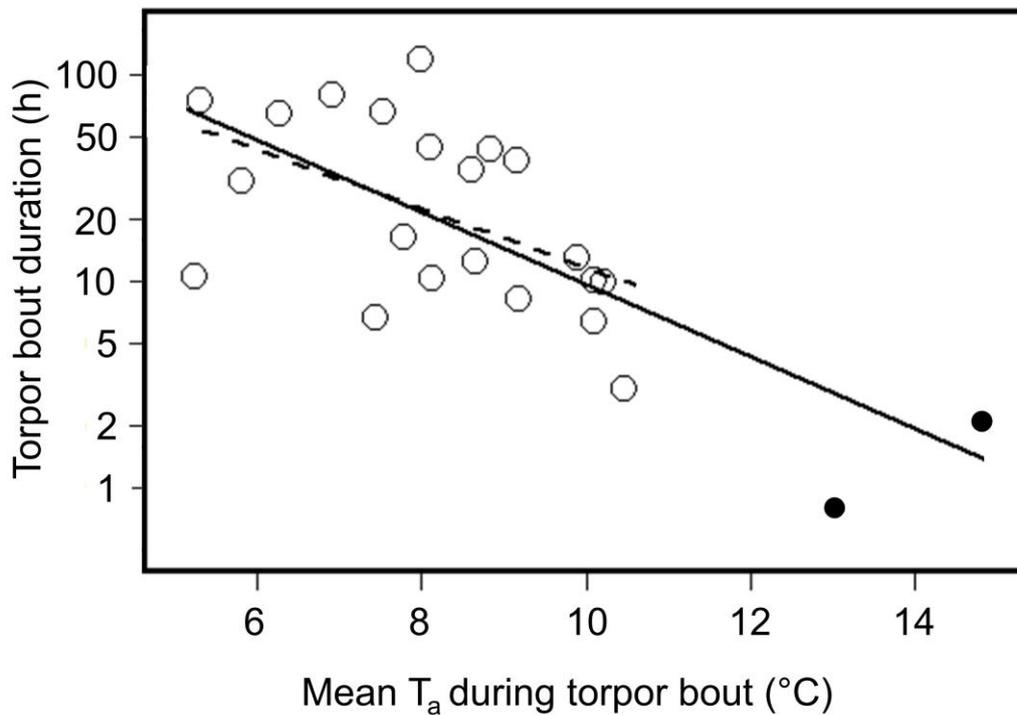
Mean  $T_a$  is the average of all mean ambient temperatures ( $T_a$ ); max  $T_a$  and min  $T_a$  are the mean of all respective daily maxima and minima  $T_a$ ; range  $T_a$  is the mean of all daily ranges in  $T_a$  (max.  $T_a$  – min.  $T_a$ ). Values are means  $\pm$  SD, communal  $N=18$  days, solitary  $N=12$ , \* denotes a significant difference between values (Student's  $t$  test;  $P<0.01$ ), <sup>#</sup>=0.098.

During summer and winter, 97.6% of torpor bouts occurred in solitary roosts (42/43) and only once in a communal roost. The HI for all bats was significantly higher (i.e. more heterothermic) when they roosted in solitary roosts (19.5±4) compared to communal roosts (3.2±3.6,  $P<0.01$ ,  $t=-15.7$ ,  $df=35.8$ ). During winter, bats used torpor on 95.3% of days and on 11.1% of observation days during summer (Fig. 2). Every individual entered torpor at least once in winter, while only one individual did so during summer. Torpor bout durations ranged from 0.9 to 2.2 h in summer and 6.7 to 120.8 h in winter.

Multiday torpor bouts made up 47.6% of torpor bouts (10/21) during winter and were not recorded during summer. Mean daily torpor bout duration during winter was 10.1±3.7 h ( $n=4$ ,  $N=11$ ) while mean multiday torpor duration was 60.7±27.5 h ( $n=4$ ,  $N=10$ ). Torpor bout duration ( $\log_{10}$ ) decreased with increasing  $T_a$  during winter ( $P=0.02$ ,  $t=-2.6$ , ordinary least square (OLS)  $r^2=0.23$ ; Fig. 3). When torpor bout duration ( $\log_{10}$ ) and  $T_a$  from summer and



**Figure 2** Skin temperature ( $T_{sk}$ , upper trace, dotted line) and ambient temperature data ( $T_a$ , lower trace, dashed line) for two different *M. tuberculata* individuals, monitored over 3 days in a) a communal roost during summer and b) a solitary roost during winter. Black and white bars denote night and day, respectively.



**Figure 3** Torpor bout duration (hours) of *M. tuberculata* as a function of mean  $T_a$  during torpor bout in summer (black circles) and winter (white circles). Winter torpor bout duration ( $\log_{10}$ ) decreased with increasing  $T_a$  (linear mixed effect model;  $P=0.02$ ,  $t=-2.6$ ,  $N=3$  individuals,  $n=16$  torpor bouts), dashed line represents ordinary least square regression of winter only torpor bouts  $r^2=0.23$ . Summer and winter torpor bout duration ( $\log_{10}$ ) decreased with increasing  $T_a$  (linear mixed effect model;  $P<0.001$ ,  $t=-4.7$ ,  $N=4$ ,  $n=18$ ), solid line represents ordinary least square regression of summer and winter torpor bouts  $r^2=0.49$ . Note log scale used on y-axis.

winter were pooled and regressed together the  $r^2$  value of this relationship was stronger ( $P<0.001$ ,  $t=-4.7$ , OLS  $r^2=0.49$ ) compared to data from the winter-only.

Torpor bout duration did not differ between males and females ( $P=0.8$ ,  $t=-0.2$ ,  $df=9.6$ ). During winter, females had lower minimum torpid  $T_{sk}$   $11.9^{\circ}\text{C}$  than males  $14.8^{\circ}\text{C}$  ( $P=0.03$ ,  $t=-2.3$ ,  $df=17$ ), but were not exposed to significantly colder  $T_a$  ( $P=0.7$ ,  $t=0.3$ ,  $df=32.7$ ). The HI during days when bats did not use torpor was negatively correlated with the mean  $T_a$  ( $P<0.01$ ,  $t=-5.9$ ,  $df=13$ , OLS  $r^2=0.69$ , slope=-0.44) and the previous night's mean  $T_a$  ( $P<0.01$ ,  $t=-4$ ,  $df=13$ , OLS  $r^2=0.53$ , slope=-0.54,  $n=4$ ,  $N=18$ ). However, when removing the two winter

days when bats did not enter torpor and analysing data for summer only, the relationship between mean  $T_a$  ( $P=0.01$ ,  $t=-2.9$ ,  $df=12$ , OLS  $r^2=0.37$ , slope=-0.37) remained significant but not the relationship between previous night's mean  $T_a$  ( $P=0.067$ ,  $t=-2$ ,  $df=12$ , OLS  $r^2=0.23$ , slope=-0.32,  $n=3$ ,  $N=16$ ).

We found that  $E_{diff}$  was positively correlated with HI ( $P<0.01$ ,  $t=3.8$ ,  $df=13$ ,  $n=4$ ,  $N=18$ ,  $r^2=0.45$ ), i.e. more heterothermic behaviour lead to greater energy savings. The  $r^2$  of this relationship increased when we analysed just the summer data ( $P<0.01$ ,  $t=5.1$ ,  $df=13$ ,  $n=3$ ,  $N=16$ , OLS  $r^2=0.65$ ). We found that  $E_{diff}$  increased with decreasing mean  $T_a$  ( $P=0.02$ ,  $t=-2.6$ ,  $df=13$ ,  $n=4$ ,  $n=18$ , OLS  $R^2=0.3$ ) and the  $r^2$  of this relationship increased when we analysed summer only data ( $P=0.01$ ,  $t=-3.0$ ,  $df=12$ ,  $n=3$ ,  $N=16$ , OLS  $r^2=0.39$ ).

The circular distribution of arousal times after torpor bouts was significantly non-random (Rayleigh  $Z=19.8$ ,  $P<0.001$ , mean vector=0.93,  $n=5$ ,  $N=23$ ). Mean time of arousal was  $18:13\pm 1:31$  h and arousals began  $8.2\pm 102.8$  min ( $n=5$ ,  $N=23$ ) before sunset. During winter, mean  $T_a$  on nights when bats aroused from torpor was higher ( $7.9\pm 2.1^\circ\text{C}$ ) than on nights when bats remained torpid ( $6.7\pm 1.7^\circ\text{C}$ ,  $P=0.045$ ,  $t=-2.1$ ,  $df=40$ ). None of the other weather variables we recorded differed between days bats remained torpid or aroused.

## **Discussion**

We demonstrate that *M. tuberculata* exhibit seasonal roost choice, with a preference for communal roosts during summer and solitary roosts during winter. This agrees with our first hypothesis. We also found support for our second hypothesis, providing the first evidence that *M. tuberculata* is flexible in its seasonal energetic strategy, using prolonged torpor during winter and short bouts of torpor during summer and winter. In addition, torpor bout duration correlates negatively with mean  $T_a$ . We found mixed support for our final hypothesis, as we

did not find that females performed longer torpor bouts than males, but we did observe that females had lower minimum torpid  $T_{sk}$ . Our data indicate that individuals are more energetically stressed during winter than summer and suggests that they use buffered roosts and torpor to cope with presumed energy shortages.

Roost choice changed seasonally with 83% of nights spent in communal roosts during summer compared to 7% during winter. The thermal characteristics we recorded indicate that communal roosts are more thermally stable than  $T_a$  and buffer against extreme cold. In other parts of New Zealand, *M. tuberculata* prefer thermally buffered communal roosts with large cavities during summer (Sedgeley, 2003, 2006). Large cavities may allow for more conspecifics and the potential for greater social thermoregulation (Willis, Voss, & Brigham, 2006; although see Dausmann & Glos, 2015). Roosts that are buffered and large enough for social thermoregulation may be ideal for reproductive females to leave young of the year which may be more susceptible to drastic  $T_a$  fluctuations. However, disturbance by roost mates can be detrimental, and impromptu arousals by roost mates may lead to unwanted energy expenditure which may be especially dangerous when energy reserves are low (Speakman et al., 1991; Turner et al., 2014). The preference for solitary roosts during winter may be driven by energetically stressed individuals avoiding the potential costs associated with communal living.

The preference by bats for solitary roosts during winter may reflect a need for buffered microclimates. For all hibernating animals there is a "set-point" temperature ( $T_{set}$ ) below which thermoregulation is initiated, metabolic rate increases and torpor bout duration decreases, and therefore, energy costs increase (Heller & Colliver, 1974). In response to freezing  $T_a$  some hibernating individuals must perform costly emergency arousals (Humphries, Thomas, et al.,

2003). To protect against temperatures below  $T_{set}$  many species use insulated nests/roosts (Buck & Barnes, 1999; Casey, 1981; Schmid, 1998). It is likely that during winter, individuals must seek out roosts to facilitate and enhance energy savings from torpor, but buffered against  $T_a$  below  $T_{set}$ . Although we could not determine the  $T_{set}$  of *M. tuberculata*, we did record minimum torpid  $T_{sk}$  of 8.6°C. We recommend future studies using open-flow respirometry to determine *M. tuberculata*'s  $T_{set}$  and behavioural tests to examine preferences in roost microclimate.

To balance the physiological costs of torpor with the energetic benefits, the torpor optimization hypothesis predicts that individuals with large energy stores should express less torpor than those with limited reserves. *Mystacina tuberculata* seem to employ both short and multiday torpor bouts during winter. During winter, bats used torpor more often, and these torpor bouts are deeper and more prolonged compared to summer. Similar to other insectivorous bats (Park, Jones, & Ransome, 2000; Stawski & Geiser, 2010) torpor bout duration during winter correlated with  $T_a$  and individuals performed longer torpor bouts in response to colder  $T_a$ . Temperatures < 10°C are likely to constrain insect abundance which reduces foraging success and makes torpor a less risky energetic strategy compared to foraging (Hope & Jones, 2012; Park et al., 2000). Despite relatively cold  $T_a$  during winter in 2015, warmer mean night  $T_a$  between 9-10°C were not uncommon, which may provide conditions for greater insect abundance, and therefore, the opportunity to forage successfully. During summer, individuals likely have larger fat stores compared to winter, and therefore, do not need to rely on torpor due to improved fuel availability and insulation.

If torpor is employed, energy savings increase with a fall of  $T_{sk}$ . We found no sex differences in torpor bout duration during winter. However, we did find that

female minimum torpid  $T_{sk}$  was lower than males despite no significant differences in the mean or minimum  $T_a$  they experienced. This agrees with predictions of the torpor optimization hypothesis for species with sex specific life history differences. Jonasson and Willis (2011) showed that male bats use their energy stores more rapidly than females during hibernation and their body mass declined (by as much as 130%) faster relative to that of females. Male *M. lucifugus*, unlike females, do not need to conserve fat to fuel post-emergence pregnancy (Jonasson & Willis, 2011). Female *M. tuberculata* are likely under greater pressure to enter the reproductive season with larger fat reserves to initiate pregnancy. Conversely, males are likely under less pressure to conserve energy during winter, and spend more energy than females during torpor, and potentially decrease costs of torpor by maintaining higher minimum  $T_{sk}$ .

Weather conditions appear to influence the thermal strategies of normothermic individuals and our data lend further support to the torpor optimization hypothesis. We found that the HI for normothermic individuals correlated with mean  $T_a$ , indicating that individuals were more heterothermic during colder days. Moreover, HI positively correlated with daily  $E_{diff}$ . Individuals using a greater degree of heterothermy would save more energy compared to individuals maintaining a stable  $T_b$ . Though only an estimate, our model predicts that a 15 g individual in a 10°C day-roost defending a  $T_b$  of 39.4°C would spend 1.4 kJ while at 25.5°C it would spend only 0.78 kJ. It would be useful for further studies in the laboratory to calculate metabolic rate under controlled conditions to better calculate the precise energy savings.

During winter, arousals from torpor were significantly non-random and occurred on significantly warmer nights. This suggests that individuals, while torpid, are able to use environmental cues from within their roost to assess

ambient conditions. Greater horseshoe (*Rhinolophus ferrumequinum*) and Natterer's bats (*Myotis nattereri*) maintained a circadian pattern to arousals and synchronized arousals with sunset, likely to take advantage of mild winter nights and potential prey abundance (Hope & Jones, 2012; Park et al., 2000). Aerial insect abundance can increase dramatically with mild  $T_a$  (Jones, Duvergé, & Ransome, 1995), providing opportunities to forage especially near dusk when flying insects are most abundant (Racey & Swift, 1985). Our results suggest that during winter, individuals may track  $T_a$  passively and arouse in response to nights which provide lower thermoregulatory costs and higher probability of successful foraging.

## **Conclusions**

We show that *M. tuberculata* has a highly flexible seasonal energetic strategy despite relatively low seasonal variation in  $T_a$ . During summer and winter, roosts are used that buffer against  $T_a$  fluctuations, and this is most important during winter. Our data suggest that, in the wild, *M. tuberculata* are facultative torpor users. While we cannot definitively state that *M. tuberculata* are hibernators, they clearly employ a continuum of heterothermic responses during summer and winter; from shallow heterothermy to multiday torpor bouts. Longer torpor bouts were correlated with colder  $T_a$  and were interrupted by arousals at sunset during significantly warmer evenings presumably for individuals to take advantage of milder conditions. These data agree with the torpor optimization hypothesis and it appears that individuals attempt to balance the physiological costs associated with torpor induction and cessation against energy savings of metabolic suppression in torpor. Our data also indicate that small mammals in warm temperate climates respond to relatively small changes in seasonal  $T_a$  with a similar energetic strategy as more cold temperate species. It will be interesting

to compare populations of *M. tuberculata* from the cold temperate South Island of New Zealand to determine roost preference and thermoregulatory response to more extreme seasonal changes in  $T_a$ , and the extent of the species' phenotypic plasticity and energetic flexibility.

# **Chapter 3: Stressful Summers? Torpor Expression Differs Between High and Low Latitude Populations of Bats**

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## **Abstract**

Variation in weather and food availability impacts the energy budgets of endotherms, with some species using torpor as an energy-saving strategy during periods of negative energy balance. We evaluated how latitudinal differences in energy balance relate to variation in torpor expression and roosting sociality between populations. We monitored summer skin temperatures ( $T_{sk}$ ) of individuals from 2 populations of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) separated by 6° of latitude using temperature telemetry. Although mean summer  $T_a$  was only <1°C lower for the higher-latitude than lower-latitude population, individuals living at the higher-latitude site used torpor on 36% of observation days compared to 11% for lower-latitude bats. None of the recorded weather variables affected the propensity to enter torpor or torpor bout duration; however, the minimum torpid  $T_{sk}$  of bats positively correlated with daily minimum  $T_a$ . Roosts occupied by solitary bats were warmer than  $T_a$ , and temperatures within them fluctuated less than  $T_a$ . Higher-latitude individuals roosted solitarily (38%) more frequently than lower-latitude individuals (17%) and individuals from both populations exclusively used torpor while roosting solitarily. Arousals from torpor by higher-latitude bats coincided with sunset and not daily  $T_a$  maxima suggesting that bats were not fully exploiting advantages of passive rewarming. Site-specific roost choice and torpor patterns were apparent between *M. tuberculata* populations during summer, demonstrating that small differences in  $T_a$  differentially affect energetic strategy. The thermoregulatory behavior of species inhabiting latitudinal gradients in climate is highly plastic, likely to meet the specific challenges of their environment.

## **Introduction**

Due to their high surface-to-volume ratios, small endotherms (i.e., mammals and birds) are especially susceptible to low ambient temperatures ( $T_a$ ) and quickly lose

heat to the environment. Maintaining a high and stable body temperature ( $T_b$ ) is energetically costly and, to mitigate costs, many clades of small endotherms employ torpor (Grigg et al., 2004; Ruf & Geiser, 2015; Wang, 1989), characterized by decreases in metabolic rate (MR), which leads to reduced  $T_b$  (Geiser, 2004).

Winter is associated with lower  $T_a$  and decreased food availability and is typically a period of negative energy balance. Torpor is a commonly used energy-saving strategy at this time (for a review, see Geiser, 2004). However, torpor is not exclusively a winter behavior, as small mammals also may employ torpor during summer when under energetic constraints (Bieber & Ruf, 2009; Levy & Dayan, 2011; Stawski & Geiser, 2010; Turbill, 2006; Turbill, Law, & Geiser, 2003). All individuals using torpor must arouse to normothermia at some point (Ruf & Geiser, 2015). Timing of arousals is critical, as although arousals may be brief, the process constitutes the most energetically expensive phase of the torpor-arousal cycle (Geiser, 2004; Thomas et al., 1990; Wang, 1989). Some species synchronize arousals with their predicted daily active phase (Hope & Jones, 2012; Park et al., 2000), whereas others synchronize them with daily  $T_a$  maxima, which harnesses passive rewarming and reduces arousal costs (Geiser, 2004).

Ambient temperature typically decreases with latitude and lower values of  $T_a$  tend to coincide with lower torpid metabolic rate (MR), and longer torpor bouts (e.g., Dunbar & Brigham, 2010; Fenn, Zervanos, & Florant, 2009). Environmental differences can influence divergence among populations across latitudinal gradients and also should influence the frequency and intensity of expression of torpor (Dunbar & Brigham, 2010; Fenn et al., 2009). When exposed to identical  $T_a$ , woodchucks (*Marmota monax*) captured from higher latitude expressed torpor more frequently than individuals from lower latitude (Fenn et al., 2009). Although

responses of individuals from different latitudinal populations to similar  $T_a$  have been studied in the laboratory, few studies have examined this in the field (e.g., Dunbar & Brigham, 2010; Fenn et al., 2009; Stawski, 2012; Zervanos, Maher, Waldvogel, & Florant, 2010).

The New Zealand lesser short-tailed bat (*Mystacina tuberculata*) provides a useful model to examine variation in thermoregulatory behavior in small mammals, as the geographic range of this species covers 12° of latitude ranging from subtropical to temperate habitats. Lesser short-tailed bats roost in tree cavities throughout the year that vary in degree of thermal insulation (Sedgeley, 2006). Communal roosts are commonly used during late spring and summer by males and females; however, individuals occasionally roost alone (solitary roosts) during summer (Czenze, Brigham, Hickey, & Parsons, 2017a). During winter, individuals rarely use communal roosts and instead roost solitarily in thermally buffered trees (Czenze et al., 2017a; King, 1990). During late summer, males spend a third of the night singing to attract females, and this likely impacts foraging time and places males under greater energetic pressure than females (Toth 2016). In addition, mated females delay pregnancy and give birth the following spring (King, 1990). Some species, including *M. tuberculata*, with sex-specific differences in seasonal reproductive costs, exhibit differences in seasonal energy expenditure (Becker, Tschapka, Kalko, & Encarnação, 2013; Czenze et al., 2017a; Jonasson & Willis, 2011; Humphries et al., 2003; Michener, 1992). Lower-latitude *M. tuberculata* use short torpor bouts during summer (Czenze et al., 2017a), but there are no data comparing thermoregulation by lower- and higher-latitude populations.

We used temperature-sensitive radio transmitters to assess differences in summer thermoregulatory patterns between free-ranging *M. tuberculata* from

populations separated by 6° of latitude. Our first hypothesis was that latitudinal differences in energy balance would lead to variation in torpor expression and roosting sociality between populations. Specifically, we predicted that, compared to the lower-latitude population, individuals from the higher-latitude population would express more frequent, prolonged, and deeper torpor bouts in thermally buffered solitary tree roosts to conserve energy. We also predicted that higher-latitude individuals would be more likely to time their arousals to coincide with either sunset or daily  $T_a$  maxima to harness passive rewarming and maximize energy savings. Finally, we examined the hypothesis that the timing of reproductive investment would lead to intersexual differences in summer  $T_{sk}$  patterns. We predicted males, whose summer reproductive investment is higher than that of females, would use deeper or longer torpor bouts than females.

## **Methods**

All procedures were approved by the University of Auckland Animal Ethics Committee (AEC-R1374), were conducted under permit 39083-FAU issued by the New Zealand Department of Conservation, and followed guidelines set by the American Society of Mammalogists for use of wild mammals in research (Sikes, 2016). Our higher-latitude study site was located in the Eglinton Valley of Fiordland National Park (44°58 S, 168°00 E), South Island, New Zealand during the 2016 Austral “summer” (January – April). Our lower-latitude study site was located in Pikiariki Ecological Area of Pureora Forest Park (38°26’S, 175°39’E), central North Island, New Zealand; we used data on  $T_{sk}$  collected during January – April 2015 by Czenze et al. (2017a). Due to its inland location, Pureora Forest Park is colder than most of the North Island and its annual fluctuations in  $T_a$  are more similar to the South Island than other forests in the North Island (Table 1).

**Table 1** Ambient thermal characteristics in Pureora Forest and Eglinton Valley, New Zealand, during summer 2015 and 2016, respectively.

	Mean day $T_a$	Mean night $T_a$	Mean max $T_a$	Mean min $T_a$	Mean range $T_a$	Max $T_a$	Min $T_a$	Max range $T_a$
Pureora Forest	14.7±2	12.4±2.3	17.1±2.3	10.6±2.6	6.5±2.3	22.3	2.7	11.5
Eglinton Valley	13.6±2.9	11.6±2.8	16.7±4.3	9.8±3.1	6.9±4.1	29.3	1.8	18.4

All mean  $T_a$  variables differed by  $<2^\circ\text{C}$ . Mean day  $T_a$  is the average of all daily mean  $T_a$  from sunrise to sunset; Mean night  $T_a$  is the average of all nightly mean  $T_a$  from sunset to rise; Mean max  $T_a$  and Mean min  $T_a$  are the mean of all respective daily maxima and minima  $T_a$ ; Mean range  $T_a$  is the mean of all daily ranges in  $T_a$  (max.  $T_a$  - min.  $T_a$ ); Max  $T_a$  and Min  $T_a$  are the respective absolute maxima and minima  $T_a$ ; Max range  $T_a$  is the largest recorded daily range in  $T_a$ . All values are means ( $^\circ\text{C}$ )  $\pm$  standard deviation ( $N = 82$  days).

Pikiariki is a mature podocarp-hardwood forest. The Eglinton Valley is a temperate southern beech forest dominated by red (*Nothofagus fusca*) and silver beech (*N. menziesii*). Bats occupied both solitary tree roosts (housing only 1 individual) and communal tree roosts (housing upwards of 400 individuals at a time). We classified roosting sociality by counting emergences from roosts for 1 hour after dusk.

We caught bats in harp traps and mist nets. Individuals were weighed to the nearest 0.5 g using a Pesola spring scale (Pesola AG, Schindellegi, Switzerland), and sex was determined. Forearm length was measured to the nearest 1 mm.

We clipped a small patch ( $<1\text{ cm}^2$ ) of fur between the shoulders and attached a temperature-sensitive radio-transmitter (0.9-1 g, BD-2NT; Holohil Systems Ltd, Carp, Ontario, Canada) using a latex cement (TORBOT Group, Inc., Cranston, Rhode Island) to reproductive adult males and non-reproductive adult females. Radio-transmitters recorded  $T_{sk}$ ; for small bats,  $T_{sk}$  provides a reasonable approximation of  $T_b$  (Audet & Thomas, 1996; Barclay et al., 1996; Willis & Brigham, 2003). On average, the body mass of bats fitted with transmitters was

14.6  $\pm$ 1.7 g and thus transmitters represented between 5.1 and 7.5% of body mass. Individual bats were tracked to day roosts each morning using a scanning receiver (Australis 26K Scanning Receiver, Titley Electronics, Ballina, New South Wales, Australia) connected to a 3-element Yagi antenna. A data-logging receiver (Lotek SRX\_400, SRX\_600, or SRX\_800; Lotek Engineering Inc., Newmarket, Ontario, Canada) was deployed at roosts and recorded individual  $T_{sk}$  at 10-minute intervals whenever a tagged bat was present. We recorded solitary roost  $T_a$  from functional transmitters that individual bats had shed inside known solitary roosts. During severe river flooding, we were unable to travel to and identify specific roosts. However, we were still able to record  $T_{sk}$  from the 2 individuals we were tracking.

To determine duration of torpor bouts, we classified thermoregulatory phases following Jonasson and Willis (2011): 1) warming: an abrupt increase in torpid  $T_{sk}$  (3 consecutive recordings) that eventually stabilized at a “normothermic” level; 2) normothermic: the time between the end of the warming phase and the initiation of cooling; 3) cooling: readily obvious as a decline in  $T_{sk}$  (3 consecutive recordings) preceding steady-state torpor; And 4) torpid:  $T_{sk}$  (3 consecutive recordings) following the cooling phase and preceding the warming phase. We excluded data from the first 24 h after releasing tagged bats to avoid the influence of disturbance to individuals on our results. We recorded the date and time of each arousal from the  $T_{sk}$  trace and converted the time of arousal to radians. We then used Rayleigh’s test for circular distributions to determine if the daily distribution of arousal times was significantly different from random.

We recorded  $T_a$ , barometric pressure ( $P_{bar}$ ), and roost temperature ( $T_{roost}$ ) of 1 communal roost using data loggers (HOBO Micro Station Data Logger - H21-002, Onset Computer Corporation, Bourne, Massachusetts). Ambient temperature

was recorded in the shade, 2 m above the ground, and the logger inside the occupied communal roost was placed 1 m inside a cavity entrance in a location known to be used by bats.

We used Levene's test to test for homogeneity of variance, and compared morphometric differences between sex and site, and differences in arousal time compared to  $T_a$  max time and sunset time, using either Student's T-test or, if data was heteroskedastic, Welch's 2-sample T-Test. To determine whether sex predicted expression of torpor, we used linear mixed effect models (packages 'nlme', 'lme4' and 'MuMIn', R Core Team 2009). We conducted model selection by comparing models using maximum likelihood tests until only significant variables remained, and tested linearity by examining residuals. We used torpor bout duration and minimum  $T_{sk}$  as response variables, sex, and site as fixed effects, and individual as a random effect. We also used mixed effect models with torpor bout duration and minimum  $T_{sk}$  as response variables, and 3 climate variables that have been associated with bat activity in past studies:  $T_a$ ;  $T_{roost}$ , which may be buffered and less variable compared to  $T_a$ ; and change in  $P_{bar}$ , which reflects passing weather fronts. We used a generalized linear mixed effect model with a binomial distribution, weather variables as fixed effects, and individual as a random effect to test if weather variables predicted whether bats did or did not use torpor.

All analyses were conducted in R studio version 0.98.945 (R Development Core Team 2009) and values are reported as means  $\pm$  1 standard deviation (SD), with  $n$  representing the number of animals and  $N$  the number of events or arousals. In all linear mixed models,  $P$  represents the significance of the full model and  $R^2$  represents the ordinary least square (OLS) partial regression of the variable of interest. We assessed significance at the  $P < 0.05$  level.

## Results

At our higher-latitude site, all bats roosted in trees. Mean daily  $T_{roost}$  of roosts occupied by solitary individuals was higher than mean  $T_a$  (Table 2), mean minimum  $T_{roost}$  was higher than minimum  $T_a$ , and  $T_{roost}$  range was less than  $T_a$  range. The roosts occupied by solitary individuals did not differ in any thermal characteristics from occupied communal roosts.

**Table 2** Occupied solitary roost thermal characteristics compared to ambient conditions in Eglinton Valley during summer using Student's  $T$ -test.

	Mean $T_a$	Max $T_a$	Min $T_a$	Range $T_a$
Solitary	17.7±2.0	19.3±1.5	15.3±3.4	4.0±3.3
Ambient	14.3±1.2	19.5±3.4	11.2±1.9	8.3±5.1
$T$ -value	-4.6	0.18	-3.4	2.4
$df$	16.5	15.1	19.6	18.8
$P$ -value	<0.01*	0.85	<0.01*	0.02*

Solitary roosts chosen by bats (*M. tuberculata*) were warmer and more buffered than  $T_a$ . Mean  $T_a$  is the average of all Mean  $T_a$ ; Max  $T_a$  and Min  $T_a$  are the mean of all respective daily maxima and minima  $T_a$ ; Range  $T_a$  is the mean of all daily ranges in  $T_a$  (max.  $T_a$  - min.  $T_a$ ). Values are means (°C) ± standard deviation  $n = 3$  (roosts),  $N = 12$  (days). \*indicates statistical significance at  $P < 0.05$ .

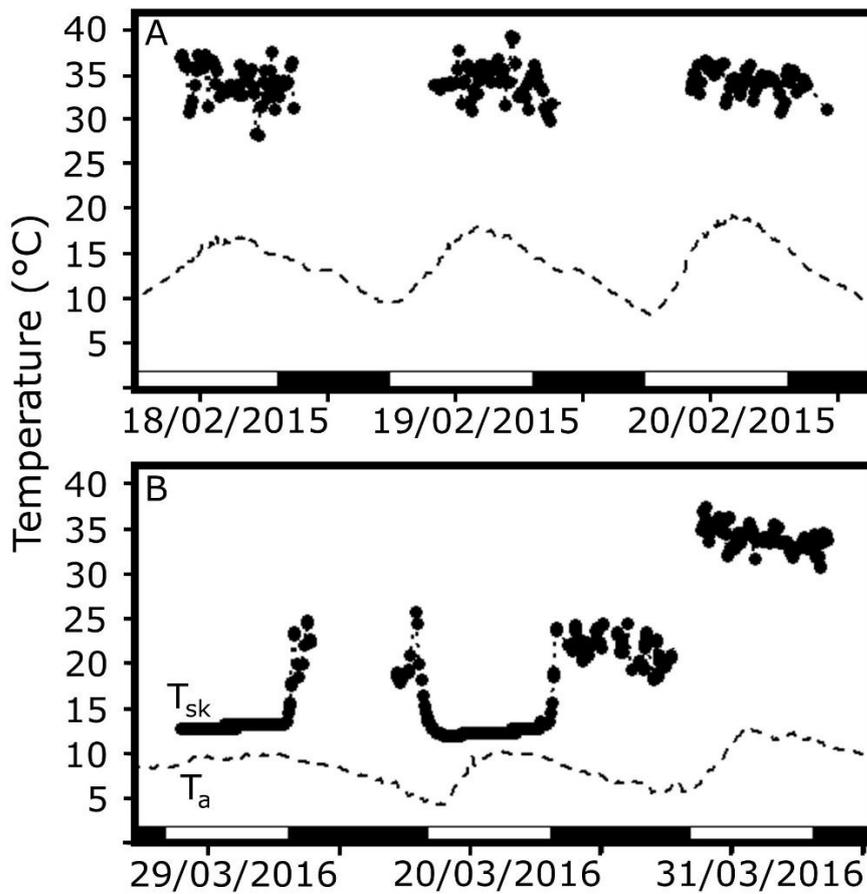
In total, we attached transmitters to 12 individuals (lower latitude = 2 males, 1 female; higher latitude = 5 males, 4 females) and recorded 62 bats-days of  $T_{sk}$  data from 11 individuals (6 males, 5 females; we did not record any data from 1 higher-latitude male).

We did not observe multiday torpor bouts at either site; however, individuals at both sites used short torpor bouts (i.e. <24 h; higher latitude = 8/8 individuals, lower latitude = 1/3 individuals). Bats used torpor more frequently at higher latitude (34%, 15/44 days) compared to lower latitude (11%, 2/18 days;  $T_{8.9} = 2.7$ ,  $P = 0.02$ , Fig. 1). At the higher-latitude site, mean torpor bout duration

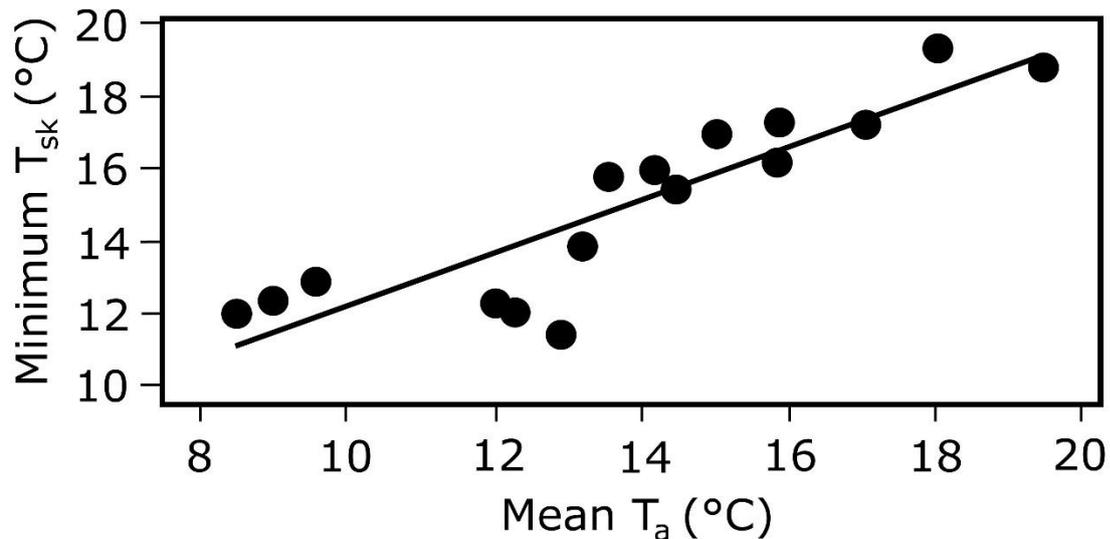
ranged from 6.5 to 11.9 h with a mean of  $9.7 \pm 1.8$  h ( $n = 7, N = 15$ ), which was 6.5-fold longer than at the lower-latitude site ( $1.5 \pm 0.9$  h,  $n = 1, N = 2$ ).

At the higher-latitude site, none of the weather variables (mean  $T_a$ , change in  $P_{bar}$ ) we recorded differed on the days bats expressed torpor ( $13.1 \pm 3.1^\circ\text{C}$ ,  $0.9 \pm 6.0$  mb) versus days when they remained normothermic ( $12.6 \pm 2.7^\circ\text{C}$ ,  $-0.34 \pm 5.0$  mb). Furthermore, torpor bout duration was not related to any of the weather variables we recorded. However, on the days bats used torpor, minimum torpid  $T_{sk}$  was positively correlated with mean  $T_a$  ( $T_9 = 7.2, P < 0.01, R^2 = 0.8, n = 8, N = 17$ , Fig. 2).

At our higher-latitude site, mean sunset  $T_a$  ( $13.3 \pm 3^\circ\text{C}$ ) was lower than max  $T_a$  ( $16.4 \pm 4^\circ\text{C}$ ;  $T_{27.1} = 2.5, P = 0.02$ ). On days when at least 1 bat used torpor,  $T_a$  max occurred at  $15:29 \pm 2$  h ( $n = 8, N = 17$ ), and average time of  $T_a$  max was  $4.2 \pm 2.3$  h before arousals. The mean time of arousal was  $18:13 \pm 1:31$  h, arousals began  $40 \pm 89.5$  min ( $n = 8, N = 17$ ) before sunset and the circular distribution of arousals was non-randomly distributed around sunset (Rayleigh  $Z = 8.1, P < 0.001$ , mean vector = 0.69,  $n = 8, N = 17$ ). Furthermore, the mean arousal time and mean  $T_a$  max time differed ( $T_{22.4} = -7.6, P < 0.01$ ), but mean arousal time and mean sunset time did not ( $T_{25.2} = 2, P = 0.053$ ).



**Figure 1** Skin temperature ( $T_{sk}$ , upper trace, dotted line) and ambient temperature data ( $T_a$ , lower trace, dashed line) for individual *M. tuberculata* monitored over 3 days. Torpor was less common in Pureora Forest Park, North Island (A) compared to Eglinton Valley, South Island, New Zealand (B). Black and white bars at the bottom of the graphs denote night and day, respectively.



**Figure 2** Minimum skin temperature ( $T_{sk}$ ) on days bats (*M. tuberculata*) used torpor as a function of  $T_a$  for individuals from Eglinton Valley, New Zealand. Minimum torpid  $T_{sk}$  decreased with decreasing  $T_a$  ( $P < 0.01$ ,  $T_8 = 7.2$ ,  $n = 7$ ,  $N = 16$ ,  $R^2 = 0.8$ ).

All higher-latitude bats used torpor exclusively while in solitary tree roosts and bats in solitary roosts used torpor on 91% (10/11) of days. Higher-latitude bats roosted solitarily on 38% (8/21) of observation days compared to lower-latitude bats 17% (3/18 days). The mean percentage of days spent solitarily was higher for higher-latitude individuals (52%;  $n = 5$ ,  $N = 21$ ) compared to lower-latitude individuals (9%;  $T_{5.9} = 2.3$ ,  $P = 0.046$ ,  $n = 3$ ,  $N = 18$ ).

At our higher-latitude site, neither minimum torpid  $T_{sk}$  ( $T_5 = -1.2$ ,  $P = 0.3$ ) nor torpor bout duration ( $T_5 = 1.5$ ,  $P = 0.2$ ) differed between males and females.

## Discussion

All mean summer  $T_a$  variables we recorded differed by  $< 2^\circ\text{C}$  between sites, yet the thermoregulatory behavior of individuals differed between the 2 populations. In agreement with our first prediction, higher-latitude individuals roosted solitarily more often, and used torpor more frequently than lower-latitude bats. While we found no direct evidence that individuals were more likely to use

torpor on colder days, minimum  $T_{sk}$  was correlated with mean  $T_a$  during torpor. We found individuals did not take advantage of passive rewarming and instead synchronized arousals with sunset. We found no support for our second hypothesis that sexual differences in the timing of reproductive investment would lead to differences in seasonal energy use. Individuals used torpor more frequently at our higher-latitude site compared with our lower-latitude site, suggesting that higher-latitude individuals have greater demands to suppress metabolism and conserve energy.

Our data suggest that higher-latitude bats face a greater pressure to save energy than their lower-latitude counterparts. The difference in mean  $T_a$  between sites was only 1°C, but despite the subtle difference, higher-latitude individuals expressed torpor more often than lower-latitude individuals. Furthermore, torpor bouts were longer at our higher-latitude site, and every higher-latitude individual used torpor at least once compared to only 1 lower-latitude individual. The subtle difference in temperature therefore may reflect a tipping-point in thermal stress for the higher-latitude bats. Our sites were separated by only 6° of latitude, but populations of mammals separated by comparable distances can differ in their thermoregulatory traits (Fenn et al., 2009; Dunbar and Brigham, 2010). Differences in torpor expression may indicate adaptation to site-specific energetic constraints as a result of genetic change (selection), or phenotypic plasticity. Alternatively, differences between populations could reflect genetic differences as a result of varying responses to similar environmental cues. There also could be other climatic, behavioral, or dietary factors influencing torpor expression that we did not consider. Future work should elucidate whether intraspecific variation in thermoregulatory behavior is a result of genetic traits or phenotypic plasticity by

exposing bats from populations across New Zealand to a range of  $T_a$  to determine if individuals are capable of acclimatizing to new conditions.

While bats at both sites roosted exclusively in trees, solitary tree roosts were used on 38% of days at our higher latitude site compared to 17% at our lower latitude site. Torpor bouts during summer only occurred in solitary tree roosts, and bats in solitary roosts used torpor on 91% (10/11) of days. In other tree-roosting species, roost choice influences  $T_{sk}$  and energy expenditure (Stawski, Turbill, & Geiser, 2008; Willis & Brigham, 2005), and individuals select roost microclimates depending on weather conditions and their energy supply (Boyles, Dunbar, Storm, & Brack, 2007). During winter, lower-latitude bats used torpor in thermally buffered solitary tree roosts (Czenze et al., 2017a). We observed a similar pattern during the summer at our higher-latitude site, and although communal and solitary tree roosts did not differ in microclimate, they were both more stable and warmer than  $T_a$ .

The microclimatic similarity between occupied communal and solitary roosts raises the question of why torpor is more commonly used by bats roosting alone. In tree-roosting lemurs (*Cheirogaleus medius*), individuals hibernating socially do not gain energy savings through social thermoregulation when compared to solitary individuals (Dausmann & Glos, 2015). Larger group size may be less energetically favorable, as impromptu arousals mediated by roost mates may waste energy (Dausmann & Glos 2015; Speakman et al., 1991; Turner et al., 2015). Furthermore, individuals within communal roosts also may be more heavily parasitized than individuals in solitary roosts (Czenze & Broders, 2011). Thus, individuals may choose solitary roosts to decrease the costs associated with communal living. While bats from our higher-latitude site appeared to prefer solitary roosts compared to lower-latitude individuals, more work is required to

confirm this as our data were collected in different years, and our sample size was small.

As  $T_a$  falls further below the thermal neutral zone, thermoregulation incurs greater metabolic costs, and this should trigger a greater torpor response (Park et al., 2000; Rambaldini & Brigham, 2008; Turbill, Körtner, & Geiser, 2003; Twente & Twente, 1965). While we did not find that torpor bout duration was correlated with  $T_a$ , we found that minimum  $T_{sk}$  positively correlated with  $T_a$ . This correlation between minimum  $T_{sk}$  and  $T_a$ , but not torpor bout duration, is similar to the pattern of summer torpor use by *Nyctophilus bifax* (Stawski & Geiser, 2010a, b). Energy savings increase with reduced  $T_b$ , and our data suggest that although duration of torpor was not affected by  $T_a$ , individuals may have maximized their energy savings by allowing  $T_{sk}$  to approach  $T_a$ .

Similar to other studies (Brigham, Körtner, Maddocks, & Geiser, 2000; Christian & Geiser, 2007; Rintoul & Brigham, 2014), we did not detect weather-dependent differences among the days that bats entered torpor or remained normothermic. However, thermoregulation also is influenced by food availability (see Vuarin & Henry, 2014 for a review). Decreased insect activity and therefore food availability for bats negatively affects an individual's energy balance and makes torpor a more profitable strategy compared to foraging (Hope & Jones, 2012; Park et al., 2000). At our higher-latitude site, the mean nightly  $T_a$  was only slightly lower than at our lower-latitude site (0.8°C); however, at similar  $T_a$  twice as many insects were captured at a tropical site in comparison to a subtropical site (Stawski, 2010). It may be that, similar to *N. bifax*, higher-latitude populations of *M. tuberculata* are under greater energetic pressures to use torpor due to lower food availability. It would be useful to experimentally manipulate

insect (i.e., prey) availability, in low- and high-latitude forests to determine the effect on thermoregulation by bats.

Higher-latitude individuals did not appear to take full advantage of passive rewarming by arousing with daily  $T_a$  maxima and instead aroused in synchrony with sunset. Passive rewarming is more common in species that take advantage of  $T_a$  fluctuations by roosting in rock crevices, under exfoliating bark, or by basking and exposing themselves to direct solar radiation (Gesier et al., 2004). Using thermally labile tree roosts, *N. gouldi* passively increase  $T_b$  by 20°C before actively rewarming (Turbill, 2006). While the maximum daily  $T_a$  range at our higher-latitude site was over 18°C, the mean difference between sunset  $T_a$  and max  $T_a$  was only 3°C. Using Thomas et al.'s (1990) equation to estimate arousal costs, a 3°C increase in  $T_a$  would save an individual 98 J during arousal but, since average max  $T_a$  time occurs 5 h before sunset, the same individual would spend an additional 8 kJ defending normothermia compared to a torpid individual arousing at sunset. It is likely that the subtle difference between sunset  $T_a$  and max  $T_a$  does not provide enough energetic benefit for individuals to arouse at max  $T_a$  when balanced against the cost of remaining normothermic for an additional 5 h before emerging at sunset.

We found no evidence that, during the summer singing season, male *M. tuberculata* bear higher reproductive costs and thus require torpor to a greater extent than females. During the summer reproductive season, males sing for 34% of the night (Toth, 2016), and while singing males should have less foraging time than females, our data suggest they do not appear to be under greater pressure to conserve energy during the day. Future studies should focus on comparing singing and non-singing males during the reproductive season to elucidate

whether differences in reproductive investment lead to appreciable differences in thermoregulatory patterns among males.

### **Conclusions**

In summary, we show that *M. tuberculata* has a flexible strategy for conserving energy. Higher-latitude individuals use torpor more regularly, and for longer periods than bats from lower latitudes. While the propensity to enter torpor during summer does not appear to be correlated with lower  $T_a$ , torpor bout depth and  $T_a$  do seem to be related. During summer, torpor is used in solitary tree roosts that are warmer and buffered relative to  $T_a$ , and solitary roosts are preferred by higher-latitude bats more than lower-latitude bats. Our data suggest that during the summer singing season reproductive males are no more heterothermic than non-reproductive females. Our data are consistent with the hypothesis that small mammals inhabiting latitudinal gradients can exhibit behavioral flexibility and have different energetic strategies in different parts of their range. Further, energetic strategies appear to, in part, depend on small population-specific differences. It would be of interest to compare populations of *M. tuberculata* from the subtropical portion of their range to determine roost preference and thermoregulatory response to less extreme seasonal changes in  $T_a$ , and further assess the extent of this species' phenotypic plasticity and energetic flexibility.

# **Chapter 4: Winter Climate Affects Torpor Patterns and Roost Choice in New Zealand Lesser Short-Tailed Bats**

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## **Abstract**

Variation in winter conditions differentially impacts thermoregulation of endotherms, with some species using torpor as an energy-saving strategy during periods of negative energy balance. We evaluated how differences in energy balance due to winter ambient temperatures ( $T_a$ ), would lead to differing torpor expression and roost choice between populations. Here, we monitored  $T_a$  and skin temperatures ( $T_{sk}$ ) of New Zealand lesser short-tailed bats (*Mystacina tuberculata*) using temperature telemetry from an inland population from Pureora, North Island and another from Hauturu/Little Barrier Island (Hauturu), an offshore island. Relative to Pureora, all recorded  $T_a$  variables were higher in Hauturu. Hauturu bats used short torpor (<24 h) more frequently (51% of observational days) than their Pureora conspecifics (33%). Furthermore, minimum  $T_{sk}$  of Hauturu bats correlated positively with mean  $T_a$  and Hauturu bats were more likely to arouse on nights with warmer sunset  $T_a$ . No correlation was found for Pureora bats. Torpor bout duration did not differ between sites. On Hauturu, bats preferred thermally unstable roosts and preferentially roosted inside dead punga/silver fern-trees (*Cyathea dealbata*). As punga thermal characteristics did not differ from  $T_a$ , Hauturu bats may take advantage of warm  $T_a$  to reduce rewarming costs. We did not observe differences in any thermoregulatory variables between sexes in Hauturu. Differences in winter  $T_a$  may differentially impact populations across a latitudinal gradient. Although bat populations in warmer climates are under less thermoregulatory stress, site and climate specific adaptations appear to be employed that affect roost choice and torpor patterns.

## **Introduction**

Metabolic energy is a principal currency for all living things, and balancing food intake versus metabolic output is critical for survival. An individual's energy balance is affected by biotic and abiotic factors such as photoperiod, food/water

availability, and ambient temperature ( $T_a$ ) (Doucette et al., 2012; Körtner & Geiser, 2000; McNab, 2002; Song & Geiser, 1997).

Subtle changes in  $T_a$  can dramatically impact endotherms' (i.e. many mammals and birds) energetic balance (Boyles et al., 2007). Small mammals, with high surface to volume ratios, rapidly lose heat to the environment and many clades of small temperate, subtropical and tropical mammals decrease energy expenditure through torpor (Geiser, 2004; Grigg et al., 2004; Ruf & Geiser, 2015; Wang, 1989). Torpor is a physiological state characterized by a controlled depression in metabolic rate which leads to a lower  $T_b$  that in turn further reduces metabolism (Geiser, 2004).

Ambient temperature influences thermoregulatory behaviour and hibernation traits among populations of hibernators (Dunbar & Brigham, 2010; Stawski & Geiser, 2011; Zervanos et al., 2010). Although temperate hibernators tend to use deep torpor to deal with prolonged low  $T_a$  and minimize energy use (e.g., Barnes, 1989) subtropical winters can be less thermally challenging and subtropical species may use shorter or shallower torpor bouts (Geiser & Stawski, 2011). Indeed, tropical populations of the eastern long eared bat, *Nyctophilus bifax*, exposed to warmer  $T_a$ , exhibit shorter torpor bouts and defend higher skin temperatures ( $T_{sk}$ ) than subtropical individuals (Stawski, 2012). Although torpor patterns of temperate species are relatively well-known, fewer studies have examined the use of winter torpor by subtropical species and species that inhabit both temperate and subtropical climates (e.g., Levin et al., 2015; Stawski, 2012; Stawski, Turbill, & Geiser, 2009)

Regardless of climate, species employing torpor appear to choose specific microclimates (i.e., roost, nest or burrow) and regularly arouse to a stable  $T_b$  (i.e. normothermia). Although arousals are brief, they form the most energetically

expensive phase of the torpor/arousal cycle, and increased  $T_a$  significantly reduces energetic costs of arousals (Currie, Noy, & Geiser, 2015; Geiser, 2004; Thomas et al., 1990; Wang, 1989). Temperate species appear to preferentially choose microclimates that buffer them from large fluctuations in  $T_a$  (Buck & Barnes, 1999; Schmid, 1998; Sedgeley, 2001). Although thermally buffered microclimates may protect from extreme low  $T_a$ , they insulate individuals from beneficial increases of  $T_a$ . Tropical species, which are less likely to experience dangerously low  $T_a$ , appear to occupy shelters that are less insulated, and harness the thermoregulatory benefits of increased  $T_a$  (Bondarenco, Körtner, & Geiser, 2016; Turbill, 2006).

The timing, duration and frequency of arousals is critically important to an individual's energy budget, and a balance between the energetic savings from torpor, and costs of arousals must be met (Humphries, Thomas, et al., 2003; Jonasson and Willis, 2011). In addition, species with differential timing of reproductive investment exhibit differences in sex-specific winter energy budgets (Humphries, Thomas, et al., 2003; Jonasson & Willis, 2011; Michener, 1992).

The New Zealand lesser short-tailed bat (*Mystacina tuberculata*) is the only extant species of family *Mystacinidae*, and is endemic to New Zealand. *Mystacina tuberculata* range from the top of New Zealand's North Island to the bottom of the South Island, as well as being found on several offshore islands, including Hauturu/Little Barrier Island. *Mystacina tuberculata* provide a good model to examine the variation of energetic strategies in small mammals, as their range includes both subtropical and temperate habitats with more subtle seasonal variation in climate than faced by most temperate mammals. *Mystacina tuberculata* roost in tree cavities that vary in thermal stability (Sedgeley, 2006). Winter activity, presumably for foraging, is positively correlated with  $T_a$  and likely reflects periods with increased prey availability (Christie, 2006; Christie &

Simpson, 2006; Daniel, 1979). Males sing during summer, females give birth the following spring (Toth, 2016), and some populations of *M. tuberculata* exhibit sex differences in seasonal thermoregulatory behaviour (Czenze et al., 2017a). Although thermoregulatory behaviour varies between seasons in the central North Island, no work has evaluated population differences during winter in subtropical and temperate populations.

We used temperature-sensitive radio transmitters to assess differences in winter thermoregulatory patterns between free-ranging *M. tuberculata* from an inland and offshore island population. Our first hypothesis was that differences in energy balance, due to winter  $T_a$ , would lead to varying torpor expression and roost choice between populations. Specifically, we predicted that higher  $T_a$  in Hauturu would cause individuals to use torpor less frequently and bouts would either be shorter or shallower. Second, we predicted that, similar to other subtropical bats, individuals from Hauturu would prefer roosts with thermally unstable microclimates to reduce costs of arousals, and would be more responsive to  $T_a$  to take advantage of warmer evenings for foraging. Finally, we examined the hypothesis that the timing of reproductive investment would lead to intersexual differences in winter  $T_{sk}$  patterns. Specifically, we predicted females, whose reproductive investment occurs after winter, would use deeper or longer torpor bouts than males.

## **Methods**

All procedures were approved by the University of Auckland Animal Ethics Committee (AEC-R1374) and were conducted under permit 39083-FAU issued by the New Zealand Department of Conservation. Our offshore island site is located was Hauturu/Little Barrier Island (Hauturu) (36°19 S, 175°07 E), an island 80 km off the East Coast of the North Island, New Zealand during the 2016 Austral

“winter” (May – July). Our inland site was located in the Pikiariki Ecological Area of Pureora Forest Park (Pureora) (38°26’S, 175°39’E), central North Island, New Zealand; we used data on  $T_{sk}$  and  $T_a$  collected during May – July 2015 by Czenze et al. (2017a).

At both sites, bats occupied mainly solitary roosts (housing only one individual) and occasionally communal roosts (housing more than one individual at a time). Roosts were classified by recording the number of individuals who emerged for one hour after dusk.

We caught bats in harp traps. Individuals were weighed to the nearest 0.5 g using a Pesola spring scale (Pesola AG, Schindellegi, Switzerland), and sex was determined. Forearm length was measured to the nearest 1 mm.

For small bats,  $T_{sk}$  provides a reasonable approximation of core temperature and is a reliable index for the study of torpor in field conditions (Audet and Thomas, 1996; Barclay et al., 1996; Willis and Brigham, 2003). We clipped a small (<1 cm<sup>2</sup>) patch of fur between the shoulders and attached a temperature-sensitive radio-transmitter (0.9-1 g, BD-2NT; Holohil Systems Ltd, Carp, Ontario, Canada) using a latex cement (TORBOT Group, Inc., Cranston, Rhode Island, USA). On average, the body mass of bats fitted with transmitters was 12.6 g ± 1.5 g and thus transmitters represented between 6.0 and 8.6% of body mass. Individual bats were tracked to day roosts each morning using a scanning receiver (Australis 26K Scanning Receiver, Titley Electronics, Ballina, New South Wales, Australia) connected to a 3-element Yagi antenna. A data-logging receiver (Lotek SRX\_400, SRX\_600, or SRX\_800; Lotek Engineering Inc. Newmarket, Ontario, Canada) was deployed at roosts and recorded individual  $T_{sk}$  at 10-min intervals whenever a tagged bat was present. Unsafe terrain prevented travel to one specific roost, however, we were still able to record  $T_{sk}$  for the individual who inhabited it. In

Hauturu, we recorded roost temperature ( $T_{roost}$ ) of a *pohutukawa* (*Metrosideros excelsa*) from a functional transmitter that an individual bat had shed inside the tree. After inspection, we determined the transmitter had been shed inside the tree approximately 1.5 m above cavity entrance.

Thermoregulatory phases were readily identifiable from visual inspection of  $T_{sk}$  traces. Although we did not use specific  $T_b$  cutoffs (i.e. 30°C) to identify thermoregulatory stages, we did use objective guidelines derived from Jonasson and Willis (2011) and previously used for *M. tuberculata* (Czenze et al., 2017a): 1) warming: an abrupt increase in torpid  $T_{sk}$  (3 consecutive recordings) that eventually stabilized at a “normothermic” level; 2) normothermic: the time between the end of the warming phase and the initiation of cooling; 3) cooling: readily obvious as a decline in  $T_{sk}$  (3 consecutive recordings) preceding steady-state torpor; And 4) torpid:  $T_{sk}$  (3 consecutive recordings) following the cooling phase and preceding the warming phase. We excluded data from the first 24 h after releasing tagged bats to avoid the influence of disturbance to individuals on our results.

In Hauturu, we recorded  $T_a$ , barometric pressure ( $P_{bar}$ ), and  $T_{roost}$  of a punga/silver fern-tree (punga; *Cyathea dealbata*), previously inhabited by a solitary individual, using data loggers (HOBO Micro Station Data Logger - H21-002, Onset Computer Corporation, Bourne, Massachusetts, USA). Ambient temperature was recorded in the shade, 2 m above the ground, and the logger inside the punga was placed 1 m inside the entrance.

We performed logistic regression using a generalized linear mixed effect model (GLME; packages ‘nlme’ version 3.1-127, ‘lme4’ version 1.1-10 and ‘MuMIn’ version 1.15.6 in R version 3.1.0; R Development Core Team 2009) to analyse data with a dichotomous response variable while controlling for pseudoreplication

associated with multiple recordings from individuals. We used a GLME to examine differences in the proportion of torpor bouts which were or were not >24 h with site and sex as explanatory variables, and individual as a random effect. We also used a GLME to determine whether individuals would or would not arouse from torpor in response to weather variables that have been associated with bat activity in past studies:  $T_a$  and change in  $P_{bar}$ , which reflects passing weather fronts and is correlated with both insect and bat activity (Jones et al., 1995), and individual as a random effect. Finally, we used a GLME to determine if  $T_a$  and change in  $P_{bar}$  predicted roost type (i.e. tree cavity or punga) used by bats, with individual as a random effect.

We used linear regression using linear mixed effect models (LME; packages 'nlme' version 3.1-127, 'lme4' version 1.1-10 and 'MuMIn' version 1.15.6 in R version 3.1.0; R Development Core Team 2009) to analyse data with a continuous response variable while controlling for pseudoreplication. We conducted model selection by comparing models, starting with a saturated model including the interaction of all explanatory variables (sex,  $T_a$ , and site), using maximum likelihood tests until only significant variables remained. We used LMEs with torpor bout duration or minimum  $T_{sk}$  as response variables, sex,  $T_a$ , and site as explanatory variables, and individual as a random effect. In all linear mixed models,  $P$  represents the significance of the full model and  $R^2$  represents the total proportion of variance described by explanatory variables and random effects (*sensu* Nakagawa & Schielzeth, 2013).

All analyses were conducted in R version 3.1.0 (R Development Core Team 2009) and values are reported as means  $\pm$   $SD$  with  $n$  representing the number of animals and  $N$  the number of events/arousals. We assessed significance at the  $P < 0.05$  level.

## Results

All  $T_a$  variables we recorded, except daily  $T_a$  range, were higher in Hauturu compared to Pureora (Table 1).

**Table 1** Ambient thermal characteristics in Pureora Forest compared to Hauturu/Little Barrier Island during winter 2015 and 2016 respectively.

	Mean max $T_a$	Mean min $T_a$	Mean day $T_a$	Mean night $T_a$	Mean range $T_a$	Max $T_a$	Min $T_a$	Max range $T_a$
Pureora	9.3±2.6	4.4±2.8	7.6±2.5	6.2±2.7	4.9±2.1	10.1	-1.2	10.1
Hauturu	15.4±1.8	11.6±2.3	14±2	12.1±2.4	6.9±4.1	18.2	6.8	6.4

Winter  $T_a$  was higher in Hauturu than Pureora. Mean max  $T_a$  and Mean min  $T_a$  are the mean of all respective daily maxima and minima  $T_a$ ; Mean day  $T_a$  and Mean night  $T_a$  are the mean of all respective mean  $T_a$  from sunrise-sunset and sunset-sunrise; Mean range  $T_a$  is the mean of all daily ranges in  $T_a$  (max.  $T_a$  – min.  $T_a$ ); Max  $T_a$  and Min  $T_a$  are the respective absolute maxima and minima  $T_a$ ; Max range  $T_a$  is the largest recorded daily range in  $T_a$ . Values are in (°C), means ± SD  $N=64$  (days).

In total, we attached transmitters to 11 individuals (Pureora=3 males, 2 female; Hauturu=4 males, 2 females) and recorded 104 bats-days of  $T_{sk}$  data from 9 individuals (5 males, 4 females; we did not record data from 1 Pureora and 1 Hauturu individual, both males).

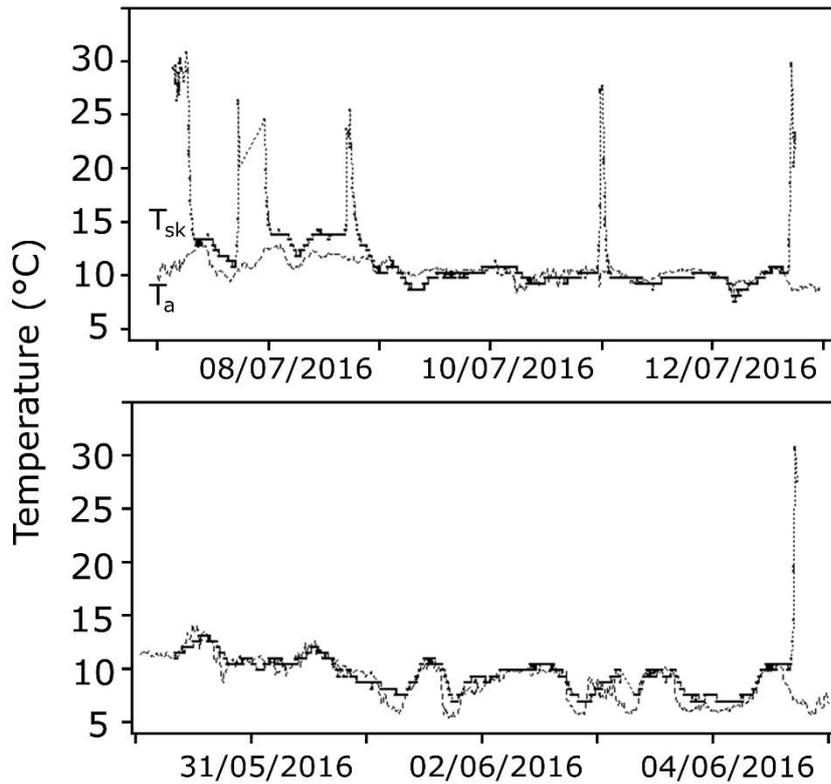
We recorded short (<24 h) and multiday torpor bouts in Hauturu and Pureora. Bats in Hauturu used torpor on 98% of observation days (60/61), and on 95% of observation days (41/43) in Pureora. Bats used short torpor more often in Hauturu (51% (31/61 days)) compared to Pureora (33% (14/43 days);  $Z=2.1$ ,  $n=9$ ,  $N=59$ ,  $P=0.04$ ). In Hauturu, torpor bout duration ranged from 6.2 to 128.4 h. Mean short torpor bout duration was  $9.1\pm3.4$  h ( $n=5$ ,  $N=28$ ), and mean multiday torpor bout duration was  $68.1\pm30.2$  h ( $n=4$ ,  $N=9$ ; Fig 1.), which was not different than multiday torpor bout duration in Pureora ( $60.7\pm27.5$  h ( $n=4$ ,  $N=10$ );  $T=-0.6$ ,  $df=5$ ,  $P=0.6$ ). Further, when we combined short and multiday

torpor bouts we found no difference in torpor bout duration between Hauturu ( $22 \pm 28.9$  h) and Pureora ( $34.2 \pm 32.9$ ;  $T=1.5$ ,  $df=5$ ,  $P=0.17$ ).

In Hauturu, the lowest  $T_{sk}$  of torpid bats we recorded was  $4.7^\circ\text{C}$  compared to  $8.6^\circ\text{C}$  in Pureora. Mean minimum  $T_{sk}$  of torpid bats was not affected by sex. However, mean minimum  $T_{sk}$  of torpid bats was related to the interaction between site and mean  $T_a$  (Table 2; Fig. 2), so we analysed sites separately. In Hauturu, minimum  $T_{sk}$  of torpid bats ( $T=7.1$ ,  $df=32$ ,  $P<0.01$ ,  $R^2=0.71$ ,  $n=5$ ,  $N=38$ ) was positively correlated with corresponding  $T_a$ , but this was not true in Pureora ( $T=1.0$ ,  $df=16$ ,  $P=0.3$ ,  $R^2=0.02$ ,  $n=4$ ,  $N=21$ ; Fig 2). Mean minimum  $T_{sk}$  of torpid bats was lower in Hauturu ( $11.1 \pm 3.0^\circ\text{C}$ ) compared to Pureora ( $12.3 \pm 2.4^\circ\text{C}$ ).

In Hauturu, sunset  $T_a$  best predicted whether or not an individual would arouse from torpor ( $Z=3.5$ ,  $n=5$ ,  $N=61$ ,  $P<0.01$ ) but not in Pureora ( $Z=1.6$ ,  $n=4$ ,  $N=43$ ,  $P=0.12$ ).

In Hauturu, multiday torpor bout duration increased with decreasing mean  $T_a$  ( $T=-4.2$ ,  $df=7$ ,  $P<0.01$ ,  $R^2=0.76$ ; Fig. 3). However, short torpor bout duration was not correlated with mean  $T_a$  ( $T=1.9$ ,  $df=26$ ,  $P=0.08$ ,  $R^2=0.27$ ).

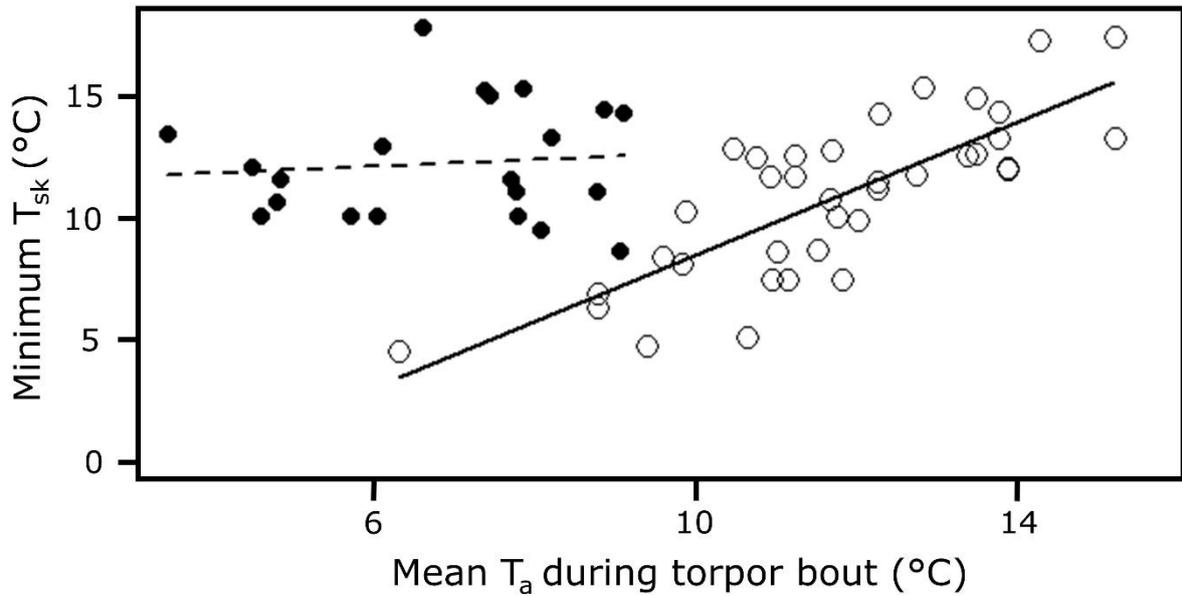


**Figure 1** Representative traces for skin temperature ( $T_{sk}$ , upper trace, dotted line) and ambient temperature ( $T_a$ , lower trace, dashed line) for 2 individual *M. tuberculata* in Hauturu/Little Barrier Island.

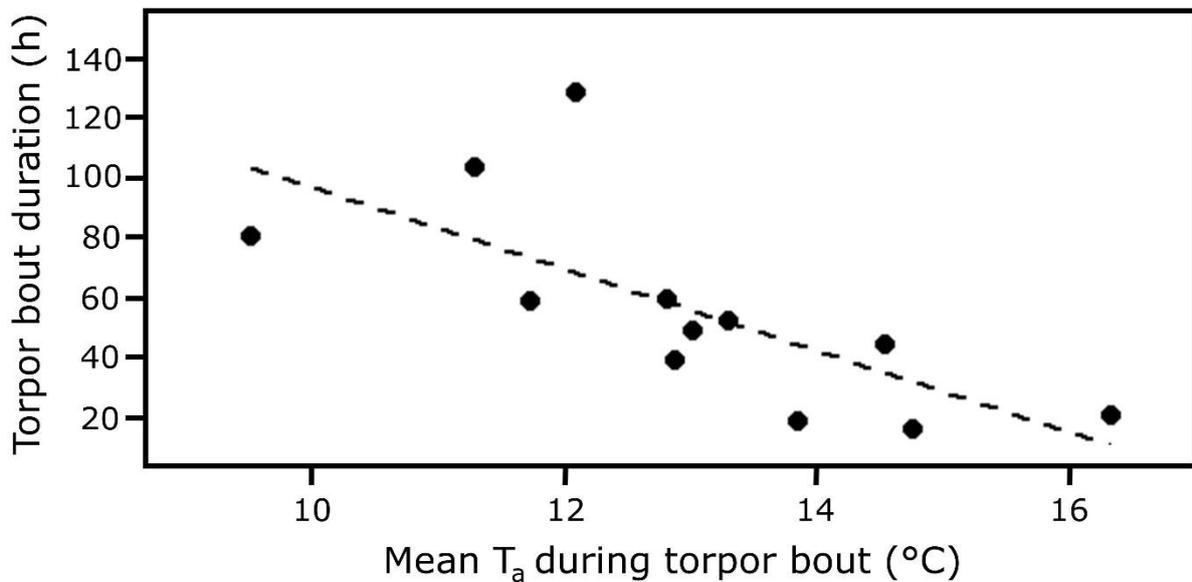
**Table 2** Significant predictors (site, mean ambient temperature ( $T_a$ ), and the interaction between the two) from a linear mixed effect model of minimum skin temperature ( $T_{sk}$ ) of torpid lesser short-tailed bats (*Mystacina tuberculata*) from Pureora and Hauturu/Little Barrier Island, New Zealand.

Predictor variable	Regression coefficient	<i>d.f.</i>	<i>T</i> value	<i>P</i> value
(Intercept)	-6.43±2.85	48	-2.26	0.03
Site (Pureora)	16.87±3.99	7	4.23	<0.01
Mean $T_a$	1.23±0.188	48	6.55	<0.01
Site (Pureora): Mean $T_a$	-1.0±0.36	48	-2.76	<0.01

Decreasing  $T_a$  during winter was associated with decreased minimum  $T_{sk}$  in Hauturu but not in Pureora. Regression coefficient is  $\pm$  standard error; *d.f.* represents degrees of freedom; *n* (individuals)=9; *N* (observations)=59.



**Figure 2** Minimum skin temperature ( $T_{sk}$ ) for days bats used torpor as a function of  $T_a$  for individuals from Hauturu/Little Barrier Island (white circles) and Pureora (black circles). Minimum  $T_{sk}$  decreased with decreasing  $T_a$  for days bats used torpor in Hauturu (white circles;  $T=7.1$ ,  $df=32$ ,  $P<0.01$ ,  $R^2=0.71$ ,  $n=5$ ,  $N=38$ ) but not Pureora (black circles;  $T=1.0$ ,  $df=16$ ,  $P=0.3$ ,  $R^2=0.02$ ,  $n=4$ ,  $N=21$ ). Solid line represents the regression of Little Barrier Island individuals, and dashed line represents regression of Pureora individuals.



**Figure 3** Multiday torpor bout duration (h) as a function of  $T_a$  for individuals from Hauturu/Little Barrier Island. Torpor bout duration decreased with increased  $T_a$  ( $T=-4.2$ ,  $df=7$ ,  $P<0.01$ ,  $n=4$ ,  $N=12$ ,  $R^2=0.76$ ) dashed line represents regression of days bats remained torpid.

Bats in Pureora roosted exclusively in tree cavities, but Hauturu bats roosted in tree cavities on 20% of days (11/56), non-tree roosts, such as sloughed nīkau palm (*Rhopalostylis sapida*) fronds, on 7% of days (4/56), and hollow punga on 73% of days (41/56). In Hauturu, none of the temperature variables we recorded, except  $T_{range}$ , differed between the punga roost and  $T_a$ . Only max  $T_{roost}$  and  $T_{range}$  differed between data collected from pohutakawa (tree roost) and punga (non-tree roost) (Table 3).

In Hauturu, torpor bout duration was not different between tree and non-tree roosts ( $T=1.4$ ,  $df=32$ ,  $P=0.2$ ). However, minimum  $T_{sk}$  was lower in non-tree roosts ( $T=4.0$ ,  $df=50$ ,  $P<0.01$ ) than tree roosts.

In Hauturu, multiday torpor bout duration ( $T=3.3$ ,  $df=2$ ,  $P=0.08$ ), and minimum  $T_{sk}$  of torpid bats did not differ between the sexes ( $T=0.6$ ,  $df=3$ ,  $P=0.6$ ), and females did not use multiday torpor bouts more than males ( $Z=-1.7$ ,  $n=5$ ,  $N=39$ ,  $P=0.09$ ).

**Table 3** Ambient vs non-tree roost ( $N=40$  days) and tree vs non-tree ( $N=8$ ) roost thermal characteristics in Hauturu/Little Barrier Island. Punga roosts did not differ from ambient temperatures ( $T_a$ ).

	Mean $T_a$	Max $T_a$	Min $T_a$	Range $T_a$
Ambient	13.2±1.9	15.1±1.7	11.3±2.1	3.7±1.2
Non-Tree	13.2±2.4	14.8±2.4	11.6±2.5	3.1±1.1
$T$ -value	0.1	-0.6	0.7	-2.3
$Df$	74	70.0	76.4	77
$P$ -value	0.9	0.5	0.5	0.03*
Tree	8.3±2.1	9.3±2.2	7.4±2.0	1.9±1.0
Non-Tree	10.9±3.0	12.7±2.7	9.4±3.1	3.4±0.8
$T$ -value	0.06	2.7	1.4	3.4
$Df$	12.3	13.1	11.7	13.3
$P$ -value	0.2	0.02*	0.2	<0.01*

Mean  $T_a$  is the average of all mean  $T_a$ ; Max  $T_a$  and Min  $T_a$  are the mean of all respective daily maxima and minima  $T_a$ ; Range  $T_a$  is the mean of all daily ranges in  $T_a$  (max.  $T_a$  – min.  $T_a$ ). Values are in ( $^{\circ}\text{C}$ ), means  $\pm$   $SD$ . \* Represent significant differences based on Student’s T test.

## Discussion

Although the sites are only 232 km away from each other, mean winter  $T_a$  in Hauturu was 6.2 $^{\circ}\text{C}$  higher than in Pureora, and *M. tuberculata* differed in expression of torpor between sites. We found mixed support for our first prediction that expression of torpor should vary between sites. We did not record differences in frequency of torpor, or torpor bout duration between sites. However, Pureora bats were more likely to use multiday torpor bouts than Hauturu bats. In accord with our second prediction relating to roost choice, Hauturu bats preferentially chose roosts that did not differ from ambient conditions. Unlike Pureora bats, we found that the minimum  $T_{sk}$  positively correlated with corresponding  $T_a$  for Hauturu bats. Furthermore, multiday torpor bout duration was negatively correlated with

mean  $T_a$  for Hauturu bats. Sunset  $T_a$  was the best predictor of arousal for Hauturu bats, but not for Pureora bats. In Hauturu, we found no evidence of sex differences in any thermoregulatory behaviour. Although bats did not use torpor (short and multi-day combined) more in Pureora than Hauturu, Hauturu bats used multiday torpor less frequently, suggesting that Hauturu bats have less need to suppress metabolism to cope with elevated thermally induced energetic demands.

Hauturu bats were exposed to a mean winter  $T_a$  nearly double that of their Pureora counterparts. Although we did not observe any differences in torpor variables, we did find that Hauturu bats used short torpor more often than Pureora bats. Prolonged torpor is associated more with physiological costs like dehydration stress and accumulation of metabolic wastes (Ben-Hamo, Munoz-Garcia, Williams, Korine, & Pinshow, 2013; Németh, Nyitrai, Németh, & Altbäcker, 2010; Thomas & Geiser, 1997; Thomas & Cloutier, 1992). Shorter torpor bouts are associated with warmer/unpredictable climates (Lovegrove, 2000), and individuals can save energy during rest phases and take advantage of milder conditions during active phases. During winter, Hauturu bats may prefer short torpor due to higher  $T_a$ , and therefore lower energy constraints, compared to Pureora bats, which are exposed to predictably lower  $T_a$  and face greater pressure to employ prolonged torpor bouts and risk the accumulation of physiological costs. Alternatively, or in addition to, higher night  $T_a$  in Hauturu may provide conditions for increased prey availability, foraging success, and therefore decreased demands for multiday torpor.

Minimum  $T_{sk}$  correlated positively with  $T_a$  in Hauturu but not in Pureora. Energy savings are negatively correlated with  $T_{sk}$  until a minimum "set-point" temperature ( $T_{set}$ ) below which thermoregulation is initiated, metabolic rate increases, torpor bout duration decreases, and energetic costs increase (Heller and Hammel, 1972). In free-ranging Australian Gould's wattled bat (*Chalinolobus*

*gouldii*), minimum  $T_{sk}$  was positively correlated with  $T_a$  (Stawski & Currie, 2016). Ambient temperature may be a signal of food availability for insectivorous species as aerial insect abundance increases with  $T_a$ , especially when  $T_a$  exceeds 10°C (Jones et al., 1995). In some climates, increased thermoregulatory costs, in concert with decreased prey availability, make torpor a better strategy compared to foraging (Hope and Jones, 2012; Park et al. 2000). In Pureora, mean night  $T_a > 10^\circ\text{C}$  occurred on only 7% of observation nights (3/43) compared to 92% of observation nights (56/61) in Hauturu. Below a threshold  $T_a$ , insects likely become predictably absent and, therefore, foraging success decreases. Although the notion of temperature mediated effects on prey availability and bat behavior is speculation, bats captured at Hauturu regularly defecated during handling, suggesting they were foraging and well fed.

In Hauturu, like in Pureora, bats employ short and multiday torpor bouts during winter (Czenze et al., 2017a). Similar to other insectivorous bats (Park et al. 2000; Stawski and Geiser 2010), torpor bout duration during winter is negatively correlated with  $T_a$ . Further, in Hauturu, but not in Pureora, arousals from torpor occurred on evenings with significantly warmer sunset  $T_a$ . Thermoregulatory behaviour is influenced by food availability (see Vuarin and Henry, 2014 for a review), and torpid individuals use environmental cues to assess ambient conditions and food availability (Wojciechowski, Jefimow, & Tegowska, 2007). Sunset  $T_a$  may provide cues regarding foraging opportunities as insect prey is likely more abundant soon after dusk (Racey & Swift, 1985; Rydell, Entwistle, & Racey, 1996). Indeed, Natterer's (*Myotis nattereri*) and Greater horseshoe bats (*Rhinolophus ferrumequinum*) synchronize arousals with sunset to take advantage of warm  $T_a$  and increased prey availability (Park et al. 2000; Hope and Jones 2012). We observed that bats track  $T_a$  passively and arouse in response to nights

that likely provide lower thermoregulatory costs and a higher probability of successful foraging. We are unable to say what an individual did during the time we lost its signal after an arousal; however, the presence of faeces suggests individuals were foraging. Therefore, it seems likely that individuals may be arousing on warmer nights, to take advantage of presumably higher prey availability.

In Hauturu, individuals preferentially chose thermally unstable roosts. In Pureora, bats use roosts buffered against sub-zero  $T_a$  (Czenze et al., 2017a). Roosting difference is likely a preference by Hauturu bats for dead hollowed punga trunks and, despite their availability, punga roosts were never used by Pureora bats. Hauturu punga roosts were cylindrical “chimney” structures with an exposed top. Punga  $T_{roost}$  variables did not differ from  $T_a$ , except for mean daily  $T_{range}$  which was 0.5°C lower. Furthermore, the tree cavity roost we recorded had higher mean  $T_{roost}$ , maximum  $T_{roost}$ , and  $T_{range}$  relative to punga. Roost choice facilitates thermoregulatory behaviour and, to defend against temperatures below  $T_{set}$ , many species prefer insulated nests/roosts (Buck & Barnes, 1999; Casey, 1981; Humphries, Thomas, et al., 2003; Schmid, 1998). Although thermally stable roosts prolong torpor, thermally variable roosts may enhance arousals via passive rewarming and reduce costs during the most demanding phase of torpor (Chruszcz & Barclay, 2002; Currie et al., 2015; Rintoul & Brigham, 2014; Stawski et al., 2009). In a comparison of three sympatric bats, brown long-eared bats (*Plecotus auritus*), a heterothermic species, prefer roosts with low thermal stability and low mean minimum  $T_{roost}$ , whereas the other species use more thermally stable roosts (Otto, Becker, & Encarnação, 2016). During winter, Pureora individuals seek warm, thermally stable roosts to avoid dangerously low  $T_a$  (Czenze et al., 2017a). Hauturu bats, which experience less extreme  $T_a$ , can choose more thermally

variable roosts and harness the benefits of faster cooling rates or reduced arousal costs.

In cold climates, energetically constrained individuals may be thriftier with fat reserves (Jonasson and Willis, 2011). During winter, in colder parts of their range, female *M. tuberculata* have lower minimum  $T_{sk}$  than males, presumably to conserve fat to fuel post-emergence pregnancy (Czenze et al. 2017a). In contrast, bats from warmer climates forage more regularly during winter, and sex differences in thermoregulatory behaviour are not apparent (Hope and Jones, 2012; Park et al. 2000). In Hauturu, we did not detect differences between the sexes in any of the thermoregulatory variables, yet we did at Pureora. The mean winter night  $T_a$  in Hauturu was 12.1°C, almost double that of the Pureora, likely increasing the probability of successful foraging. If Hauturu females can augment their fat reserves via winter foraging, they would be under less pressure to save energy.

## **Conclusions**

*Mystacina tuberculata* has a varied strategy for conserving energy at different latitudes during winter. Although many of the thermoregulatory variables did not differ between sites, bats from Hauturu appear to be more influenced by ambient conditions than Pureora bats. This is most evident in their minimum  $T_{sk}$ , and arousal times, all of which are influenced by  $T_a$ . Population differences in roost choice are apparent. Individuals from Hauturu chose thermally unstable roosts and we show the first evidence that *M. tuberculata* prefer punga roosts in their more northern offshore island range. Finally, in this warmer location, the less severe demands on energy removes sexual differences in torpor behavior observed at other sites. Our data provide further evidence that differences in seasonal  $T_a$  at sites separated by latitude impact the energetic strategies within

populations of small mammalian species.

**Chapter 5: Demographics, Season, and Location Affect Dietary Preferences of New Zealand Lesser Short-Tailed Bat (*Mystacina tuberculata*)**

## **Abstract**

Variation in the diet of generalist insectivores can be affected by a number of site-specific traits including weather, habitat type, and season, as well as demographic traits like reproductive status and age. Compared to the Northern hemisphere, few studies have used molecular tools to examine seasonal and population differences in insectivorous bat diets from the Southern hemisphere. I used molecular analysis to compare the diets of three distinct New Zealand populations of lesser short-tailed bats (*Mystacina tuberculata*). I used molecular sequencing techniques to amplify a 157 base pair fragment of the mitochondrial cytochrome C oxidase subunit 1 gene from faecal matter. Summer diets were compared between Eglinton Valley in the South Island and Pureora Forest in the North Island. Winter diets were also compared between Pureora and Hauturu/Little Barrier Island populations, which also permitted seasonal diet comparisons for the Pureora population. In contrast to previous morphology-based diet studies, Lepidoptera and Diptera accounted for approximately 90% of amplicons identified from faecal matter. Assuming similar template stabilities and amplification efficiencies, the proportion of orders represented within prey and the Simpson diversity index, differed between sites and seasons within the Pureora population. For the Pureora population, the Simpson diversity index was also higher in summer than winter and was higher in Pureora when compared to Eglinton. Summer Eglinton samples also revealed that young-of-the-year diets were more diverse than any other demographic group. Lactating females had the lowest dietary diversity during summer in Pureora. In Hauturu, I found a significant negative relationship between mean ambient temperature and prey richness. My data suggest that *M. tuberculata* are much less dependent on terrestrial foraging than previously reported. I show that molecular analyses can provide finer identification of prey items, and these reveal additional layers of dietary complexity. This

provides novel insights into foraging behaviour and ecological interactions within different habitats. My study is the first from the Southern hemisphere to use molecular techniques to examine spatial-temporal variation in the diet of a generalist insectivore that inhabits a contiguous range with several habitat types and climates.

## **Introduction**

To maintain homeostasis, individuals must balance energetic transactions (i.e., energy spent vs. energy gained). Expended energy is partitioned between maintenance, somatic growth, thermoregulation, locomotion and reproduction. While foraging is an expenditure it is also the source of energy gain. An individual's energy balance is influenced by biotic and abiotic factors such as photoperiod, food/water availability, digestibility and abundance of prey, and ambient temperature ( $T_a$ ) (Doucette et al., 2012; Körtner & Geiser, 2000a; McNab, 2002; Song & Geiser, 1997).

Endotherms (i.e., mammals and birds) face a heavy energetic burden, as the majority of their output is lost as metabolic heat that acts to maintain normothermic body temperatures ( $T_b$ ). Due to surface area to volume ratio laws, active small insectivorous endotherms are likely under even greater pressures during winter than similarly sized herbivorous species, as insect populations are more sensitive to weather. For example, many volant insects cannot fly, have limited flight capacity, or are dormant at low  $T_a$  (Jones et al., 1995).

Small endothermic species with ranges distributed across climatic zones likely experience location-dependent influences to their energetic balance (Dunbar & Brigham, 2010; Stawski & Geiser, 2011; Zervanos et al., 2010). Both energy expenditure (i.e., thermoregulatory costs and foraging costs) and energy intake (i.e., prey availability and dietary selection) likely differ between summer and

winter, and higher latitude versus lower latitude climates. For aerial insectivores, foraging costs appear to be negatively correlated with  $T_a$  (Humphries & Careau, 2011; Klüg-Baerwald, Gower, Lausen, & Brigham, 2016). Additionally, insect diversity correlates with many factors including plant diversity and latitude (Rohde, 1992; Zhang et al., 2016), and prey abundance at similar  $T_a$  may be higher at lower versus higher latitude sites (Stawski, 2010). Individuals of the same species that inhabit different climates are likely to differ in energetic expenditure and/or intake (Dunbar & Brigham, 2010).

Aspects of an adult bat's life history, such as pregnancy and lactation, and hibernation come with different energetic burdens, and species may alter their diet accordingly. Many bat species synchronise lactation with peaks in summer insect diversity (Clare, Symondson, Broders, et al., 2014; Clare, Symondson, & Fenton, 2014; Levin, Yom-Tov, & Barnea, 2009). Greater mouse-tailed bats (*Rhinopoma microphyllum*) capitalise on the brief periodic nuptial flights of fat-rich ants (*Camponotus* spp.), which coincides with lactation and pre-hibernation fattening, and almost exclusively feed on these ants when available (Levin et al., 2009). Further, young-of-the-year (YOY) of several insectivorous bat species exhibit a broader diet when compared to adults (Adams, 1996; Adams, 1997; Hamilton & Barclay, 1998; Rolseth, Koehler, & Barclay, 1994).

The New Zealand lesser short-tailed bat (*Mystacina tuberculata*) is a small forest dwelling species, the only extant member of the family *Mystacinidae*, and is endemic to New Zealand. The species ranges from Omahuta-Puketi Forest in the North Island (35° 13' 38.5" S 173° 38' 18.31" E ) to Whenua Hou/Codfish Island in the South (46° 46' 23.9" S 167° 37' 55.7" E ) (Carter & Riskin, 2006). Although *M. tuberculata* is omnivorous, they use a combination of aerial hawking and terrestrial foraging to capture arthropods, which make up the majority of their

diet (Arkins et al., 1999; O'Donnell, Christie, Corben, Sedgeley, & Simpson, 1999; Parsons, 1997; Webb et al., 1998). Microscopic prey identification indicates that *M. tuberculata* predominantly feed on five orders of arthropods: Coleoptera, Lepidoptera, Diptera, Blattodea, and Orthoptera (Arkins et al. 1999). Furthermore, diet appears to change seasonally, with higher proportions of volant insects consumed during summer.

On Little Barrier Island/Hauturu (Hauturu), where the climate is less seasonal, *M. tuberculata* have the highest dietary diversity during summer (Arkins et al., 1999). Although seasonal variation in diet is apparent and *M. tuberculata* is purported to be an opportunistic forager, these conclusions were based on data for one population and used traditional morphological techniques that may have a bias towards the detection of harder-bodied, larger prey. Molecular techniques are increasingly used to identify prey and are effective when applied to a generalist foraging species (Clare, Fraser, Braid, Fenton, & Hebert, 2009). Although molecular tools have been applied to examine seasonal and population differences for several bat species in the Northern hemisphere, comparatively few studies have used them to examine bats from the southern hemisphere (Bohmann et al., 2011; Burgar et al., 2014; Law, Gonsalves, Chidel, & Brassil, 2016).

*Mystacina tuberculata* presents a model species for examination of spatial-temporal variation in diet because it is a small generalist insectivore with a wide habitat range and presumably different insect communities. Using faecal samples collected from bats I tested several hypotheses. First, I assessed the variability of *M. tuberculata* diet across New Zealand, to test the hypothesis that spatial-temporal variation in resource use is an important form of nutritional or dietary flexibility. Specifically, I predicted that: 1) during the same season, populations from lower latitudes will eat a more diverse diet. 2) Bats will have a more diverse

diet during summer compared to winter. 3) Within a season, prey abundance and diversity will be correlated with  $T_a$ . My second hypothesis was that prey consumption will differ due to demographic differences in energy demands, such as that between lactating females and non-reproductive adults. Furthermore, I predicted that YOY would consume a more diverse diet assuming they have naïve foraging behaviour.

## Methods

All procedures were approved by the University of Auckland Animal Ethics Committee and were conducted under New Zealand Department of Conservation Wildlife Act Authorization Number 39083-FAU. My study was conducted at three sites; 1), the Pikiariki Ecological Area of Pureora Forest Park (Pureora; 38°26' S, 175°39' E), central North Island, New Zealand, during November 2014 – October 2015; 2), the Eglinton Valley of Fiordland National Park (Eglinton; 44°58' S, 168°00' E), South Island, New Zealand during January – April 2016. 3) Hauturu/Little Barrier Island (Hauturu; 36°19' S, 175°07' E), 80 km off the East coast of the North Island, New Zealand during May – July 2016).

Pureora Forest Park is a mature podocarp-hardwood forest containing Kahikatea (*Dacrycarpus dacrydioides*), Matai (*Prumnopitys taxifolia*), Miro (*Prumnopitys ferruginea*), Rimu (*Dacrydium cupressinum*), and Totara trees (*Podocarpus totara*). The forest is characterised by a low canopy with a dense understory and it is bordered by exotic pine plantations and pastoral land. The Eglinton Valley is dominated by a temperate southern beech forest consisting of red (*Nothofagus fusca*) and silver beech (*N. menziesii*). Hauturu is the only large forested area in New Zealand relatively unaffected by introduced browsing mammals. Pohutukawa (*Metrosideros excels*), Kohekohe (*Dysoxylum spectabile*), Puriri (*Vitex lucens*), Taraire (*Beilschmiedia tarairi*), Kauri (*Agathis australis*),

Northern Rata (*Metrosideros robusta*), Tawheowheo (*Quintinia serrata*), Tawari (*Ixerba brexioides*) and Southern Rata (*Metrosideros umbellata*) trees are common.

Bats were captured using harp traps and mist nets. Individuals were held singly in cloth bags for up to 1 hour. Faecal samples (all pellets collected per bat) were stored at -20°C in 1.7 ml micro-centrifuge tubes.

Individuals were weighed to the nearest 0.5 g using a Pesola spring scale (Pesola AG, Schindellegi, Switzerland; mean body mass =  $14.6 \pm 1.7$  g,  $n=369$ ). I recorded sex and measured forearm length to the nearest 1 mm (mean forearm length =  $42.5 \pm 1.5$  mm,  $n=369$ ). Adult females were classified as non-reproductive (no obvious bare patches around the nipples), pregnant (determined through gentle abdominal palpation), lactating (large bare nipples and milk produced when pressed), and post-lactating (nipples visible but no milk could be expressed). YOY bats were distinguished from adults by the lack of ossification of the metacarpal-phalangeal joint on the third digit (Racey, 1974).

At each study site, I recorded  $T_a$  every 10 min using data loggers (HOBO Micro Station Data Logger - H21-002, Onset Computer Corporation, Bourne, MA, USA,  $\pm 0.2^\circ\text{C}$ ) placed 2 m above the ground in the shade.

The QIAamp Stool Mini Kit (Qiagen, UK) was used to extract DNA from faecal samples from individual bats following the manufacturer's instructions, but including modifications suggested by Zeale, Butlin, & Barker (2011) and Clare, Symondson, & Fenton (2014). PCR and sequencing were performed by the Genome Centre (Queen Mary University of London) in brief: Amplification of a 157bp fragment of the mitochondrial cytochrome c oxidase subunit 1 was performed using primers ZBJ-ArtF1c and ZBJ-ArtR2c (Zeale et al., 2011) adapted to include Fluidigm tags CS1 and CS2. Each 10 $\mu\text{L}$  PCR reaction contained 5  $\mu\text{L}$  of

Qiagen multiplex PCR (Qiagen, CA) master mix, 3  $\mu$ L of water, 0.5  $\mu$ L of each 10  $\mu$ M primer and 1  $\mu$ L of eluted DNA. PCR amplification was as follows: 95°C, 15 min; 50 cycles of 95°C, 30 s; 52°C, 30 s; 72°C, 30 s and 72°C, 10 min. Amplicon QC was performed using a DNA D1000 TapeStation (Agilent Technologies, CA, USA) and quantification was performed using a QuBit dsDNA HS Assay Kit (Invitrogen, Life Technologies, Thermo-Fisher Scientific, MA, USA). Sequencing was performed bi-directionally with single indexes following manufacturer's protocols on the MiSeqv2 Chemistry 300 cycle run (Illumina Inc., CA, USA).

Reads were merged in Mothur (Schloss et al., 2009) and then processed using the Galaxy platform (<http://main.g2.bx.psu.edu/root>, Blankenberg, Kuster, & Coraor, 2010; Giardine, Riemer, Hardison, & Burhans, 2005; Goecks & Nekrutenko, 2010). Primer sequences were removed and all sequences that were longer or shorter than the target amplicon length of 157bp were filtered out. Sequences were collapsed into unique haplotypes and then singleton sequences were excluded from further analyses. Sequences were clustered into molecular operational taxonomic units (MOTUs) (Floyd, Abebe, Papert, & Blaxter, 2002) a representative sequence of each MOTU was picked for analysis with the QIIME pick otu and uclust methods (<http://qiime.sourceforge.net/>, Caporaso, Kuczynski, Stombaugh, & Bittinger (2010)). MOTUs were clustered using a similarity threshold of 92% (see Clare, Chain, Littlefair, Cristescu, & Deiner (2016) for the appropriateness of MOTU cluster levels for diet and food web analysis). Reference sequences were used to identify MOTUs to the order level using BLAST analyses and a reference database of > 600,000 DNA barcodes extracted from Genbank with a wider taxonomic profile (including potential contaminants bacteria, fungi, mammals etc.). MEGAN version 5.6.3. (Huson, Mitra, Ruscheweyh, Weber, & Schuster, 2011) was used to screen out unknowns, unidentified sequences and

those not resolved to order with the LCA parameters: Min score=150.1, Max expected=0.001, Top percent=10.0, Min support=1, LCA percent=100.0, Min complexity=0.2. The identified MOTUs were used for statistical analysis of diet and network analysis.

For ecological analysis, I split the data into "winter" (1 May – 1 August) and "summer" (1 January – 1 April) and examined differences between sites (Hauturu vs. Pureora winter; Eglinton vs. Pureora summer) and seasonally (Pureora winter vs. Pureora summer). To avoid potential confounding variables, ecological analyses were restricted to adult males and non-reproductive adult females. For ecological analysis, I removed MOTUs for orders that bats do not intentionally eat (e.g. nematodes). I conducted ecological analyses in PAST (Hammer, Harper, & Ryan, 2001) on order-level data and compared Simpson's diversity indices among locations and seasons with *P*-values estimated by bootstrapping with 2000 replicates. I compared the proportion of occurrence of each order in the diet (proportion = number of MOTU in an order/ total number of MOTU, where MOTU is a proxy for species) among locations and sampling periods using a  $Chi^2$  frequency test with *P*-values computed using a Monte Carlo simulation with 2000 replicates using R (v. 3.4.0; R Core Team 2009) .

I compared MOTU richness (number of MOTU present in a faecal sample) between sites using a Kruskal-Wallis H test (if data were non-parametric), or a one-way ANOVA (if data were parametric), followed by a posthoc Tukey HSD test to generate specific *P*-values using R (v. 3.4.0; R Core Team 2009). I found no difference in MOTU richness at any site between YOY males and YOY females, so they were pooled for further analysis. In addition, I found no differences between adult males and non-reproductive adult females at any site, so they were also pooled for further analysis. I used linear models with MOTU richness as a

dependent variable and demographic (e.g., YOY, non-reproductive adult, lactating adult female) date, and mean night  $T_a$  as independent variables. I conducted model selection by comparing models, starting with a saturated model including the interaction of all explanatory variables, using maximum likelihood tests until only significant variables remained. I assessed significance at  $P < 0.05$ .

## Results

Analyses were conducted on faecal matter collected between 14 November 2014 and 28 June 2016 from 243 individual bats (adult male: 106; lactating females: 55; non-reproductive adult females: 56; young-of-the-year females: 15; young-of-the-year males: 11).

I found 1105 unique MOTU from 36 orders (Table 1). The diet of bats at all sites and in both seasons was dominated by MOTU identified as Lepidoptera (63% - 81%) or Diptera (8% - 18%). Using order-level taxonomy only, the proportion of orders consumed differed between winter sites ( $X^2=13.18$ ,  $P < 0.01$ ; Fig. 1), summer sites ( $X^2=9.19$ ,  $P=0.03$ ), and seasonally in Pureora ( $X^2=26.4$ ,  $P < 0.01$ ).

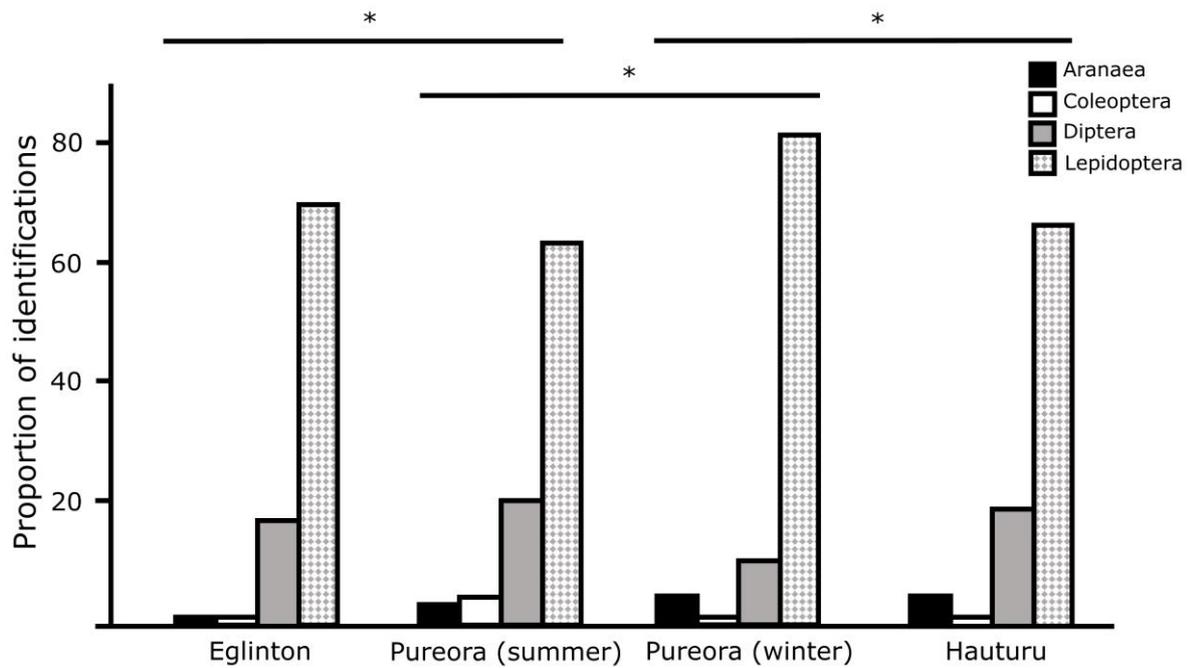
Using order-level taxonomy only, Simpson diversity indices approached significance for winter sites (LBI=0.52, Pureora=0.41,  $P=0.05$ ), differed between summer sites (Pureora=0.56, Eglinton=0.47,  $P < 0.01$ ), and seasonally in Pureora (Winter=0.41, Summer=0.56,  $P < 0.01$ ).

Dietary MOTU richness (number of distinct MOTU in a sample) of non-reproductive adults varied between sites ( $df=3,83$ ,  $F=6.5$ ,  $P < 0.01$ ; Fig. 2). Post-hoc analysis revealed differences between winter sites ( $P < 0.01$ ) with Hauturu bats having greater MOTU richness than bats in Pureora. Pureora bats had higher MOTU richness in summer than in winter ( $P=0.03$ ), but not compared to individuals from Eglinton during summer ( $P=0.9$ ).

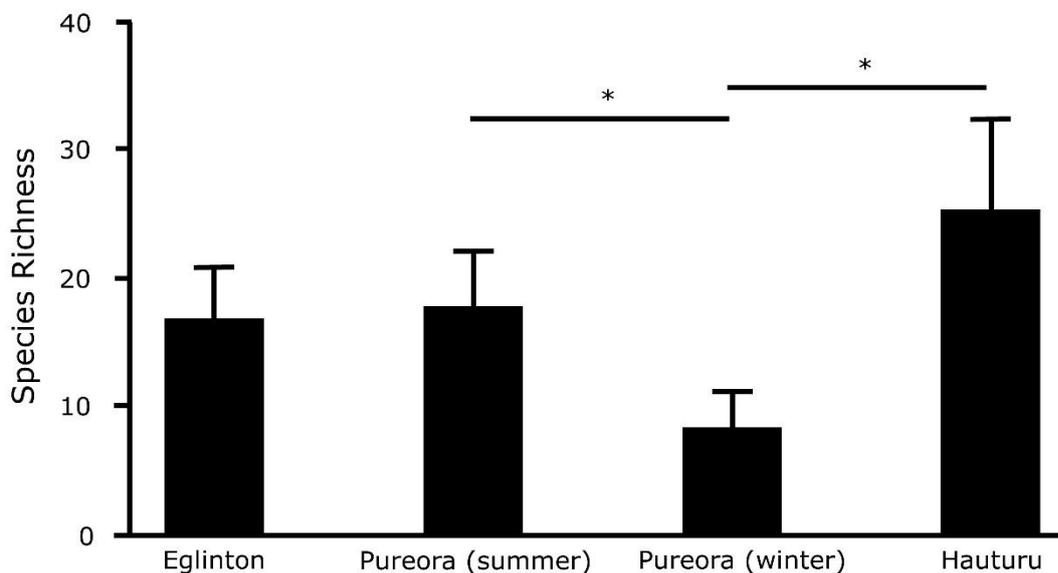
**Table 1** Order-level taxonomy of diversity in *Mystacina tuberculata* faeces collected from Pureora, Eglinton, and Hauturu.

Order	No. MOTU	Frequency
Adinetida	9	18
Alcyonacea	1	1
Astigmata	1	1
Calanoida	1	1
Isopoda	1	1
Leptothecata	1	3
Megaloptera	1	1
Mysida	1	1
Nudibranchia	1	1
Oribatida	3	3
Pantopoda	1	1
Philodinida	1	1
Rhabditida	11	21
Siphonaptera	2	8
Siphonophorae	1	1
Tylenchida	1	1
Amphipoda*	1	1
Araneae*	48	120
Blattodea*	6	90
Coleoptera*	45	187
Collembola*	9	13
Decapoda*	8	9
Diptera*	203	545
Ephemeroptera*	5	25
Euphausiacea	2	5
Hemiptera*	16	28
Hymenoptera*	12	12
Lepidoptera*	685	3168
Mantodea*	1	1
Neuroptera*	4	17
Opiliones*	1	1
Orthoptera*	10	27
Plecoptera*	2	11
Psocoptera*	3	12
Scolopendromorpha*	2	2
Trichoptera*	5	6

\* Signifies a probable prey order.



**Figure 1** Diversity in prey consumed by *M. tuberculata*. The proportion of each prey group in the diet varied significantly between sites and seasons. Proportion = number of MOTU in an order/ total number of MOTU. \* indicates significant differences.

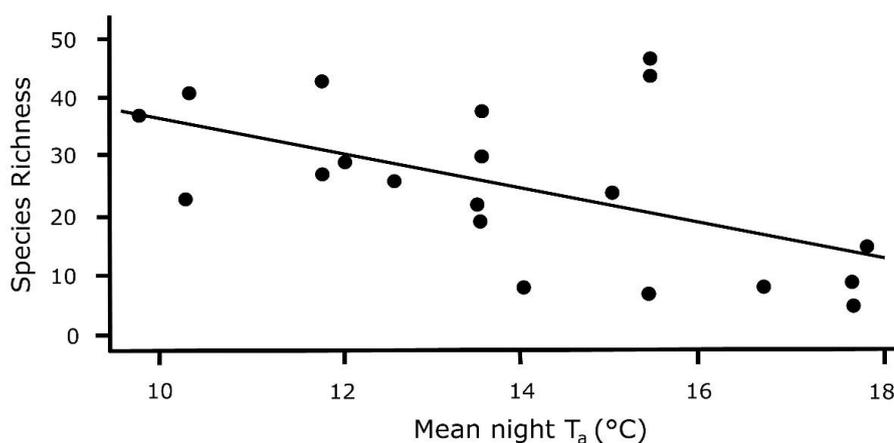


**Figure 2** Mean prey MOTU richness from *M. tuberculata* faecal samples with 95% confidence intervals, based on data restricted to ordinal-level taxonomy. \* represents a significant difference  $P < 0.05$ .

During summer in Eglinton, MOTU richness differed between demographic classes ( $df=2,36$ ,  $F=6.1$ ,  $P<0.01$ ) with higher MOTU richness in YOY relative to lactating females ( $P<0.01$ ) and non-reproductive adults ( $P=0.012$ ). There was no difference between lactating adult females and non-reproductive adults ( $P=0.6$ ).

During summer in Pureora, no differences were found between YOY and lactating females ( $P=0.2$ ), or YOY and non-reproductive adults ( $P=0.9$ ). However, a difference in MOTU richness was apparent between demographic classes ( $df=1,80$ ,  $F=4.5$ ,  $P=0.01$ ) driven by lower MOTU richness in lactating females compared to non-reproductive adults ( $P=0.01$ ).

During winter in Pureora, MOTU richness differed between demographics ( $df=1,21$ ,  $F=4.8$ ,  $P=0.04$ ), with higher MOTU richness in YOY compared to non-reproductive adults. In Hauturu, no difference was detected in MOTU richness between demographics, and this was the only site where there was a relationship between mean nightly  $T_a$  and MOTU richness ( $df=18$ ,  $T=2.7$ ,  $R^2=0.25$ ,  $P=0.014$ ; Fig 3).



**Figure 3** Prey MOTU richness from *M. tuberculata* faecal samples as a function of mean night  $T_a$  for individuals from Hauturu, New Zealand. MOTU richness decreased with increasing  $T_a$  ( $df=18, T=2.7$ ,  $n=20$ ,  $R^2=0.25$ ,  $P=0.014$ ).

## Discussion

My study is one of the first to use molecular techniques to examine spatio-temporal variation in the diet of a generalist insectivore that inhabits a contiguous range with several habitat types and climates. I found support for our first hypothesis that diet is affected by site and season. I found that prey orders consumed differed between winter and summer sites as well as seasonally and that diversity was higher in summer compared to winter. However, it was not a summer site that had the highest MOTU richness, but Hauturu during winter. Hauturu was also the only site where there was a significant relationship between mean  $T_a$  and diet. There was also support for my second hypothesis that diet differed due to demography. Young-of-the-year had a more diverse diet than any other demographic group and lactating females had the lowest dietary diversity. My data suggest that, although the faecal samples of *M. tuberculata* are dominated by Lepidoptera and Diptera, several site-specific seasonal and demographic variables influence diet.

The use of molecular dietary analysis and the consideration involved in the analyses of these data have been discussed previously (Clare et al., 2016; Pompanon, Deagle, & Symondson, 2012). There are two important issues in interpreting the data I collected. First, I detected an unexpectedly high proportion of Lepidoptera and Diptera in the diet. Traditional morphologically based analyses have suggested these bats eat more Coleoptera and terrestrial insects (Arkins et al., 1999). Molecular analyses are more sensitive to small, soft, easily digested material than traditional analyses (Clare, Kerr, Von Königslöw, Wilson, & Hebert, 2008) but cannot quantify biomass reliably. Thus, while my analysis may underestimate the importance of taxa such as Coleoptera and Orthoptera, I suspect the effect is not large. Second, I used MOTUs and a clustering threshold of 92%. This is relatively low compared to the suspected reality of species

identified (see discussion in Clare et al., 2016), but is recommended to reduce MOTU inflation (Clare et al., 2016; Flynn, Brown, Chain, MacIsaac, & Cristescu, 2015). I have used the empirical recommendations of Clare et al. (2016) to be conservative, and note that MOTUs should not be equated to “species” (see Floyd et al., 2002) but as a comparable taxonomic entity for ecological and statistical interpretations.

My study adds items to the list of prey known to be consumed by *M. tuberculata*. In addition to the orders that have been previously reported for *M. tuberculata* (Arkins et al., 1999) I found MOTU of Collembola, Decapoda, Ephemeroptera, Plecoptera, Psocoptera, Scolopendromorpha, and Trichoptera. Further, I found MOTU from several ecto- and endoparasite orders Oribatida, Siphonaptera, Astigmata, Rhabditida, Adinetida, Tylenchida.

Although *M. tuberculata* has a diverse diet, Daniel (1979) reported that *M. tuberculata* feed mainly on Coleoptera, Lepidoptera, Diptera, and Orthoptera, which Arkins et al. (1999) reported to make up 50% of the diet. My results contrast with these studies. I found that Coleoptera, Lepidoptera, Diptera, Orthoptera, and Aranea make up the majority of the diet, but Lepidoptera and Diptera account for 80 to 90% of the diversity at all sites.

All previous work analysing *M. tuberculata* diet used visual inspection and morphological analysis of partially digested prey remains in faeces to assess diet (Arkins et al., 1999; Daniel, 1979). This technique has limitations as only hard bodied prey will be recognisable after digestion, leading to an over-representation of these taxa compared to soft-bodied prey. Conversely, molecular analysis has been demonstrated to accurately identify not only hard-bodied prey but also small, soft-bodied prey (Clare et al., 2008). It is likely that previous work may have

overestimated the proportion of Coleoptera and Orthoptera in *M. tuberculata* diet and my work highlights the benefit of using molecular methods to determine diet.

I report differences in the Simpson diversity index, the proportion of orders consumed, and MOTU richness between summer sites. Individuals from Pureora had a more diverse diet than individuals from Eglinton. These sites are separated by 6° of latitude, but Czenze et al. (2017b) reported that mean summer  $T_a$  of each site was within 1°C. Therefore, it is unlikely that the differences I observed were caused by temperature differences. One explanation for the site-specific difference is the forest type. The Eglinton Valley is dominated by two tree species and has low invertebrate abundance, typical of forests in more temperate climates, and bats have larger home ranges (O'Donnell et al., 1999). Conversely, Pureora is comprised of several tree species, and individuals from Pureora have a smaller home range compared to Eglinton, suggesting a higher prey abundance and or diversity (Toth, 2016). In other parts of the world, there is a positive relationship between and plant and insect diversity (Zhang et al., 2016). This is consistent with Pureora having a more diverse forest and thus a more diverse insect community. However, more work is needed to precisely compare the insect communities in each forest.

Despite being sampled during winter, individuals from Hauturu had the second highest Simpson diversity index, which differed from Pureora individuals. Further, the proportion of orders consumed differed with Hauturu having the highest mean MOTU richness of all sites. The difference in winter climate between the two sites could partially explain the difference. Aerial insect abundance can decrease dramatically with decreasing  $T_a$  (Jones et al., 1995), and  $T_a < 10^\circ\text{C}$  has often been reported to constrain insect abundance (Park et al., 2000; Hope & Jones, 2012). During winter, mean night  $T_a$  in Hauturu ( $12.1 \pm 2.4^\circ\text{C}$ ) is higher

than Pureora ( $6.2 \pm 2.7^\circ\text{C}$ ), mean night  $T_a > 10^\circ\text{C}$  occurred on 92% of observation nights in Hauturu compared to 7% in Pureora, and  $T_a$  never dropped below  $0^\circ\text{C}$  in Hauturu but did so on 26% of nights in Pureora (Czenze et al., 2017c). Therefore, the winter conditions in Hauturu are likely to facilitate greater abundance and diversity of flying insects and, if *M. tuberculata* are feeding opportunistically, it may explain the differences in diet. Additionally, Hauturu is unique as it is free of mammalian and insect pests. The high diversity and MOTU richness may be due to the pristine nature of the island reserve as non-native flora and fauna can adversely affect insect diversity (Bezemer, Harvey, & Cronin, 2014; Burghardt & Tallamy, 2015; New, 2016). Since Pureora is essentially three distinct habitat types (e.g., non-native pines, pastoral land, and native podocarps) and bats potentially forage in each (Toth, 2016), it would be interesting to determine how much of *M. tuberculata* diet in Pureora is comprised of non-native species.

In Pureora, I found seasonal differences in the Simpson diversity index, the proportion of orders consumed, and MOTU richness. Many insect species are dormant, or inactive during winter, and arthropod consumption by bats varies with season (Kunz, de Torrez, Bauer, Lobova, & Fleming, 2011). Daniel (1979) suggested that, during winter, fewer moths are consumed by *M. tuberculata* due to cold temperature, and during April-May in Hauturu, Arkins et al. (1999) reported that Lepidoptera fragments occurred in only 3% of samples. This is in stark contrast to my data, which show that Lepidopteran fragments occurred in 95% (20/21 individuals) of Hauturu samples. Further, in Pureora, I found that the proportion of Lepidopteran MOTU increased from summer (65%) to winter (76%), while Dipteran MOTU decreased (17% to 5%). The decrease in Dipterans was mirrored by an increase in spiders (4% to 12%), suggesting that bats may be switching their foraging techniques. In captivity, *M. tuberculata* partition foraging

to 40% terrestrial, 30% aerial hawking, and 30% gleaning (McCartney, Stringer, & Potter, 2007). These findings, particularly the 40% terrestrial foraging, are not consistent with my results from a natural population. Although I cannot identify the method by which bats captured prey items, I would expect to see a greater proportion of ground dwelling insects in the diet if these proportions were correct. Therefore, 40% terrestrial foraging effort is likely an artefact of housing bats in a small enclosure and not indicative of natural behaviour. Future studies could employ accelerometers on free-ranging bats to determine the partition of foraging between terrestrial, aerial hawking, and gleaning.

On Hauturu, I found a negative relationship between  $T_a$  and mean MOTU richness with higher MOTU richness during colder nights compared to warmer nights. The thermoregulatory behaviour of bats on Hauturu is influenced more strongly by temperature than mainland bats (Czenze et al., 2017c). Although heat produced through activity is used for thermoregulation in a wide range of animals, generally, the costs of flight increase with decreasing  $T_a$  (Humphries & Careau, 2011; Klüg-Baerwald et al., 2016). Further, there is a threshold  $T_a$  where flying insects likely become absent, and Czenze et al. (2017c) argued that bats are using the warm  $T_a$  as a proxy for the increased probability of foraging success. Insect diversity falls after summer, and big brown bats (*Eptesicus fuscus*) may compensate by increasing their dietary diversity (Clare, Symondson, & Fenton, 2014). If bats choose to forage during colder nights they will spend more energy and, to mitigate these increased costs, likely cannot afford to be selective. Conversely, during a warmer evening, individuals are likely to be less energetically burdened and may invest more time foraging to capture higher-quality prey items. Further work is required to determine the nutrient content of prey items that are

selected by bats under a range of  $T_a$  and use bomb calorimetry, and respirometry to determine caloric intakes and expenditures.

Demographics appeared to play a varied role in MOTU richness depending on the site. During summer in Eglinton, YOY bats had significantly higher MOTU richness than other demographics. Although Arkins (1996) found no difference in *M. tuberculata* diet between age classes on Hauturu, adults and YOY of several other insectivorous bat species exhibit dietary differences (Adams, 1996; Adams, 1997; Hamilton & Barclay, 1998; Rolseth, Koehler, & Barclay, 1994). In some bats, YOY forage in more open areas due to poor flying skills and likely as a result have different diets to adults (Adams, 1996; 1997; Hamilton & Barclay, 1998; Rolseth et al., 1994). In addition to poor flying skills, YOY bats may capture fewer prey items than adults during the same foraging times (Anthony & Kunz, 1977). Yearling North-western Crows (*Corvus caurinus*) select a broader range of prey sizes than do adults (Richardson & Verbeek, 1987), and this pattern holds true for some bats (Borkin & Parsons, 2011; Hamilton & Barclay, 1998; Salsamendi et al., 2008). A combination of poor flying and handling skills may lead YOY *M. tuberculata* to be less "choosy". Alternatively, YOY bats may be exploring and opportunistically feeding on insects that naturally occur in each habitat. Future work could focus on dietary choice experiments to determine the extent of dietary preference for each demographic.

In Pureora during summer, lactating females had a narrower diet than other demographic classes. Energy balance and energetic demands of reproduction can affect foraging effort and diet (Anthony & Kunz, 1977; Barclay, 1989; Whitaker, Neefus, & Kunz, 1996). Energetic requirements should be greatest for the demographic with highest energy demands (i.e., reproductive females) (O'Donnell, 2001; Racey & Swift, 1985). The high energetic cost of pregnancy and

lactation is more likely to affect foraging strategies of females compared to males (Kunz, Whitaker, Jr., & Wadanoli, 1995; Swift, Racey, & Avery, 1985; Wilkinson & Barclay, 1997). Lactating little brown bats (*Myotis lucifugus*) have narrower diet breadth than other demographics as they are likely to form a “search image” to improve foraging efficiency and increase selectivity (Anthony & Kunz, 1977). Additionally, even when other prey types are available, lactating Mexican free-tailed bats (*Tadarida brasiliensis*) maintain a narrow diet likely due to water balance (Whitaker et al., 1996). By incorporating more fat-rich prey items, lactating greater mouse-tailed bats can store an important metabolic water source for when milk production is highest (Levin et al., 2009). In temperate regions, big brown bat faeces contained a higher richness of Coleoptera and Trichoptera during late fall and before hibernation, and these are high in linoleic acid, an energy-rich polyunsaturated fatty acid, (Clare, Symondson, & Fenton, 2014; Schalk & Brigham, 1995). Lactating *M. tuberculata* likely face a greater energetic burden than other demographics and may face a greater selection pressure to optimise foraging time by being more “choosy” and selecting high-quality prey items. Alternatively, a more restricted home range that optimizes foraging effort against energetic gains may be more strongly selected for in females compared to males. The home range requirements of bats are driven by their energetic requirements, which vary according to sex, age, and reproductive status (e.g., Borkin & Parsons, 2011; O’Donnell, 2001; Racey & Swift, 1985). Lactating *M. lucifugus* have 51% smaller home range than males (Henry, Thomas, Vaudry, & Carrier, 2002), and *Pipistrellus pipistrellus* (Racey & Swift, 1985), *Macrophyllum macrophyllum* (Meyer, Weinbeer, & Kalko, 2005), and *Chalinolobus tuberculatus* in the Eglinton Valley (O’Donnell, 2001) all have smaller home ranges than males. This pattern

has been attributed to the need for females to visit the roost and feed their young during the night (O'Donnell, 2001; Racey & Swift, 1985).

## **Conclusions**

I show that, unlike previous work, *M. tuberculata* incorporate a broad diversity of moths and flies in their diet. Despite their preference for these prey *M. tuberculata* exhibit site-specific differences in the proportion of prey orders consumed, and dietary diversity, suggesting that certain orders are more influential in certain sites than others. These differences are likely due to site-specific differences in habitat type and season. I also provide the first evidence of demographic differences in the diet of *M. tuberculata*, with juveniles having the broadest diet, and lactating females the most restricted. Newly available molecular techniques help to unveil new layers of dietary complexity and add finer resolution to understanding behaviours than were possible using previous techniques. Generating an insight into the diverse hunting patterns of generalists may help improve conservation efforts, highlight their crucial role in an ecosystem via stability or bio-control, and function as proxy for investigating the diversity of an ecosystem itself.

# **Chapter 6: Summary, Conclusions, and Future Directions**

## Summary

Seasonal, latitudinal, and climatic variation in weather and food availability impacts the energy budgets of small endotherms. In response to periods of negative energy balance, small insect eating bats may alter their thermoregulatory patterns, roosting behaviour, and diet. Most physiological research regarding energy balance has focused on species that experience extreme seasonal temperature variations, and knowledge is lacking for animals from less variable temperate to subtropical climates. I investigated the seasonal and latitudinal variation in thermoregulation, roost choice, and diet of lesser short-tailed bats (*Mystacina tuberculata*) from distinct populations across New Zealand.

In Chapter 2, I tested the hypothesis that differences in seasonal energy budgets, due to changes in ambient temperature ( $T_a$ ), affect roost choice and thermoregulatory patterns. My results showed the first evidence that torpor is used flexibly throughout the year by lesser short-tailed bats, demonstrating that both roost choice and season impact torpor patterns. My data suggest that, in the wild, lesser short-tailed bats use torpor facultatively, and employ a continuum of heterothermic responses, from shallow heterothermy to multiday torpor bouts. During summer, communal roost trees were used on 83% of observation days, and individuals occupying them rarely entered torpor. Conversely, during winter, solitary roosts were occupied on 93% of observation days, and 100% of individuals occupying them used torpor. During summer, bats employed torpor on 11% of observation days compared to 95% during winter. Although torpor bout duration did not differ between sexes, minimum skin temperature ( $T_{sk}$ ) was lower in females suggesting greater energy savings. Torpor bout duration correlated negatively with mean  $T_a$  with longer torpor bouts correlated with colder  $T_a$ . Torpor bouts were interrupted by arousals at sunset on warmer evenings presumably for individuals to take advantage of milder conditions, which likely had an increased

probability of foraging success. I show that lesser short-tailed bats have a highly flexible seasonal energetic strategy, and that perhaps reflective of small endotherms in general from warm temperate climates, respond to relatively small changes in seasonal  $T_a$  with a similar energetic strategy as more cold temperate species.

In Chapter 3, I tested the hypothesis that latitudinal differences in energy balance would lead to variation in roosting sociality and torpor expression between populations. I evaluated how latitudinal differences in energy balance relate to variation in torpor expression and roosting sociality and show that lesser short-tailed bats have a flexible strategy for conserving energy. Mean summer  $T_a$  differed by  $<1^\circ\text{C}$  between sites, and individuals living in the Eglinton Valley used torpor on 36% of observation days compared to 11% for Pureora bats. Roosts occupied by solitary bats were actually warmer than  $T_a$  and yet individuals from both populations exclusively used torpor while roosting solitarily. However, Eglinton Valley individuals roosted solitarily more regularly (38%) than Pureora individuals (17%). While the propensity to enter torpor during summer does not appear to be correlated with lower  $T_a$ , torpor bout depth and  $T_a$  appear to be related. Arousals from torpor by Eglinton Valley bats coincided with sunset and not daily  $T_a$  maxima suggesting that bats were not fully exploiting advantages of passive rewarming. Site-specific roost choice and torpor patterns were apparent between lesser short-tailed bat populations during summer, and energetic strategies appear to, in part, depend on population-specific differences. My data are consistent with the hypothesis that small mammals inhabiting latitudinal gradients can exhibit behavioral flexibility and use varied energetic strategies in different parts of their range, likely to meet the specific challenges of their environment.

In Chapter 4, I tested the hypothesis that differences in winter  $T_a$ , and therefore energy balance, would lead to varying torpor expression and roost choice between populations. I show that, during the most energetically challenging months, lesser short-tailed bats have a varied strategy for conserving energy at different latitudes. Although many of the thermoregulatory variables did not differ between sites, bats from Hauturu appear to be more influenced by ambient conditions than Pureora bats. Relative to the Pureora, winter  $T_a$  was higher in Hauturu. Hauturu bats used short torpor (<24 hours) more frequently (51% of observation days) than their Pureora conspecifics (33%). Torpor bout duration did not differ between sites. I found no correlation between  $T_a$  and the use of torpor by Pureora bats, but minimum  $T_{sk}$  of Hauturu bats was positively correlated with mean  $T_a$ . These bats were more likely to arouse on nights with warmer sunset  $T_a$ . Individuals from Hauturu used more thermally unstable roosts and I provide the first evidence that lesser short-tailed bats prefer to use punga as roosts in this part of their range. Despite their availability, Pureora bats never roosted in punga, instead exclusively selecting tree cavities. Although bat populations in warmer climates are under less thermoregulatory stress, site and climate specific adaptations appear to be employed that affect roost choice and torpor patterns. My data provides further evidence that differences in seasonal  $T_a$  between sites impact the energetic strategies within populations of small mammals.

In Chapter 5, I tested the hypothesis that spatial-temporal variation in resource use is an important form of dietary flexibility when assumed resource availability fluctuates. Molecular techniques revealed that variation in the diet of generalist insectivores can be affected by a number of site-specific traits including weather, habitat type, and season, as well as demographic traits such as reproductive status and age. Unlike previous diet studies, which have manually

identified invertebrate prey based on morphology after it has travelled through the gut, I found that lesser short-tailed bats are more dependent on moths and flies, which comprised up to 90% of diet. Despite these preferences, lesser short-tailed bats exhibit site-specific dietary differences. The proportion and diversity of prey orders consumed differed between winter and summer sites as well as seasonally. Prey diversity was higher in summer compared to winter, and higher in Pureora compared to Eglinton Valley, likely due to site-specific differences in habitat type and season. Demographic differences are apparent in the diet of lesser short-tailed bats with young of the year having the broadest diet, and lactating females the narrowest. In Hauturu, a significant negative relationship exists between mean  $T_a$  and prey richness. Molecular analysis allows finer identification of prey items and helps reveal new layers of dietary complexity and enhanced resolution about foraging to ecological behaviour that was impossible using previous techniques e.g., faecal analysis.

### **Conservation Implications**

Seasonal changes in climate and weather are shifting temporal abundance of resources and populations must adjust accordingly or face a lack of food, reduced active season, or population declines (e.g., Post & Forchhammer, 2008). Some species are better able to tolerate climate change via phenotypic, phenological, and/or physiological flexibility (Inouye, Barr, Armitage, & Inouye, 2000; Lane, Kruuk, Charmantier, Murie, & Dobson, 2012; Lučan, Weiser, & Hanak, 2013; Ozgul et al., 2010; Sparks, Jeffree, & Jeffree, 2000; Visser et al., 2006), which is critical if they depend on a short peak of food abundance. Caribou have not been able to adjust to climate change and have experienced declines in survival and offspring production due to the growing asynchrony between reproductive timing and plant phenology (Post & Forchhammer, 2008). Phenological mismatches can also

negatively impact insectivores as insect activity is mediated by  $T_a$ ; some birds time their reproduction take advantage of the seasonal abundance of these insects (e.g., Visser et al. 2006).

Climate change is predicted to reduce precipitation and predictability of rainfall in the subtropics where water balance is likely especially important for survival (Walther et al., 2002). The survival probability of adult female little brown bats (*Myotis lucifugus*) and young-of-the-year were higher in wet years with high cumulative summer precipitation (Frick, Reynolds, & Kunz, 2010). The impact of reduced drinking water availability is likely compounded for reproductive females, who may visit watering areas 13 times more than non-reproductive females (Adams & Hayes, 2008). Reduced precipitation also affects insects, and, globally, Lepidopterans are experiencing a range contraction and population decline (see Wilson & Maclean, 2011 for a review). Warmer, drier active seasons may be challenging in more tropical habitats (Downs, Zungu, & Brown, 2012). This may coincide with over 30,000 Australian bats dying of hyperthermia during the last 20 years (Welbergen et al. 2008).

How climate change impacts New Zealand, and specifically lesser short-tailed bats, may depend on the population. Unpredictable precipitation may impact the northern populations more negatively during summer by decreasing water/food availability. Lesser short-tailed bat diet differs depending on season and location; however, up to 90% is comprised of Diptera and Lepidoptera, and population declines in either could negatively impact bats. By determining which parts of New Zealand are likely to face the greatest declines in water availability, and thus insect population declines, conservation managers could predict and model which populations of bats may be under the greatest threat of climate change.

The higher latitude populations of lesser short-tailed bats may respond

differently to climate change. Milder winters and prolonged active seasons in the South Island could potentially benefit populations, especially if insects become more abundant. However, if the energetic costs of defending higher body temperatures, and increased arousal frequency typically associated with higher  $T_a$  (Geiser & Kenagy, 1988), outweigh the potential increase in insect availability, bats may still face a negative energy balance. Regardless of the population, lesser short-tailed bats appear to respond to weather cues, to some extent, via roost choice and thermoregulatory patterns. Therefore, it is likely that this variability will buffer them from the direct effects of climate change (Boyles, Seebacher, & Smit, 2011; Geiser & Turbill, 2009). It would appear that the greatest threat of climate change to the bats would be the indirect effects on their insect prey.

Although classified as nationally vulnerable, the population of the northern lesser short-tailed bats is thought to be stable, especially on Hauturu where all invasive predators have been eradicated (O'Donnell, Christie, Lloyd, Parsons, & Hitchmough, 2012; O'Donnell; Christie; Hitchmough; Lloyd; Parsons, 2010). Even while roosting in trees, bats are vulnerable to predation and it is worth highlighting that on Hauturu bats chose roosts that would offer even less protection. The extent to which bats across New Zealand roosted in punga before the introduction of invasive mammals remains a mystery, but, especially in areas where invasive predators have been removed, conservation managers should be aware that dead punga may serve as roosting habitat and take care to protect these vulnerable roosts.

Bats are notoriously difficult to relocate as their volant nature allows them to return to their original homerange more easily than terrestrial mammals. Relocation was attempted in 2005 with 20 juvenile bats that were thought to be more likely to remain in the new site than adults (Ruffell & Parsons, 2009;

Weinberger, Bontadina, & Arlettaz, 2009). Four months after soft release, none of the females appeared to be reproductive, potentially due to a combination of captive rearing and aviary over-use (Ruffell & Parsons, 2009).

Proper food resources are critical for relocation to be successful and translocated reptiles were less likely to move out of their new home-range when food was provided (Ebrahimi & Bull, 2012). Supplemental feeding was not identified as being a causal factor in the disease of lesser short-tailed that were fed mealworms (Ruffell & Parsons, 2009). However, the island may not have contained enough invertebrate prey items to sustain the new colony. My thesis provides conservation managers with dietary data from across populations and seasons, and the information on which to base future surveys of potential translocation islands to determine if they have the suitable prey items.

### **Future Directions**

While I am confident that my thesis has provided useful data and comparisons of energy balance among populations of lesser short-tailed bats, my research has also raised several additional questions that would benefit from future studies. I allude to many of these in the thesis, but several are worth an explicit discussion here.

Perhaps the most glaring current knowledge gap is information on the actual metabolic rate of lesser short-tailed bats. Throughout the thesis I recommend using open-flow respirometry to measure metabolic rate. By understanding specific metabolic rates we can calculate the precise energy savings of individuals in torpor, or calculate specific energy benefits afforded to individuals by choosing insulated vs. uninsulated roosts. Respirometry will also let future researchers compare the energy expenditure of singing vs. non-singing males to elucidate whether differences in reproductive investment lead to appreciable

differences in thermoregulatory patterns among males. Respirometry could also answer whether intraspecific variation in thermoregulatory behaviour is a result of genetic traits or phenotypic plasticity by exposing bats from populations across New Zealand to a range of  $T_a$  to determine if they are capable of acclimatising to new conditions. Unfortunately, many of these studies are better suited to the laboratory; however, there are still several avenues available to study free-ranging lesser short-tailed bats.

I made several seasonal comparisons between sites, but several site/season combinations remain unstudied. I did not have the opportunity to study the summer thermoregulatory patterns and roosting preferences of individuals from Hauturu, but the potential for new insights about this population of bats, and the species as a whole, should appeal to future researchers. Although significantly more challenging to record, winter torpor patterns of individuals from Eglinton would allow future researcher to examine torpor expression in response the coldest and longest winter in the species' range. Finally, effort should be made to study the population on Whenua Hou/Codfish Island. This population, like Hauturu, is free of introduced mammal pests and future researchers could elucidate if life history traits, like punga roosting, are common in populations without predation risk. This would lead to insights about how lesser short-tailed bats may have lived before the arrival of humans, and how predation risk has led to the evolution of their behaviour.

The final avenue of future research I want to highlight is foraging and diet. I recommend that future studies employ accelerometers on free-ranging bats to determine the partition of foraging between terrestrial, aerial hawking, and gleaning. I suggest that the importance of terrestrial foraging by lesser short-tailed bats may be overstated, and accelerometers may be able to provide future

researchers with the tools to determine foraging effort. I could not determine if individuals were preferentially selecting prey items or simply eating what was available, nor could I quantify the proportion of insects in an individual's faecal sample. Future studies could sample insects from bat habitats to determine if dietary selection occurs and use qPCR to determine what prey items comprise the majority of the diet. Further, future work could determine the macronutrient content of the prey items and also determine fatty acid content to determine the energetic gains related to specific prey items.

In the future, the use of respirometry, temperature sensitive radio-telemetry, accelerometry, and calorimetry could lead to detailed and specific energetic models for populations of lesser short tailed-bats across New Zealand. These models could be used to bridge the gap between the fields of behavioural ecology and ecophysiology, which was always the broad goal of the research I report in my thesis.

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