TOWARDS A MECHANISTIC UNDERSTANDING OF ELEVATIONAL RANGE OCCUPATION AND TERRITORIAL BEHAVIOUR IN TROPICAL MONTANE SONGBIRDS

Thesis submitted for the degree of

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AUTHOR DECLARATION

I declare that all work is entirely my own except in the following cases. In Chapter 3, the R code for fitting non-linear mixed models to metabolic rates to estimate thermoregulation was originally written by Craig White (Monash University) and adapted by me for my data. In Chapter 2 and Chapter 5, habitat data were collected primarily by a habitat survey team led by Ric Barker (Queens University Belfast) as part of annual survey plots. In Chapter 3 and Chapter 4, approximately a quarter of winter data on basal metabolic rates and behavioural focal watches on Black-headed Nightingale-Thrushes *Catharus mexicanus* were also collected by a field assistant (Martin Suanjak). In Chapter 5 approximately one third of the avian survey data were collected by field ornithologists (James Kennerley and Aiden Burrows) undertaking annual surveys at the field site.

The contents of this thesis were written by Samuel E.I. Jones with the incorporation of comments and intellectual input from Steve Portugal, Robin Freeman and Joe Tobias

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Probably like a lot of field biologists, I was always at my most content during fieldwork. The following quote is one that has always stuck with me ever since I first had the privilege of working in tropical forests, and reflects my feelings when following around Nightingale-Thrushes, birding, and wandering.

“I only went out for a walk and finally concluded to stay out till sundown, for going out, I found, was really going in.” - John Muir

ABSTRACT

Tropical mountains are some of the most biodiverse environments on earth. Typical of these gradients are the distinct elevational ranges of species along them, where related species occupy narrow elevational distributions that infrequently overlap. The mechanisms maintaining these patterns remain poorly understood, however, hampering our understanding of the processes that have created them, and our ability to accurately project changes along them in the face of warming climates. Similarly poorly known is how, or whether, life-history characteristics of tropical species mechanistically relate to their physiology/energetics. It has been hypothesised, however, that the lower metabolic rates of tropical species arise either from adaption to warmer, more stable tropical environments, or from the direct energetic costs associated with innate species characteristics (such as their behavioural traits).

This thesis addresses these two topics by studying tropical montane songbirds in Mesoamerica. Firstly, I assess the drivers of elevational range segregations along tropical mountains in line with three longstanding hypotheses; physiological specialisation to elevation-specific microclimates, habitat preferences and interspecific competition. Secondly I explore how the physiology of montane songbirds differs between seasons, and between species that occupy different elevational ranges. In addition, for one species I assess whether physiological shifts drive territorial behaviour, consistent with the suggestion that the pace of life of tropical birds is innately linked to their energetic expenditure.

In the first data chapter (**Chapter 2**) I test the dual role of interspecific competition (through playback experiments) and habitat preference (via survey plots) in maintaining the elevational segregation between two species of Nightingale-Thrush (*Catharus* sp.). I find that the lower elevation species (Black-headed Nightingale-Thrushes *Catharus mexicanus*; BHNT) displays competitive dominance over its high elevation congener Ruddy-capped Nightingale-Thrushes *C. frantzii;* RCNT but that the two species are also found in divergent habitats at their elevational range limits.

In the next data chapter (**Chapter 3**), I explore the physiology of BHNT, RCNT and three other species by investigating seasonal differences in metabolic rates (in response to manipulated temperature ranges) and haemoglobin concentrations, assessing whether physiological traits are related to elevation. I find seasonal differences are variable between species; BHNT increase metabolic rates in the summer (breeding season), whereas other sympatric species have consistent metabolic rates across the year. I also find distinct cold-tolerance traits in the higher-elevation RCNT, although these are more likely to be the result of historic isolation to high elevations, rather than contemporary adaption.

In **Chapter 4** I then seek to interpret the mechanisms underpinning the seasonal shifts in metabolic rates in BHNT. I test whether the increased metabolic rates in summer are functionally related to territorial activity via field experiments and a novel experimental design exploring the metabolic responses to conspecific song. I find that BHNT display seasonal differences in their metabolic responses to conspecifics, consistent with changes in territorial aggression in field experiments. Together this suggests that increases in BMR in the summer are related to increased territorial activity, probably as a result of energy management strategies.

Lastly, in **Chapter 5** I combine data from previous chapters on thermal physiology and competition, with point count data, structural habitat attributes and temperature lapse rates across elevation to predict point level abundances of four species. In doing so I test three classic hypotheses driving elevational range segregation in tropical montane species. I find that no species is restricted by thermal microclimates, contrary to a longstanding hypothesis, which posits that thermal specialism is the most important factor restricting elevational ranges, but that the roles of habitat and competition were mixed for other species. This suggests that the determinants of species elevational ranges may be complex and species-specific.

Taken together, the four studies in this thesis suggest that the physiology of montane songbirds is unrelated to thermal microclimates and that a variety of biotic factors work in combination to set their elevational range limits. Moreover, I also provide some of the first evidence relating seasonal physiological shifts in a tropical bird to life-history characteristics.

TABLE OF CONTENTS

LIST OF FIGURES

Supplementary figures are not listed.

LIST OF TABLES

Supplementary tables are not listed.

1 INTRODUCTION

1.1 TROPICAL MOUNTAINS; DIVERSITY AND SPECIES DISTRIBUTIONS

Despite representing a relatively small portion (~25%) of the world's terrestrial surface, mountains and their foothills contribute disproportionately to global biodiversity (Rahbek *et al.* 2019a, b). Recent estimates suggest that mountainous regions (hereafter defined by geomorphological aspects such as elevation and slope that control key features of montane environments such as variation in local climate and atmospheric pressure- for full definition see Rahbek *et al.* (2019c)) support ~87% of the worlds species of birds, mammals and amphibians, with large proportions of endemism (e.g. 54% of global amphibian diversity are endemic to mountains) (Rahbek *et al.* 2019b). Tropical mountains have even higher species concentrations still, and are among the most species rich systems on the planet (e.g. Rahbek *et al.* 2006, Kreft & Jetz 2007). For example, the Tropical Andes Biodiversity Hotspot alone maintains ~20% of all plant species, despite representing <1% of earth's land area (Myers *et al.* 2000). This exceptional biodiversity is well represented in the recognition of tropical mountains as 'hotspots' of global importance for biodiversity (Myers *et al.* 2000, Brooks *et al.* 2006, Le Saout *et al.* 2013).

Although there is widespread recognition of the high species richness of tropical mountains, this richness has proven difficult to explain by broad scale macroecological studies (Rahbek *et al.* 2019b). Tropical mountains are often outliers in studies explaining richness patterns by current or past climate (Jetz & Rahbek 2002, Jetz *et al.* 2004, Rahbek *et al.* 2006), in turn suggesting that their high diversity may be a product of various historical and contemporary ecological processes (Cadena *et al.* 2011). By extension, because mountains are topographically diverse (e.g. Elsen & Tingley 2015) studies at broad spatial scales are often at insufficient resolution to disentangle potential drivers of richness along tropical mountains (McCain 2009a). For instance, along tropical mountains, changes in elevation, habitat and climate occur over small scales (e.g. ~ 1-10 km Jankowski *et al.* 2013b), whereas many global analyses of patterns of species richness are conducted at substantially larger scales (e.g. 110 km^2 to 1° × 1° latitude-longtitude Orme *et al.* 2005, Hawkins *et al.* 2007).

1.1.1 Environmental conditions and biotic turnover on tropical mountains

One of the most important and distinct features of tropical mountains are their climate regimes, where temperature decreases rapidly with increasing elevation at a rate far greater than with increases in latitude (Körner 2007, Rahbek *et al.* 2019b). For example, a typical adiabatic lapse rate of mean temperature along a tropical montane gradient of ~5-6 °C/1000 m (Terborgh & Weske 1975, Chen *et al.* 2009, Freeman 2015a) would require a shift of several thousand km in latitude from the equator to display the same temperature change (e.g. Graae *et al.* 2011). Such changes in climate with increasing elevation are important because temperature isotherms are brought into closer proximity along mountain slopes (Janzen 1967, Ghalambor *et al.* 2006, Körner 2007). In addition, the relative lack of seasonal climatic oscillation in tropical latitudes means that the climatic conditions present at different elevations remain relatively stable across the year (e.g. Janzen 1967, Londoño *et al.* 2017).

In turn, these patterns of climatic zonation combine with topographic or geological features of tropical mountains to subsequently influence turnover between vegetation communities (Jankowski *et al.* 2009, Fadrique *et al.* 2018). For example, high elevations in the tropics are typified by montane cloud forests, where the lower pressure at high elevations causes mist and high humidity, subsequently driving biotic composition (Bubb *et al.* 2004). Vegetation changes considerably with elevation on tropical mountains creating rapid transitions between habitats that differ in their structure and composition (e.g. Grubb *et al.* 1963, Lovett *et al.* 2006). Indeed, one of the most famous examples of this zonation between vegetation communities was characterised by Alexander von Humboldt along Ecuador's Mt Chimborazo constituting one of the oldest datasets on elevational ranges of plant species (von Humboldt & Bonpland 2009, Morueta-Holme *et al.* 2015).

Corresponding with these climatic and biotic shifts along tropical mountains, are high levels of faunal beta diversity and species turnovers (e.g. Terborgh 1977, Patterson *et al.* 1998, Jankowski *et al.* 2009, McCain 2009b). High beta diversity – where the proportion of site level species diversity is low in comparison to regional diversity (Whittaker 1975) – is created along tropical montane gradients by species generally occupying narrow elevational ranges. Such ranges are often mutually exclusive between related species, creating a pattern of belt-like distributions along mountainsides which make up the overall species richness patterns of the mountain that can vary with region (Rahbek 1997, McCain 2009b, Quintero & Jetz 2018). This pattern of elevational zonation has been displayed across numerous species groups (e.g. birds, mammals and butterflies; Diamond 1973, Terborgh 1977, Patterson *et al.* 1998, McCain 2004, Jankowski *et al.* 2009, Pyrcz *et al.* 2009), typically manifesting in related species with parapatric distributions. This elevational range patterning has generally been suggested to be a result of range expansion of species that have evolved in allopatry (secondary contact) (Patton & Smith 1992, García-Moreno & Fjeldså 2000, Cadena *et al.* 2011, Caro *et al.* 2013, Freeman 2015b, Cadena & Céspedes 2019) suggesting that community assembly along tropical mountains (at least in the species studied) is the result of colonisation processes.

These two salient features of tropical mountains – rapidly changing abiotic and biotic features and high degrees of species turnover– have attracted ecologists to them as natural systems to study the determinants of species ranges (e.g. Terborgh 1971, Diamond 1973, Jankowski *et al.* 2013a). Tropical montane systems are well suited for such studies because they are replicated across the globe, and thus incorporate a wide variety of environmental gradients with extensive taxonomic coverage of species (McCain 2009a, Mahli *et al.* 2010). However, while the contemporary biological importance and general patterns of distributions in tropical mountains is well recognised, the processes maintaining this species turnover remain poorly resolved (Jankowski *et al.* 2013a).

1.1.2 Abiotic and biotic drivers of elevational range segregation

Arguably the most influential abiotic explanation offered for the tight elevational distributions of tropical species is that proposed by Daniel Janzen (1967). His theory '*Why mountain passes are higher in the tropics*' predicted that because tropical species experience low seasonal variation in environmental temperature in comparison to temperate montane species, they should evolve narrow physiological tolerances with low acclimation potential to climates outside of this range. Thus, tropical montane species should have lower dispersal ability in comparison to temperate species because elevations outside of their native range would pose costly physiological barriers to dispersal that limit gene flow and increase allopatric speciation (Janzen 1967, Ghalambor *et al.* 2006).

While patterns of elevational ranges in tropical species (within narrow thermal regimes) consistent with Janzen's theory have been displayed (e.g. McCain 2009b, Cadena *et al.* 2011), few empirical tests of physiological tolerance to thermal ranges have been undertaken. Those that have, have offered mixed support; for example, narrow thermal tolerances limiting elevational dispersal have been shown in freshwater invertebrates (Polato *et al.* 2018), but not in birds (Freeman 2015c, Londoño *et al.* 2017). Indeed, an emerging consensus, particularly for endotherms, is that thermal boundaries are unlikely to acutely limit elevational ranges because a variety of biophysical and physiological attributes (e.g. insulation, size, body temperature and evaporative cooling capacity) buffer endotherms from climatic conditions (Porter & Gates 1969, Jankowski *et al.* 2013a). Instead, it is generally hypothesised that the 'cold' upper limits of elevational ranges are more likely to be limited by abiotic drivers, whereas the 'warm' lower limits are limited by biotic factors (MacArthur 1972).

Efforts to date that have assessed the biotic factors underlying species turnover have focussed principally on competitive interactions between species, or habitat preferences. Competitive interactions may set the limits of elevational distributions between closely related species by competitive exclusion, where competition between species at their range edges (e.g. interference competition; Grether *et al.* 2017) limits elevational dispersal (Diamond 1973). Similarly, habitat selection may set elevational limits by species-specific ecological requirements (e.g. at changing ecotones; Terborgh 1977). Evidence in support of both hypotheses has been offered through studies on avian community composition and habitat turnover (e.g. Jankowski *et al.* 2009, 2013b), experimental assays on interspecific aggression at range limits (e.g. Jankowski *et al.* 2010, Pasch *et al.* 2013, Freeman *et al.* 2016, Boyce & Martin 2019, Chan *et al.* 2019), and behavioural and niche divergence in patterns of parapatric birds (Freeman *et al.* 2019). A central challenge remains, however, in that these determinants have largely been assessed in isolation.

In practice, biotic and abiotic factors are not mutually exclusive and likely to work in unison to create patterns of elevational turnover (Jankowski *et al.* 2013a). For example, in a study examining the elevational range limits of singing mice (*Scotinomys* sp.) in Central America, competitive exclusion was the principal factor limiting upslope dispersal for the lower elevation species, while physiological preference for cooler climates limited downslope dispersal in the higher elevation species (Pasch *et al.* 2013). Moreover, other potentially important biotic factors limiting elevational ranges such as predation or pathogens remain largely unexamined in tropical mountains (Jankowski *et al.* 2013a).

1.1.3 Tropical mountains in a changing world

Understanding the processes that drive elevational ranges of tropical species is becoming increasingly urgent in the face of climate change. Projections suggest that species communities in tropical montane environments will undergo considerable transformation as a result of shifts in the distributions of habitats and species (Parmesan 2006, Colwell *et al.* 2008, Sheldon *et al.* 2011). Species responses to climate change are not uniform, however (see Chen *et al.* 2010, I-Ching *et al.* 2011), and may depend on the topographic nature of specific mountains (Elsen & Tingley 2015) and the elevational distributions of species (e.g. high elevation species may be disproportionally affected; Freeman *et al.* 2018b). Indeed, projections suggest that current tropical montane climates may disappear by 2100 (Williams *et al.* 2007).

These predicted changes are particularly worrying considering that tropical species may be particularly sensitive to even small increases in temperature, at least for ectotherms (Tewksbury *et al.* 2008, Deutsch *et al.* 2008, Huey *et al.* 2009). A similar effect has been suggested for tropical endotherms (Khaliq *et al.* 2014), however, the validity of some of these data have been strongly questioned (McKechnie *et al.* 2016). Nonetheless, there is mounting empirical evidence displaying upslope shifts in species communities on tropical mountains across taxa. For example, in birds (Forero-Medina *et al.* 2011, Freeman & Class Freeman 2014, Rosselli *et al.* 2017, Neate-Clegg *et al.* 2018), plants (Feeley *et al.* 2010, Morueta-Holme *et al.* 2015, Fadrique *et al.* 2018), and invertebrates (Chen *et al*. 2009, 2010, Moret *et al*. 2016). By extension, climate change has also driven extinctions of tropical mountain top species (Pounds *et al*. 1999, Freeman *et al*. 2018a).

A combination of ‘three strikes’ 1) substantial changes in montane climates, 2) potential high sensitivity to change, and 3) small elevational ranges of tropical montane species, render tropical mountains particularly vulnerable to global change (Jankowski *et al*. 2013a). However, there are substantial challenges in making informed projections for how species will respond to global change because for most tropical montane taxa the necessary data on the drivers of distributions do not exist (Mahli *et al*. 2010, Jankowski *et al*. 2013a).

1.2 AVIAN METABOLIC RATES AND THE SLOW PACE OF LIFE OF TROPICAL BIRDS

Metabolism – the process by which resources are taken from the environment and converted into energy – is a fundamental biological process that determines the demands that a species places on its environment to survive (Brown *et al.* 2004). The rate at which these processes occur – metabolic rate – subsequently represents the rate of most biological activities (e.g. Wikelski *et al.* 2003, Lovegrove 2003). As such, metabolic rates represent the physiological processes that underpin life-history characteristics and the pace of life of different species (Ricklefs & Wikelski 2002, Auer *et al.* 2017).

The most widespread measurement of metabolic rate in birds is the basal metabolic rate (BMR); the lowest level of energetic expenditure, at rest (Lighton 2008). Strictly speaking, measurements of BMR require specific criteria, where a species is post-absorptive (not digesting food), in its natural resting circadian phase (e.g. at night for most species), not-moulting or in a reproductive state, and within its thermoneutral zone (TNZ) (e.g. McKechnie & Wolf 2004, Mathot & Dingemanse 2015). The TNZ is bounded by a lower and upper critical temperature (LCT/UCT) where a bird increases metabolic heat production (LCT) or increases evaporative water loss (UCT) in order to regulate body temperature (thermoregulation) (**Figure 1.1**). BMR is thus standardised, repeatable and widely used as a comparative measure in avian physiology (McKechnie & Wolf 2004).

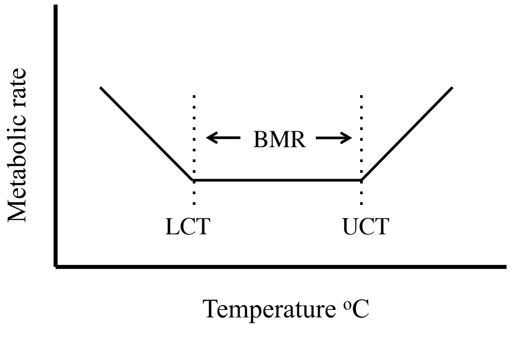

Figure 1.1 Metabolic rate as a function of temperature, displaying typical traits measured in avian physiology; basal metabolic rate (BMR) and lower (LCT) and upper critical temperature (UCT) bounding basal metabolism, respectively.

In addition to BMR, other measurements such as field metabolic rate (FMR; measuring daily energy expenditure), or peak metabolic rate (PMR; measuring the highest output of metabolic performance either through cold exposure or exercise) capture different aspects of energy expenditure (Lighton 2008). Consistent relationships between each of these measurements (particularly with FMR) have not always been displayed (e.g. Ricklefs *et al.* 1996, Portugal *et al.* 2016), however, BMR is widely considered as a proxy for metabolic intensity (e.g. White *et al.* 2007, McKechnie 2007, McKechnie *et al.* 2015). For the purposes of this section, 'metabolic rate' refers to all measures of metabolic power output, unless a specific measure is directly referred to.

In accordance to the basic principal that larger animals require more energy than smaller animals (e.g. Kleiber 1932), the strongest predictor of avian metabolic rates is body mass (Lasiewski & Dawson 1967, McKechnie & Wolf 2004). However, avian metabolic diversity arises from a number of other sources of phenotypic variation after accounting for the influence of mass (Anderson & Jetz 2005, McKechnie *et al.* 2006, Mcnab 2009). For example, Passerines (which comprise over half of the worlds extant bird species) have higher BMR than non-Passerines (Lasiewski & Dawson 1967, Londoño *et al.* 2015). Similarly, migratory species have higher BMR than non-migrant species (Jetz *et al.* 2008a, Bushuev *et al.* 2017), thought to be a phenotypic adaption to the increased metabolic demands incurred through flight (Mcnab 2009).

One of the most prevalent explanations for the variation in avian metabolic rates, however, is environmental adaption (Chown *et al.* 2004), and global analyses have suggested that environmental factors influence metabolic rates as a product either proximately or directly related to environmental temperature (Anderson & Jetz 2005, White *et al.* 2007, Jetz *et al.* 2008a, Araújo *et al.* 2013, Khaliq *et al.* 2014). Colder environments place higher energetic demands on birds owing to the increased thermoregulatory costs of maintaining body temperatures, resulting in higher metabolic rates (Mcnab 2012). In contrast, warmer or more seasonally stable temperatures reduce the metabolic demands of heat production, resulting in lower metabolic rates (Weathers 1979, Wasser 1986). For example, polar and high elevation species often have higher metabolic rates (e.g. Swanson & Liknes 2006) in comparison to species inhabiting arid environments (e.g. Tieleman & Williams 2000, Tieleman *et al.* 2003) or warmer luxuriant climates (e.g. Bartholomew *et al.* 1983, Wiersma *et al.* 2007b). In addition, many cold adapted species have lower LCTs as an adaptive trait in order to reduce the energetic costs expended on thermoregulation (Araújo *et al.* 2013, Khaliq *et al.* 2015).

1.2.1 Phenotypic flexibility in metabolic rates

There is increasing recognition that metabolic rate is not static and that substantial phenotypic plasticity exists within avian metabolism (McKechnie 2007). Phenotypic plasticity in metabolic rates may manifest in two ways (which are not necessarily mutually exclusive). The first of these is developmental plasticity, where irreversible changes arise as a product of developmental processes (Piersma & Drent 2003). Developmental plasticity in metabolic rates is a consequence of environmental conditions during development ('reaction norms' see Stearns 1989). For example, wild-born birds have higher mass scaling exponents than captive born birds, likely as a result of more controlled conditions during development (e.g. consistent, high quality food) (McKechnie *et al.* 2006). The recognition of developmental plasticity is intriguing because it raises the question of whether flexibility in metabolic traits more generally arises from adaption to local conditions, or as a result of their genetic differences (McKechnie *et al.* 2006). For example, in rearing Stonechats *Saxicola* sp. from tropical and temperate populations in identical conditions, Wikelski *et al.* (2003) found that tropical birds still had lower metabolic rates than their temperate conspecifics, suggesting that their metabolic rates were genetically intrinsic.

The second aspect of phenotypic plasticity is phenotypic flexibility, where shifts in metabolic rates are short-term and reversible at the individual level (Piersma & Drent 2003). Phenotypic flexibility is generally considered to be an adaptive response to increasing energetic demands of different parts of the annual cycle (see Swanson 2010). Well-described examples of phenotypic flexibility are increases in metabolic rates during migration in long distance migrant species (e.g. up to 100% increase in BMR in Red Know *Calidris canutus*; Piersma *et al.* 1995), or during winter in many resident species at north temperate latitudes (e.g. 30% increases in BMR in Downy Woodpecker *Picoides pubescens* Liknes & Swanson 1996). In cases such as these, BMR is typically up-regulated in order to meet the energetic requirements for enhanced metabolic endurance (e.g. for long distance flight or increased energy for thermoregulation, respectively; Swanson 2010). Metabolic rates do not always positively covary with energetically costly activities, however, and energy can be re-allocated to meet the energetic demands of workload, such that BMR can also decrease through energy compensation (Nilsson 2002, Mathot & Dingemanse 2015). Taken together, such energy trade-offs are critical for birds, because they form the basis of how life-history characteristics and energy usage shape the pace of life.

1.2.2 Metabolic rates of tropical birds and the slow pace of life

An additional pattern of variation in avian metabolic rates is the lower metabolic rates of tropical, compared to temperate species. Comparative studies have demonstrated that the BMR of tropical birds is between 14-23% (study dependent) lower than that of temperate species after controlling for the effects of mass and phylogeny (Wiersma *et al.* 2007a, b, Londoño *et al.* 2015, Bushuev *et al.* 2017) as well as lower exercise and cold induced PMR (Wiersma *et al.* 2007a). In particular, one of these studies (with a dataset of over 450 species; Londoño *et al.* 2015), also compared the relationship between latitude (between Peru and Panama) and elevation on BMR in tropical species, displaying two important findings. Firstly, BMR did not differ between species within the tropics, indicating that low metabolic rates are a consistent trait across tropical regions. Secondly, and most importantly, these authors found that the BMR of tropical species did not differ dependent on elevation, despite significantly cooler conditions at higher elevations. The latter finding in particular suggests that lower metabolic rates of tropical species are an intrinsic characteristic, contrasting with earlier suggestions that variation in avian metabolic rates at lower latitudes is a result of phenotypic adaption to environmental temperatures (White *et al.* 2007, Jetz *et al.* 2008a).

A number of characteristics of tropical birds suggest that their intrinsic traits may manifest in lower energy expenditure. For example, tropical birds are typified by traits such as lower fecundity, longer incubation periods, longer periods of nestling development, higher adult survival rates and more sedentary behaviours (Hau *et al.* 2000, Ghalambor & Martin 2001, Jetz *et al.* 2008b, Steiger *et al.* 2009, Boyce &

Martin 2017), contrasting with the opposite traits in temperate species. Taken together these traits characterise the ‘slow’ paced life-histories of tropical species, where energy is allocated to competing life functions, and self-maintenance is prioritised over reproductive output (Pianka 1970, Ghalambor & Martin 2001). Direct tests of the metabolic demands of these slow paced traits in tropical birds are rare (e.g. Wagner *et al.* 2013), although tropical birds have smaller metabolically costly organs (e.g. heart, lungs, liver and kidneys) indicating that their low BMR is functionally related to their internal physiology (Wiersma *et al.* 2012). Similarly, tropical birds also have smaller flight muscles than temperate species, also suggesting a functional relationship between low PMR during flight (Wiersma *et al.* 2007a, 2012).

Until recently, the seasonal variation in metabolic rates in tropical species was unknown. However, in concert with the suggestion that the metabolic rates of tropical birds appear unrelated to environmental temperatures, two recent studies have also displayed substantial flexibility in metabolic rates across seasons in tropical species (Wells & Schaeffer 2012, Pollock *et al.* 2019). The variation observed in these studies was unpredictable, where some species increased metabolic rates in the ‘summer’ wet season, while others decreased metabolic rates or stayed constant throughout the year. These changes in metabolic rates were irrespective of the lack of seasonal shifts in temperature, the opposite of seasonal shifts in temperate species (which typically increase in winter; see Smit & McKechnie 2010, McKechnie *et al.* 2015).

The drivers of these species-specific shifts in metabolic rates in tropical birds are unclear but have been suggested to result from innate links between species characteristics and their metabolic rates (Anderson & Jetz 2005, McKechnie *et al.* 2015). Anderson & Jetz (2005) termed this link as the ‘metabolic niche’, where the strategy of endotherms is constrained by limitations placed on them by environmental conditions. Thus, because environmental conditions create a greater diversity of ecological niches at lower latitudes, the variety of ‘metabolic niches’ are also broad for tropical species. To date, few studies have tested energetic usage in tropical birds and none have identified mechanistic links with changes across season.

1.2.3 Methodological considerations for measuring metabolic rates

Metabolic rates (excluding FMR) – via the standard proxy of oxygen consumption – are typically measured using flow-through respirometry systems (Lighton 2008). Birds are placed in a sealed chamber and supplied a constant air stream, the excurrent gas contents (e.g. **Figure 1.2**) from which are measured against the incurrent gas contents (e.g. 20.95% ambient O_2 levels) in order to measure the rate of O_2 consumption ($\dot{V}O_2$). $\dot{V}O_2$ is then frequently converted to a joule equivalent as a unit of energy (e.g. Gessaman & Nagy 2008). The air stream to the chamber is supplied at a sufficient rate (the flow rate ml/min) at which O_2 and CO_2 levels do not drop below 0.5% of natural levels in order to prevent hypoxia/hypercapnia. To measure BMR or thermoregulatory traits, chambers are placed in a further system where temperatures can be manipulated and $\dot{V}O_2$ measured in order to establish responses to different temperatures (**Figure 1.2**).

Physiological measurements in this thesis were undertaken at different times of year, frequently involving male birds with enlarged cloacal protuberances, representative of reproductive condition. However, BMR is used throughout this thesis for all metabolic rates that otherwise met the correct criteria (e.g. within the TNZ). While strictly speaking, non-reproductive state is a requirement for BMR, it is generally expected that females are more likely to increase metabolic rate as a result of reproductive activity more than males (e.g. Swanson 2010) and consistent evidence for increases in BMR in males in reproductive state is lacking (e.g. Wikelski *et al.* 1999, Buchanan *et al.* 2001). In addition, other studies on the physiology of tropical birds have also been undertaken in breeding seasons and likely include male birds in the same condition and I use this term for purposes of comparison (e.g. Pollock *et al.* 2019).

Other measures of metabolic rates (e.g. FMR) were not considered for this study because they would involve continual recapture of birds that was not feasible at the field site. Similarly PMR (alternatively termed summit metabolism) was not measured for the same reasons because it would require control of very low temperatures (at or sub-zero) for which suitable equipment was not available. Finally, the upper limits to the TNZ (the upper critical temperature) were also not measured because they are unlikely to capture useful information pertinent to thermal restriction to elevational ranges.

Figure 1.2 Field respirometry system used during this study. White arrows display the direction of airflow pulled through the chamber (a), in which a bird is placed and to the gas analysers (b; FoxBox, Sable Systems, USA). The larger box surrounding (a) (when closed) manipulates temperature.

1.3 CUSUCO NATIONAL PARK, HONDURAS

The fieldwork for this project was undertaken in Cusuco National Park (CNP) in the Sierra del Merendón, north-western Honduras (**Figure 1.3**). CNP is a 23,440 ha area of tropical montane cloud forest encompassing an elevational range from 600 – 2300 m (see Martin & Blackburn 2009) (**Figure 1.3**). The forests of CNP comprise of four principal habitats, tropical lowland dry forest, tropical moist forest, montane cloud forest and dwarf/elfin forest (bosque enano). At lower elevations, agricultural activities, particularly forest clearance for coffee plantations, has caused widespread loss and fragmentation of much lower elevation forest (below 1000 m). The upper elevations of the park correspond with the broad elevational bands of montane cloud forest as described by Bubb *et al*. (2004).

The biodiversity of CNP has been relatively extensively studied since 2006 as part of continual monitoring effort operated during an annual expedition coordinated by Operation Wallacea (see www.opwall.com) (e.g. Martin & Blackburn 2009, 2014, Jocque & Field 2014, Martin *et al*. 2016, Neate-Clegg *et al*. 2018, Hoskins *et al*. 2018). The biological importance of CNP is internationally recognised, ranking as the worlds 123rd most irreplaceable protected area from an analysis of over 173,000 protected areas, particularly for the diversity in its herpetofauna (where six species are endemic to CNP) (Le Saout *et al*. 2013). Research in CNP is conducted from several research camps, from which a transect network radiates (each approximately 2 km in length). These transects were originally established by local guides along natural features including valley bottoms or ridges that could be relatively easily traversed.

The avifauna of CNP comprises of ~283 species (SEIJ unpublished data), of which ~21% are Mesoamerican endemics (Martin *et al*. 2010). Similar to most tropical mountains, species richness decreases with elevation (Martin & Blackburn 2009, Neate-Clegg *et al*. 2018) and the avian community is typified by elevation specific species turnovers (**Figure 1.4**). The elevational ranges of numerous species have also moved upslope over the past decade, expected to be a response to warming climates (Neate-Clegg *et al*. 2018). This study focuses on five songbird species that inhabit the forest understorey- Ruddy-capped Nightingale-Thrushes *Catharus frantzii* (RCNT), Black-headed Nightingale-Thrushes *C. mexicanus* (BHNT), Grey-breasted Wood

Wrens *Henicorhina leucophrys* (GBWW), Chestnut-capped Brushfinches *Arremon brunneinucha* (CCBF) and Common Bush Tanagers *Chlorospingus flavopectus* (COBT), with a particular focus on (BHNT) (**Figure 1.5**).

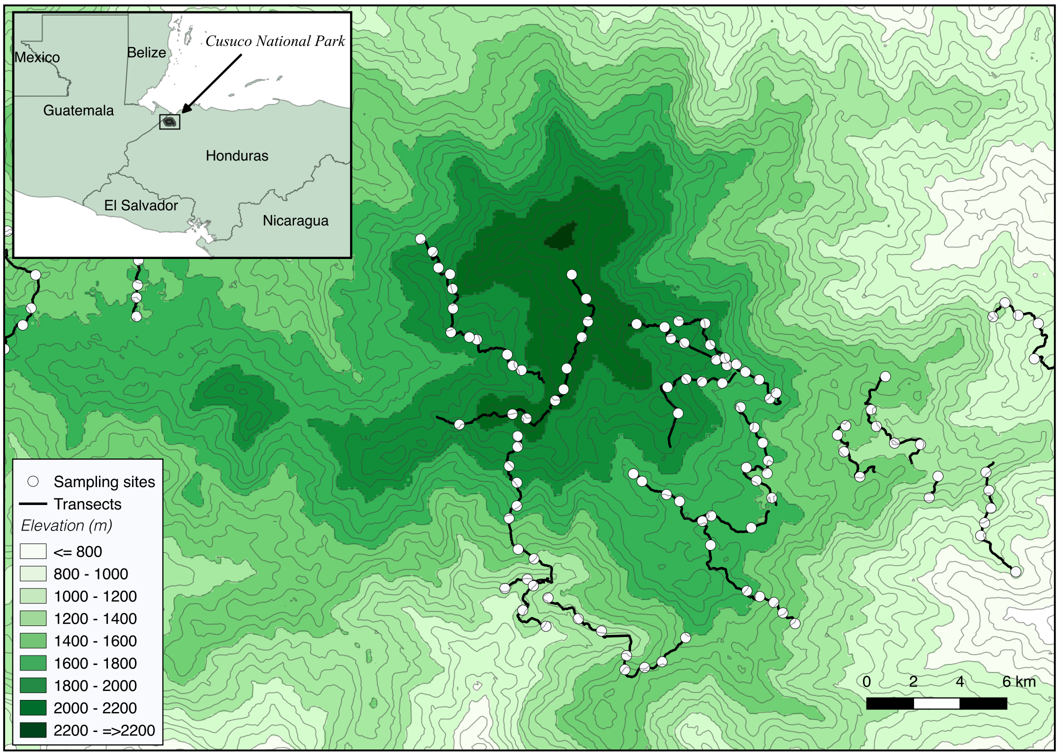

Figure 1.3 Cusuco National Park, Honduras, displaying elevational distributions of study transects and sampling sites. Some additional sampling sites on the western side of the park are not displayed.

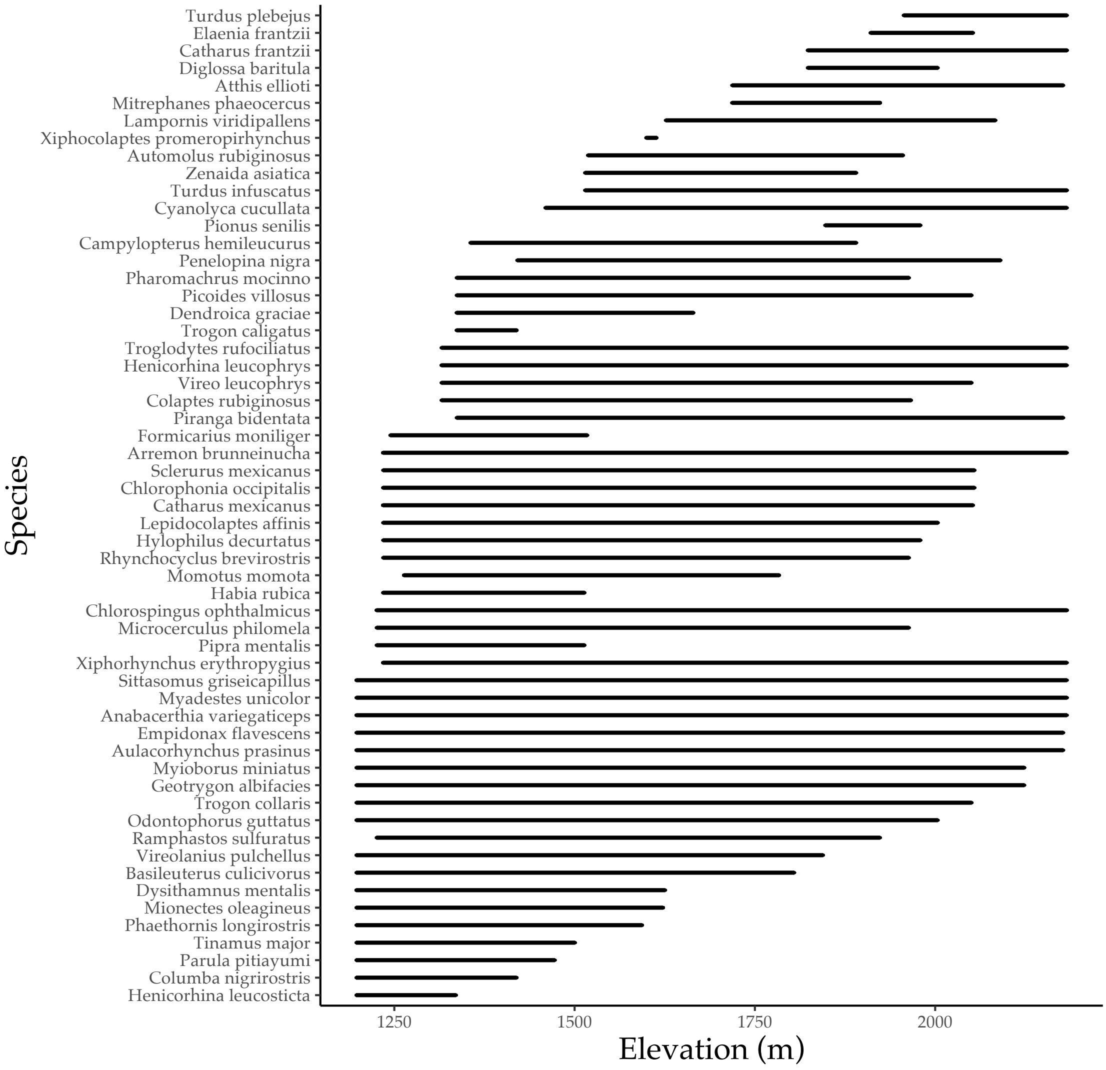

Figure 1.4 Approximate elevational ranges of a subset of 58 species in Cusuco National Park, Honduras across an elevational range of 1190-2183 m that typify elevation-species turnovers. Data presented are from unpublished surveys in 2016, where species with fewer than 5 records have been removed for purposes of presentation.

1.3.1 Ethical considerations for physiological measurements

During this study, birds were captured and handled extensively by use of mist-netting, frequently specifically targeting territorial birds with playback. Bird handling was done with extensive prior experience (British Trust for Ornithology 'C' ringing licence number (with mist-netting endorsement): C/5927). Any birds that showed undue stress on capture were released with no data taken. Because birds were captured both during the breeding season and retained overnight for physiological procedures throughout the course of this study, careful consideration was given to breeding birds. Female birds with edematous brood patches (indicative of nest tending) were not considered for overnight respirometry measures, but were measured for blood physiology. All birds that were captured for the respirometry system were captured within three hours of dusk, roosted overnight and released at the specific site of capture the following morning. Birds were kept in dark quiet conditions usually for part of the night within the respirometry chamber, but before/after respirometry sessions, in cloth bird bags. Birds were frequently kept overnight for in excess of twelve hours, however, this is in their natural resting circadian phase of the species, and at a time they are naturally roosting. Accordingly, birds were naturally fasting during this time.

For one aspect of this study, blood was sampled to measure total blood haemoglobin concentrations (g/dL). Blood was sampled following ethical procedures described by (Owen 2011), where the alar vein (on the underside of the wing) was pricked with a sterile single use hypodermic needle (26g, 5/8" BD Microlance, Ireland). After a small amount (~ 10uL) of blood was drained, light pressure was applied to the vein with a cotton wool while the wing was closed, with blood flow typically stemming after ~2minutes.

All fieldwork and experimental procedures were undertaken under permits issued annually to Operation Wallacea through the Instituto de Conservación Forestal (ICF) in Honduras, and with ethical approval from Royal Holloway University of London.

1.4 THESIS STRUCTURE

In this thesis I explore the interplay between physiology, behaviour and elevational ranges in cloud forest songbirds in Central America. I combine fine scale data on physiology and behaviour in order to explore two core themes. Firstly, I aim to assess factors that drive the elevational ranges of tropical montane songbirds and secondly, I aim to understand seasonal shifts in the physiology of montane songbirds with a particular focus on territorial behaviour in one species (BHNT). The specific details of this process are outlined below.

In **Chapter 2** I examine the nature of interspecific interactions between two species of parapatric Nightingale-Thrushes (RCNT and BHNT, respectively). Specifically, I test the nature of interspecific competition between the two species using controlled reciprocal playback experiments directed towards territorial males of each species. I then quantify habitat differences at the contact zone between the two species to examine whether habitat preferences may locate their elevational contact zone in combination with competitive interactions.

In **Chapter 3** I explore interspecific and intraspecific differences in the physiology of five species of tropical montane songbirds (RCNT, BHNT, GBWW, CCBF and COBT). I seek to explore whether species exhibit intraspecific seasonal variation in physiological traits (metabolic rates and blood haemoglobin concentrations). I also seek to establish whether interspecific differences occur dependent on species occupying different elevational ranges. I use open flow respirometry to measure metabolic rates against manipulated temperature to estimate thermoregulation and BMR. I also compare total blood haemoglobin concentrations. Finally, I compare BMR to scaling-exponents for tropical birds to place these results in the context of the physiology of tropical birds.

In **Chapter 4** I follow on from results in **Chapter 3**, specifically focussing on BHNT. I seek to explain the nature of seasonal differences in metabolic rates (as illustrated in the results of **Chapter 3**) by increases in territorial behaviour across seasons. Specifically, I use both behavioural focal watches and playback experiments to quantify behavioural differences between seasons. To assess whether a functional link exists between metabolic rates and territorial behaviour, I then utilise a novel experimental design of playback experiments on birds inside respirometry chambers in order to test the metabolic responses of exposure to conspecific song.

In **Chapter 5** I combine data collected in **Chapters 2** and **3**, as explanatory predictors of abundance against elevational ranges of four species of songbirds in order to test three prevalent hypotheses of thermoregulatory costs, habitat specialisation and species interactions in their role in defining the elevational ranges of species along tropical mountains.

In the **Introduction** and **Discussion** sections of this thesis I have adopted a first person singular writing style. However, given the collaborative nature of the data chapters as written in manuscript format, I use a combination of first person plural and passive voice for these sections.

Figure 1.5 Male Black-headed Nightingale-Thrush *Catharus mexicanus* from Cusuco National Park.

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2 WEAK ASYMMETRIC INTERSPECIFIC AGGRESSION AND DIVERGENT HABITAT PREFERENCES AT AN ELEVATIONAL CONTACT ZONE BETWEEN TROPICAL SONGBIRDS

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2.1 ABSTRACT

Closely related tropical bird species often occupy mutually exclusive elevational ranges, but the mechanisms generating and maintaining this pattern remain poorly understood. One hypothesis is that replacement species are segregated by interference competition (e.g. territorial aggression), but the extent to which competition combines with other key factors such as specialisation to distinct habitats remains little studied. Using vegetation surveys and reciprocal playback experiments, we explored the dynamics of interspecific aggression between two Nightingale-Thrushes (*Catharus* sp.) in Central America. We show that lower-elevation Black-headed Nightingale-Thrushes *Catharus mexicanus* are aggressive towards higher-elevation Ruddy-capped Nightingale-Thrushes *C. frantzii* where they meet at contact zones. However, interspecific aggressive responses were weak and unidirectional, and the two species were associated with different habitats. We conclude that the contact zone is maintained and located primarily by habitat selection, and likely reinforced by interspecific aggression. This has important implications for understanding how montane species will respond to climate change because the pace and extent of range shifts will not depend solely on habitat shifts or interspecific competition, but instead on interactions between these two factors.

2.2 INTRODUCTION

Tropical mountains offer unique opportunities to study the drivers of community composition owing to their exceptional biodiversity and rapid changes in environmental factors within relatively small spatial scales (Whittaker 1975, Jankowski *et al.* 2013a). Striking patterns of elevational parapatry – where related taxa have contiguous, but non-overlapping elevational distributions (Bull 1991) – have long been observed throughout the tropics (e.g. Wallace 1876). The elevational ranges of species on tropical mountains are often relatively narrow, with abrupt transitions from one population to another, creating a pattern of elevational parapatry, a rapid 'turnover' whereby a given species is replaced at higher or lower elevations by another ecologically similar congener (e.g. Terborgh 1971, Patterson *et al.* 1998, Pyrcz *et al.* 2009, Jankowski *et al.* 2009). Previous theoretical and empirical studies have offered multiple explanations for these spatial patterns, including interspecific competition, climatic niche partitioning, and divergent habitat preferences (e.g. Janzen 1967, Terborgh 1977, Jankowski *et al.* 2013a). However, the primary mechanisms maintaining elevational parapatry remain unresolved.

Two longstanding hypotheses for these contact zones (where the elevational range limits of two species abut) on tropical mountains are that they are maintained by abrupt habitat transitions (e.g. ecotones; Terborgh 1985) or interspecific competition (e.g. competetive exclusion; Diamond 1973). The ecotone hypothesis proposes that the limits of a species' elevational range are caused by rapidly changing habitat transitions on elevation gradients, and are therefore simply a reflection of divergent habitat specialisation among related species (Cadena & Loiselle 2007, Jankowski *et al.* 2009). By contrast, the competition hypothesis predicts that aggressive behavioural interactions (interference competition) between related species place limits on their elevational ranges by competitive exclusion (Price & Kirkpatrick 2009, Grether *et al.* 2017).

Specialisation to different habitats provides the most parsimonious explanation for the spatial segregation of species (e.g. Wiens 1989) and has been shown to correlate with the location of contact zones on some tropical elevation gradients (Jankowski *et al.* 2013b). On the other hand, interspecific competition is often inferred from non-overlapping range limits (e.g. Terborgh 1985), yet its role as a primary mechanism maintaining contact zones has been questioned (Cadena & Loiselle 2007, Sexton *et al.* 2009). Nonetheless, previous studies have suggested that interspecific competition may be widespread in tropical bird communities (Robinson & Terborgh 1995, Jankowski *et al.* 2012), and its influence on setting geographical range limits in tropical birds has gained increasing empirical support (Remsen & Graves 1995, Pigot & Tobias 2013, Jankowski *et al.* 2013a, Freeman 2015a, 2019, Grether *et al.* 2017).

Behavioural studies focusing on pairs of related bird species in tropical mountains have shown interspecific aggression at contact zones in Costa Rica (Jankowski *et al.* 2010), Ecuador (Dingle *et al.* 2010), Colombia (Caro *et al.* 2013), Malaysia (Boyce & Martin 2019) and New Guinea (Freeman *et al.* 2016). This is generally interpreted as evidence that interspecific competition maintains spatial parapatry of populations through aggressive territorial behaviour, a form of 'behavioural interference' (Grether *et al.* 2017). In addition, interspecific aggression may be reduced when small elevational gaps separate species ranges, suggesting that aggressive behaviours intensify with direct contact in some species (Freeman *et al.* 2016). While these studies have provided empirical evidence that interspecific competition may contribute to the pattern of species turnover (Terborgh & Weske 1975, Terborgh 1985), it remains unclear whether contact zones can be maintained by interspecific interactions alone, or in concert with other factors (Jankowski *et al.* 2013a, Srinivasan *et al.* 2018).

There are several ways that competition could interact with habitat differences. For example, habitat preferences may predict interspecific dominance relationships (or vice versa), such as when a dominant congener restricts the elevational range of a subordinate species by limiting it to a specific habitat (Grether *et al.* 2017). This type of asymmetric interspecific aggression between elevational replacement bird species appears to be widespread, often when (generally smaller) higher elevation species are subordinate to their (generally larger) lower elevation replacement (Jankowski *et al.* 2010, Dingle *et al.* 2010, Freeman *et al.* 2016, Boyce & Martin 2019). These asymmetric competitive interactions theoretically restrict space or resources available for the subordinate species (e.g. habitat or foraging niches; (Price & Kirkpatrick 2009, Martin *et al.* 2017). Aggressive interactions of this kind are thought to drive or maintain divergence in habitat preferences between species pairs in the tropical and temperate lowlands (Robinson & Terborgh 1995, Martin & Martin 2001a, b), but this possibility is rarely examined in montane systems.

To assess the role of interspecific aggression and habitat selection in maintaining an elevational contact zone, we conducted vegetation surveys and playback experiments in and near the territories of two species of Nightingale-Thrush *Catharus* sp. on an elevation gradient in Central America. The two species – Black-headed Nightingale-Thrush *Catharus mexicanus* and Ruddy-capped Nightingale-Thrush *C. frantzii* (hereafter BHNT/RCNT, respectively) – are a classic example of parapatric elevational distributions with very limited spatial overlap. We used vegetation surveys to quantify the extent of habitat divergence, and behavioural responses to interspecific song playback to assess the strength and directionality of interspecific aggression.

Our approach allowed us to evaluate the influence of both habitat preference and interference competition on elevational segregation in this study system. Understanding the relationship between these factors is important for interpreting mechanisms underlying the 'escalator to extinction', whereby climate-driven upslope movements result in species extirpations (Freeman *et al*. 2018) as a product of either lower-elevation behaviourally dominant species 'pushing' a subordinate higher (Grether *et al*. 2017), or the expansion/contraction of specialised habitat types (Fadrique *et al*. 2018).

2.3 METHODS

2.3.1 *Field site and study species*

Fieldwork was undertaken in Cusuco National Park in the Merendon mountains, north-western Honduras (approximately 15.552ºN, -88.296ºW). The national park encompasses an elevational range of *c*. 700–2300 m. General vegetation types range from moist broadleaf forest at lower elevations (up to 1200 m), pine-oak and tropical montane cloud forest at mid-upper elevations (*c*. 1200–2000 m) and elfin forest on mountaintops (above 2000 m; Batke *et al*. 2014, Martin *et al*. 2016).

Both species of Nightingale-Thrush have abutting/non-overlapping spatial ranges at the study site with territories of the two species frequently adjacent. Both species are common, strongly territorial year-round residents of the forest understorey, with RCNT ‘replacing’ BHNT at higher elevations. BHNT have a broader elevational range (down to at least 1000 m), while RCNT occur up to the highest points of the park (*c*. 2250 m). The exact elevation of the contact zone varies, but is generally at *c*. 1850m. BHNT (mean ± SD; 30.4g ± 1.87, $n = 242$, SEIJ unpublished data) are, on average, slightly heavier than RCNT (27.8g ± 1.59, $n = 32$, SEIJ unpublished data). Neither species has discernible differences in densities throughout their respective elevational ranges. They are closely related, but not sister species, evolving in separate clades that diverged *c*. 3.9 million years ago: BHNT in the *mexicanus* clade; RCNT in the *bicknell-occidentalis* clade (Voelker *et al*. 2013).

2.3.2 *Elevational range limits and territory mapping*

We established elevational range limits of both species using ad-hoc transects across an elevational range from 1225-2183 m in undisturbed primary forest. This was undertaken at two research camps at the field site where elevational range limits of the two species differed slightly (**Figure 2.1**). We then mapped the contact zone by marking locations of territorial males of both species using a GPS unit (Garmin GPS MAP 62s) to an accuracy of 3-9 m. Initially, we marked the locations of singing males with increasing distance from their contact zone over a 3-week period in early June 2016 between 0530-1200 (when territorial activity is strongest). This mapping exercise was repeated on a minimum of four days, following recommendations in Gregory *et al.* (2004). Singing males that were not present on all visits were considered non-territorial and no longer considered for playback experiments. Both species defend territories through continual song bouts from obvious locations and it is unlikely that locations of territories were inadvertently removed as a result of this protocol. In areas of high territorial density, we made careful observations of counter-singing between neighbouring birds to ensure that territories were discrete. We marked locations of each territory in their approximate centre after observations of the movements of the territorial holder. Subsequently, a distance (m) from the interspecific contact zone was measured in QGIS (QGIS Development Team 2016) as the distance from the centre of each territory to the closest heterospecific.

2.3.3 Habitat assessments

After territory mapping, we made a qualitative assessment of habitat types occupied by each species and mapped the approximate borders of these habitat transitions (**Figure 2.1**). While the edges of habitat transitions where approximated, they occur quickly (frequently < 50 m) and territories of each species are adjacent to one another. As such, within the immediate spatial range of our study, a transition zone of co-occurring territories does not exist. However, we classified habitats as transition habitat in cases where obvious forest types did not change rapidly over small spatial scales (generally where topography was not as steep; see **Figure 2.1**).

We quantified potential habitat differences using vegetation data (collected over the same study period) from 37 survey plots (used for REDD+ carbon assessments) situated along study transects covering the spatial and elevational extent of all territory locations (**Figure 2.1**). Habitat assessments were undertaken in a standardised 20 × 20 m square, bisected by two separate lines at 10 m, (forming four quarters of the larger 20 m^2 plot). The following attributes were measured within each plot; tree type, tree density, tree circumference, understorey density, sapling density, leaf-litter depth and soil density. Because habitat plots were at pre-existing locations and not always immediately within territories, in occasional ambiguous cases at the range edges we only ascribed habitat to a species where habitat was/was not being used by nearby territory holders (as informed by observations during territory mapping). Because our study was not undertaken across the entire breadth of each species' elevational range, we cannot rule out the possibility that habitat traits gradually change throughout their ranges. However, because the two species are parapatric, we were more interested in the possible habitat determinants that may immediately influence this (along with interspecific aggression).

Tree type, tree density and tree circumference were measured by recording all trees (defined as any woody plant with a circumference of ≥15 cm at 1.3 m height) within the overall 20 m^2 plot. Each tree was categorised to a crude species level of 'broadleaf', 'fern', 'pine', or 'palm'. Tree types for each survey plot were then calculated as a percentage of total trees present. Tree density was measured as total number of trees present in the plot and tree circumference was a mean of all tree circumferences within the plot. To measure understorey density, a 3 m pole, marked at 50 cm increments (forming 6 bands) was placed vertically at pre-marked 1m increments along the bisecting lines. At each 1 m increment along this line, the number of foliage 'touches' per 50 cm band on the vertical pole were recorded. This gave a score of six numbers (one per 50 cm band on the vertical pole) to each 1 m increment along the bisecting line which were summed. The mean of these 40 scores (20 on each bisecting line) then gave an overall underscore density 'score' for the plot as a whole, with higher scores representing denser forest understories and lower scores more open understorey.

Sapling density, leaf-litter depth and soil density were all measured in five 2 × 2 m quadrats. These 2 m^2 quadrats were placed in the centre of the quarters formed by the bisecting lines, and one in the central cross of the bisecting lines. Sapling density was measured by counting the number of woody saplings < 15 cm in circumference at 1.3 m height, in each of the quadrats. The aggregate sapling count for all five quadrats was then divided by 20 to give a density per m^2. Leaf litter depth was measured by pushing a steel ruler through the leaf-litter until it hit topsoil. The depth was then measured as the height of the highest dead leaf in each quadrat, and averaged across the five measures. Soil density was measured by dropping a standardised 1 kg spherical cone weight (cone end facing the soil) from a height of 1.5 m onto a patch of earth cleared of leaf litter. Soil depth was measured in mm from the topsoil to the centre (deepest part) of the depression (and averaged across the five measures).

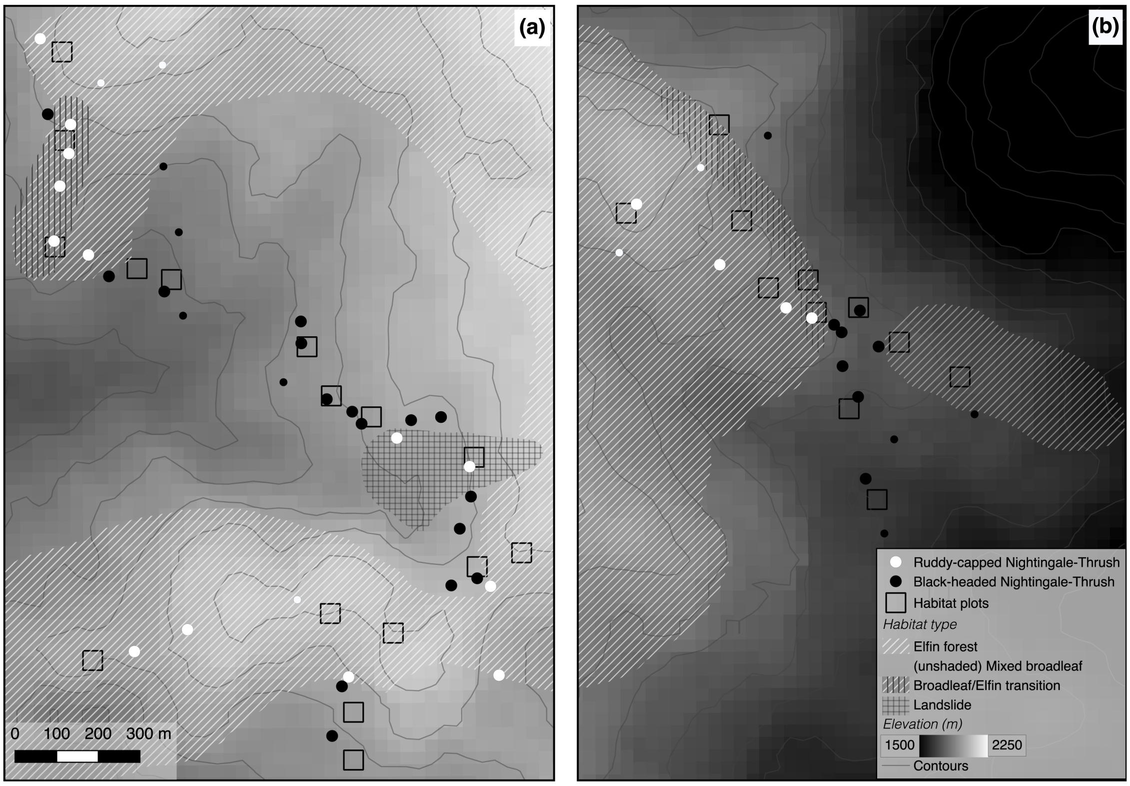

Figure 2.1 Territory locations in relation to habitat and elevation at two localities in Cusuco National Park, Honduras: (a) Cantiles camp; (b) Capuca camp. Larger points show whether playbacks were conducted on territorial Black-headed *Catharus mexicanus* or Ruddy-capped Nightingale-Thrushes *C. frantzii* and smaller points show additional territories of each species at which playback experiments were not conducted. Mapped habitat boundaries away from the immediate vicinity of habitat plots are approximated. Eight playback locations and 10 habitat plots were situated outside the areas shown.

2.3.4 Playback experiments

We conducted a total of 47 playback experiments (*n* = 29 BHNT; *n* = 18 RCNT) between June-August (2016), the time of year at which both species are breeding and territorial defence is at its strongest (Howell & Webb 1995, Taylor & Komar 2010). *Catharus* thrushes are strongly territorial, counter-singing against neighbouring territorial birds and chasing off intruders. Accordingly, playback experiments simulating territorial invasions by rival males are an appropriate test of territorial aggression (Jankowski *et al*. 2010, Freeman & Montgomery 2015).

We recorded natural territorial songs of BHNT (*n* = 14) and RCNT (*n* = 9) at the study site to use as experimental stimuli, using a directional microphone rig (Sennheiser ME66 microphone/Olympus LS-11 recorder). Recordings were free from prominent background song and occasionally edited (amplified and filtered) for clarity. Each recording was numbered arbitrarily and used in the experiments in numerical sequence to minimise pseudoreplication following recommendations by Kroodsma (1989). We recorded vocalisations of two other sympatric species, Slate-throated Whitestarts *Myioborus miniatus* (*n* = 5) and Collared Trogons *Trogon collaris* (*n* = 2), to use as control trials. These species were treated as controls because they are ecologically, vocally and phylogenetically divergent mid-storey foragers, which appear not to interact with Nightingale-Thrushes. The majority of these playback lures (recorded by SEIJ) have been archived on the online avian sound repository xeno-canto (recording set: https://www.xeno-canto.org/set/5584).

We began experiments by placing a wireless UE Roll speaker (hereafter 'speaker'), in the approximate centre of the focal territory at a height of *c*. 1m above the forest floor. The speaker was wirelessly paired to an mp3 player (iPod touch), which was controlled from a concealed position *c*. 10m away. Experiments were conducted during the peak of territorial activity both early (0600-1200 h) and late in the day (1500-1730 h). If a bird was engaged in a counter-singing bout with neighbouring territories on arrival to the focal territory, the experiment was postponed for at least two hours. No experiments were conducted during rain or windy conditions.

Each playback experiment comprised three separate 8 min trials. Each trial was made up of 3 mins of playback through the speaker, followed by 5 mins of observation time. The control trial was always conducted first, followed by conspecific and heterospecific trials which were alternated in sequence between experiments (per species) to avoid response bias dependent upon the preceding trial. We broadcast playbacks at a volume similar to that of natural vocalisations (between 78-84 decibels). We measured five behavioural responses to quantify territorial aggression to each trial; closest approach to the speaker (m), latency to approach the speaker (s), time spent within 15 m of the speaker, latency to vocalise within 15 m of the speaker and number of vocalisations within 15 m of the speaker. The 15 m area surrounding the speaker represents the greatest distance it was feasible to observe the focal bird in the forest understorey, and ensured that all responses were indeed in response to the experimental trial.

We measured close approach to the speaker between 0 (where the responding bird stood on the speaker) and 15 m, where all approaches greater than 15 m were bounded to this upper limit. We estimated approach distances visually during trials and paced out distances from the speaker after the experiment ended to check accuracy. We measured latency to approach the speaker as time elapsed between the initiation of the playback trial to the first approach of the focal bird within 15 m of the speaker. We measured latency to vocalise as the time elapsed before the first vocalisations (calls or songs) made by the focal individual within 15 m of the speaker, with the number of vocalisations a sum of all songs and calls given by the focal individual also within this radius. Finally, we measured time spent within 15 m of the speaker as the elapsed period between first arrival and leaving this boundary (there were no incidences of responding birds leaving and re-returning). The radius surrounding the speaker was used to bound territorial responses because we wanted to ensure measured behaviours were genuine aggressive responses to the trial in question, rather than just bouts of spontaneous territorial song. Both species are vocal for extended periods during the breeding season and in pilot studies we found unbounded vocal responses artificially inflated aggressive responses to playbacks. One experiment was cancelled when both *Catharus* species responded to a playback trial as it became impossible to distinguish the cause of behavioural responses.

2.3.5 Statistical Analysis

We conducted all analyses in 'R' (R Core Team 2016). We first compared structural habitat variables (log-transformed where appropriate) between approximate habitat types with one-way ANOVA. Then, to interpret behavioural responses to playback trials, we collapsed observed responses into a single composite aggression score using a principal component analysis (PCA). Because different measurement units were used to measure these behavioural responses (metres and seconds respectively), we scaled variables using the argument scale = TRUE in the *prcomp* function. The first axis (PC1) explained the majority of the overall variance (84%; PC1 loadings: close approach to speaker = -0.45; latency to approach speaker = -0.46; time in recording area = 0.46; latency to vocalise in the recording area = -0.44; number of vocalisations in the recording area = 0.41). High PC1 scores were typified by closer and faster approaches, greater time spent in the proximity of the speaker and quicker and more extensive vocal responses in the proximity of the speaker. Accordingly, PC1 loadings per trial were considered to be a representative composite aggression score and we subsequently used these values as the response variable for statistical analyses.

Using this response variable, we constructed a linear mixed-effects model using the *lme4* package (Bates *et al.* 2014). Two fixed effects were specified; an interaction term between trial type and distance from contact zone (where trial type has six levels: control, conspecific and heterospecific responses for both species) and trial order (whether conspecific lures or heterospecific lures were played first in the experiment). Including the interaction between 'trial type' and distance is statistically equivalent to including species, trial and a species × trial interaction, but allows for a clearer examination of the model parameters (see Freeman & Montgomery 2015, Freeman *et al.* 2016). We log-transformed distance from contact zone to normalise the data distribution and to model the reduction in aggressive responses to heterospecifics with increasing distance from the contact zone. We included focal bird identity (of the territory holder) as a random effect because of the repeated measures each individual received during playback experiments (three treatments). We defined significant cases of interspecific aggression as those in which the confidence intervals (CI) for control trials and heterospecific trials were non-overlapping in close proximity to the contact zone. Additionally, if aggressive responses to heterospecifics are learned from experience, we expected the strength of response to decline with distance from the contact zone (Jankowski *et al*. 2010). While the extent to which responses are learned is not relevant to understanding elevational range limits, a learned response indicates that aggression towards heterospecific playback is not merely misdirected behaviour intended for conspecific receivers (Murray 1971). Finally, to assess whether territorial responses were influenced by the order in which heterospecific and conspecific treatments were presented during playback experiments, we used Wald-Chi square tests in the *car* package (Fox *et al*. 2013) to test for an effect of the 'trial order' term on the strength of responses.

2.4 RESULTS

2.4.1 Habitat assessments at the contact zone

We found territories of RCNT in three approximate habitat types. The majority were situated in elfin/dwarf forest typified by stunted trees, and an abundance of tree ferns (Cyatheales) and mosses (Bryophyta). Additionally, some territories were situated in a transition habitat at the edge of elfin forest with bamboo stands (Bambusoideaea), and an area of successional scrub and *Pinus* (Pinaceae) in a landslide. By contrast, BHNT exclusively occupied mixed broadleaf cloud forest typified by *Quercus* (Fagaceae), *Liquidambar* (Altingiaceae) and occasional *Pinus* (Pinaceae). The contact zone was at 1890-1964 m at one study site and 1811-1835 m at another, but in both areas the two species consistently appeared to occupy different habitats (**Figures 2.1/2.2**).

We found relatively little difference in the structural habitat attributes between habitat types except for soil and tree densities (**Table 2.1**). Soil density differences were likely driven by the complete removal of topsoil at the landslide site. Differences in tree types were more substantial, with habitats occupied by RCNT being dominated by smaller trees, primarily ferns or a mosaic of ferns/broadleaf trees (**Tables 2.1 / 2.2**).

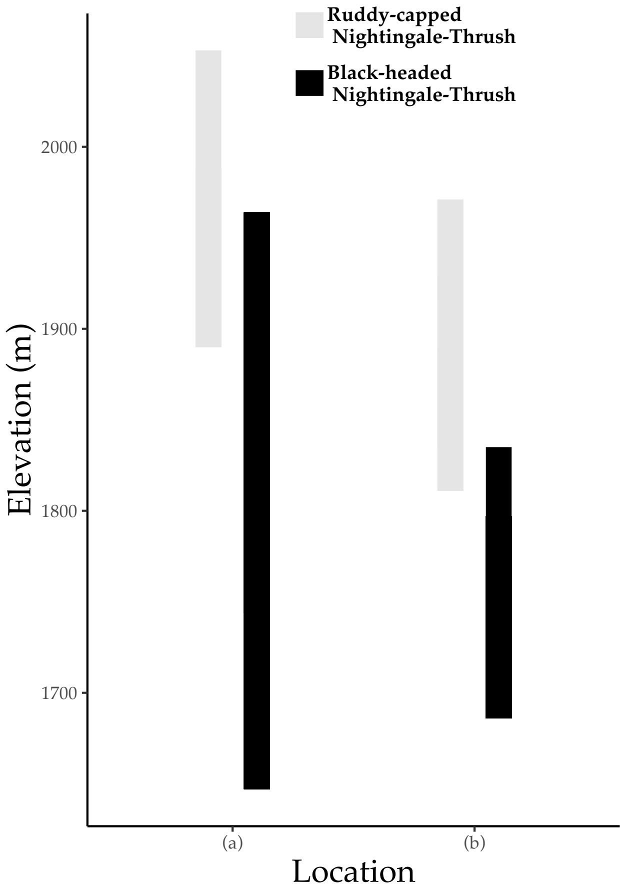

Figure 2.2 Elevational range limits of Ruddy-capped Nightingale-Thrushes *C. frantzii* (RCNT) and Black-headed Nightingale-Thrushes *C. mexicanus* (BHNT) at two localities in Cusuco National Park, Honduras (**Figure 2.1**): (a) Cantiles camp; (b) Capuca camp. Plotted elevations represent the territories mapped at two research camps and do not represent the entire elevational ranges of both species (see Methods). BHNT extend to lower elevations (*c.* 1000 m) whereas RCNT extend to higher elevations (*c.* 2300 m).

Table 2.1 Microhabitat characteristics of habitat transitions between species. Values are mean ± SD of values per plot. Numbers in brackets for habitat type and tree density/circumference represent number of habitat plots and total number of trees within habitat type, respectively. Test statistic in the final column refers to ANOVA results, with statistically significant results in bold. Soil density differences were likely driven by the complete removal of topsoil at the landslide site.

	Black-headed Nightingale-Thrush *Catharus mexicanus*	**Ruddy-capped Nightingale-Thrush** *Catharus frantzii*			
	Mixed broadleaf cloud-forest (18)	Elfin/dwarf forest (13)	Broadleaf–elfin forest transition (5)	Landslide/ succession scrub (1)	Test statistic
Tree density (20m^2)	116.0 ± 35.1 (2088)	151.1 ± 78.8 (1964)	124.0 ± 131.1 (620)	29.01 (29)	$F_{3,33} = 2.97$, $p = 0.05$
Tree circumference (cm)	49.0 ± 12.4 (2088)	41.4 ± 5.9 (1964)	44.5 ± 8.8 (620)	45.9 (29)	$F_{3,33} = 1.35$, $p = 0.28$
Understorey density (touches per m)	1.3 ± 0.4	1.5 ± 1.1	1.2 ± 0.6	1.55	$F_{3,33} = 0.27$, $p = 0.85$
Sapling density (m^2)	2.0 ± 1.0	1.4 ± 1.1	1.2 ± 1.2	1.9	$F_{3,33} = 1.24$, $p = 0.31$
Leaf-litter depth (mm)	40.0 ± 16.3	44.6 ± 15.8	54.0 ± 36.7	27.8	$F_{3,33} = 0.88$, $p = 0.46$
Soil density (mm)	29.9 ± 7.5	30.0 ± 9.1	25.3 ± 14.2	2.4	$F_{3,33} = 3.2$, $p = \mathbf{0.04}$

Table 2.2 Proportions of different tree types between habitat transitions occupied by the study species. Figures shown in brackets after habitat categories are sample sizes of habitat plots. Data shown are means (± SD) calculated across plots. Test statistic in the final column refers to ANOVA results, with statistically significant results in bold. NA indicates data were absent or insufficient for statistical analyses.

	Black-headed Nightingale-Thrush *Catharus mexicanus*	**Ruddy-capped Nightingale-Thrush** *Catharus frantzii*			
% tree type	Mixed broadleaf cloud-forest (18)	Elfin/dwarf forest (13)	Broadleaf–elfin forest transition (5)	Landslide/ scrubby regrowth (1)	Test statistic
Broadleaf	75.7 ± 16.0	50.1 ± 20.9	55.2 ± 24.3	6.9	$F_{3,33} = 7.67$, $p =$ **<0.01**
Fern	20.4 ± 16.8	50.2 ± 13.2	43.1 ± 22.7	3.4	$F_{3,33} = 9.31$, $p =$ **<0.01**
Palm	5.7 ± 4.0	5.8 ± 5.6	3.6 ± 3.5	NA	$F_{3,33} = 0.2$, $p = 0.82$
Pine	<0.01	<0.01	<0.01	89.7	NA

2.4.2 Playback experiments

Both species responded strongly to conspecific playback, generally approaching the speaker quickly, closely, and remaining within its vicinity for protracted periods while singing in response. Similarly, neither species responded aggressively to control playbacks, with only a single BHNT responding to this trial across all experiments conducted (also indicating the control stimuli species had no influence on subsequent responses; ANOVA, $F_{1,139} = 0.04$, $p = 0.84$). Aggression directed towards heterospecifics differed between species, however, where the lower elevation BHNT were territorially aggressive towards the higher elevation RCNT, but not vice versa (**Figure 2.3**). This interaction was limited to a small number of individuals (31% of BHNT experiments, $n = 9$), all but two of which were ≤ 141 m to the contact zone (rounded up here to < 150 m for the purpose of interspecific comparisons). In these instances, behavioural responses by BHNT were often as strong towards heterospecifics as to conspecifics, although numerous individuals in close proximity to RCNT did not exhibit any interspecific aggression; of 18 trials < 150 m of the contact zone, 11 (60%) did not respond to heterospecifics (**Figure 2.3**). Response rates of RCNT were even lower; only a single heterospecific trial (5.5% of 18 experiments) elicited a response from a territorial individual.

We found that 'trial order' did not have a significant effect on playback responses ($\chi^2_1 = 20.09$, $p = 0.15$), so we examined the full model for parameter estimates (**Table 2.3**). At the contact zone, the aggression responses to conspecific and heterospecific playback trials were significantly different to controls for BHNT, but were only significantly different for conspecific trials for RCNT (**Figure 2.3**). Thus, responses to heterospecifics by BHNT in close proximity to the contact zone were still significantly different to control trials, despite numerous non-responding individuals. Aggression towards heterospecific playback in BHNT declined rapidly with distance from parapatry (95% CI for the slope -1.08 / -0.11). By contrast, aggression directed towards conspecifics in both species did not differ with proximity to the edge of their own elevational ranges (95% CI for the slope: BHNT -0.17 / 0.80; RCNT -0.87 / 0.20; **Table 2.3 / Figure 2.3**).

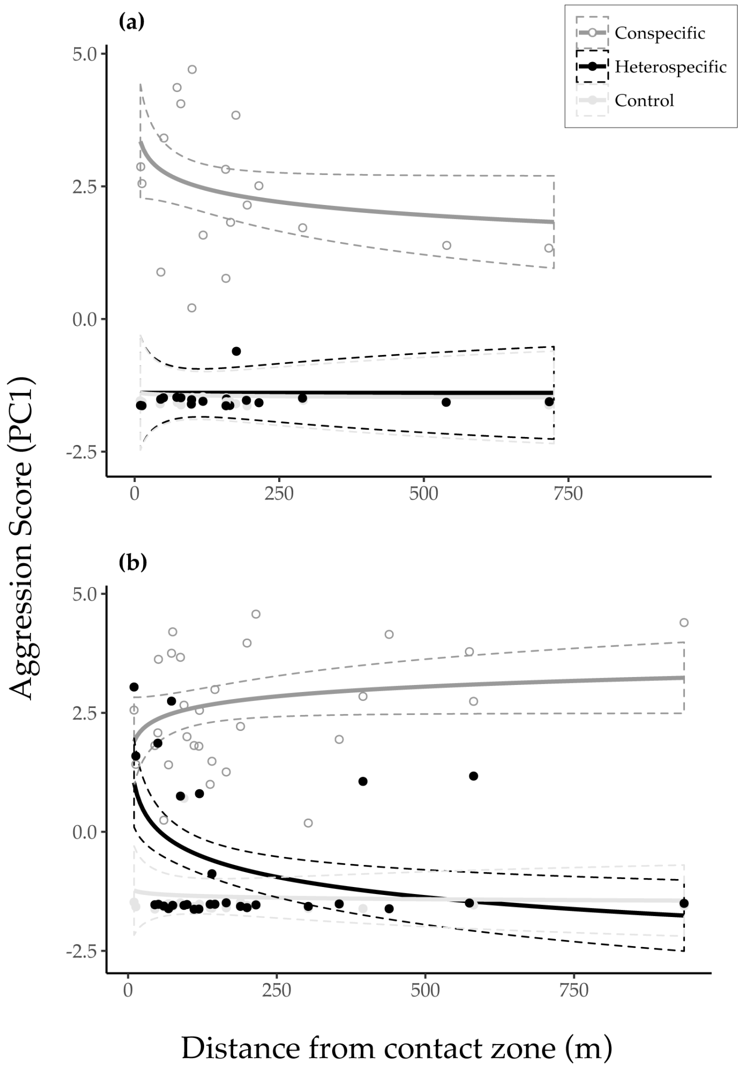

Figure 2.3 Territorial aggression (PC1 loadings) estimated from playback trials as a function of distance (over ground) from the contact zone in (a) Ruddy-capped Nightingale-Thrushes *Catharus frantzii* (RCNT) and (b) Black-headed Nightingale-Thrushes *C. mexicanus* (BHNT). Data points show responses to playback experiments of conspecific, heterospecific and control treatments (higher PC1 scores denote stronger aggression). Solid lines show GLMM model fits (log-transformed); dashed lines show confidence intervals (see Methods). The overlapping confidence intervals for control and heterospecific trials in RCNT illustrate the lack of interspecific aggression observed in experiments on this species (compare with BHNT).

Table 2.3 Parameter estimates for fixed effects from a linear mixed model of aggression responses to playback experiments for Black-headed Nightingale-Thrushes *Catharus mexicanus* and Ruddy-capped Nightingale-Thrushes *C. frantzii*. 'Intercept' represents the Ruddy-capped Nightingale-Thrush control parameter estimate. Trial type (control/conspecific/heterospecific per species) × distance to contact zone estimates (log) are approximate slope estimates for the interaction terms. Significant terms in the model are emboldened.

Parameter	**Estimate**	**SE**	**95% CI (2 d.p)**
(Intercept)	-1.34	0.99	-3.52/0.62
Ruddy-capped Nightingale-Thrush conspecific	**5.51**	**1.35**	**2.94/8.19**
Ruddy-capped Nightingale-Thrush heterospecific	-0.04	1.35	-2.60/2.52
Black-headed Nightingale-Thrush control	0.22	1.29	-2.15/2.60
Black-headed Nightingale-Thrush conspecific	**2.56**	**1.29**	**0.19/4.94**
Black-headed Nightingale-Thrush heterospecific	**3.79**	**1.29**	**1.43/6.17**
Distance to contact zone	-0.19	0. 21	-0.40/0.36
Playback trial order	-0.24	0.16	-0.54/0.05
Ruddy-capped Nightingale-Thrush conspecific × Distance to contact zone	-0.33	0.28	-0.87/0.20
Ruddy-capped Nightingale-Thrush heterospecific × Distance to contact zone	0.01	0.28	-0.51/0.55
Black-headed Nightingale-Thrush control × Distance to contact zone	-0.02	0.26	-0.51/0.46
Black-headed Nightingale-Thrush conspecific × Distance to contact zone	0.31	0.26	-0.17/0.80
Black-headed Nightingale-Thrush heterospecific × Distance to contact zone	**-0.59**	**0.26**	**-1.08/-0.11**

2.5 DISCUSSION

Our results demonstrate asymmetric interspecific aggression between two Nightingale-Thrush species, showing that lower elevation BHNT were dominant over RCNT, their higher elevation congener. The intensity of interspecific interaction was relatively weak, with many individuals present near the contact zone behaving non-aggressively (to heterospecifics). Using playback of heterospecific song, we also showed that the strength of interspecific responses declined rapidly with distance from the contact zone, suggesting that aggressive behaviour reflected a learned response to heterospecifics rather than misdirected aggression intended for conspecifics (Murray 1971). The elevational contact zone of the two species was also strongly linked to habitat differences, suggesting that their elevational ranges are segregated largely by habitat selection, perhaps in combination with interference competition.

Our results broadly mirror patterns observed in previous studies, which overall have identified eight of 13 species-pairs of tropical songbirds with elevational asymmetries in aggressive dominance, with higher elevation species being subordinate (Jankowski *et al.* 2010, Dingle *et al.* 2010, Caro *et al.* 2013, Freeman *et al.* 2016, Boyce & Martin 2019). The same pattern is consistent with other *Catharus* thrushes in temperate (Freeman & Montgomery 2015) and tropical (Jankowski *et al.* 2010) regions. While these findings indicate that aggressive interactions may play a general role in maintaining elevational range boundaries, the prevalence of asymmetric interactions suggests that they are unlikely to provide a full explanation for elevational parapatry. Evidently, dominant competitors either preferentially do not, or are unable to, occupy the elevational range of a subordinate species.

Some interspecific interactions between related bird species appear to be symmetrical, without any obvious dominance asymmetry (Tobias & Seddon 2009). However, most appear to be asymmetric, with a dominant species using and defending a preferred resource that may in turn limit the spatial distribution or niche breadth of a subordinate (Amarasekare 2002, Martin *et al.* 2017). Finer scale niche shifts in birds can occur when a subordinate species is freed from competition with a dominant one, and thus occupies a wider niche through ecological release (e.g. Alatalo *et al.* 1985, Martin & Martin 2001b). Experimental evidence for the expansion of elevational ranges through ecological release is lacking in birds, but has been shown in other tropical montane vertebrates (Pasch *et al*. 2013). Nonetheless, natural observations of range expansions in the absence of competitors has been inferred as evidence for the role of overt competitive interactions limiting elevational ranges of tropical birds (see Terborgh & Weske 1975). In our field site, neither Nightingale-Thrush species has been found to hold permanent territories outside their native elevational/habitat boundaries over a six-year period. Occasionally, solitary male BHNT have been found singing at higher elevations in elfin-forest, but this has never resulted in the establishment of permanent territories. Thus, it appears likely that habitats at higher elevations may be suitable for RCNT but not for BHNT.

It is not immediately clear what drives these habitat preferences. The most obvious difference in habitat was tree type and tree density characteristics. Potentially important factors such as diet and nesting requirements of both species appear to be similar (Howell & Webb 1995), although no detailed studies have been undertaken. RCNT can occupy primary and disturbed montane forest types (Rangel-Salazar *et al*. 2008a), though breeding performance is linked to these habitat types with greater breeding success resulting in higher population density in primary forest (Rangel-Salazar *et al*. 2008b). It is possible that asymmetries in foraging and breeding success drive divergent habitat preferences in our study species, particularly for BHNT, which appear to breed exclusively in broadleaf-dominated cloud-forest. Comparative ecological studies are needed to clarify the role of ecological specialisation, and associated fitness differences, in maintaining discrete elevational ranges or habitat niches in this system.

It is debatable whether non-overlapping spatial distributions can be maintained by interspecific aggression alone (see Martin & Martin 2001b). Even in cases of extremely strong aggression between congeners, there is usually an ecological component to segregation (Tobias & Seddon 2009). In multiple experimental trials, we found that BHNT were unresponsive to playback of heterospecific territorial song despite close proximity to the contact zone, suggesting that behavioural interference is relatively weak and perhaps unlikely to explain divergence in habitat or elevation. More rigorous examination via removal experiments may clarify this (e.g. Pasch *et al*. 2013). In addition, BHNT show substantially reduced levels of territorial aggression in the non-breeding season (see **Chapter 4**) and year-round study would clarify whether the spatial extent of co-occurrence varies by season.

Another possible mechanism for elevational parapatry in tropical mountains is physiological specialism to elevation-specific microclimates (Janzen *et al.* 1967). While it is unlikely that our study species are limited by microclimates, their elevational ranges may represent thermal conditions that are preferentially selected owing to aspects of their physiology (e.g. at different life-stages; Jankowski *et al.* 2013a). Evidence in support of elevational range restriction to thermal niches in birds is generally lacking (Freeman 2015b, Londoño *et al.* 2017), but thermal preferences in two parapatric species of Singing Mice (*Scotinomys* sp.) segregated by interspecific aggression has been reported (Pasch *et al.* 2013). Further research is needed to assess whether physiological differences can contribute to the maintenance of elevational parapatry in tropical birds.

From the perspective of environmental change, understanding the nature of interspecific interactions and habitat preferences has potentially important implications (Grether *et al.* 2017). In Cusuco NP, BHNT have shown significant upslope shifts in their mean elevational range over the past decade (Neate-Clegg *et al.* 2018). On one hand, this upslope shift may mean the dominant BHNT gradually encroach higher elevation habitats resulting in an ever-decreasing spatial range for RCNT, possibly resulting in extirpation (e.g. Freeman *et al.* 2018). On the other hand, boundaries between habitats may represent a hard limit for permanent territorial residency of BHNT and—because habitats shift relatively slowly in response to climate (Jankowski *et al.* 2013b)—potentially buffer RCNT against a gradual 'escalator to extinction' by delaying upslope shifts of the dominant congener . Whether habitat boundaries have the ability to 'buffer' these upslope shifts, however, depends on the extent to which other potentially key factors driving habitat selection are also affected by climate. For example, concurrent shifts in arthropod and bird communities have been documented on the same elevation gradients (Chen *et al.* 2009, Harris *et al.* 2012). As such, understanding the role of a variety of range determinants in combination with species interactions is necessary. This is particularly the case when considering variability in interactions measured in other studies (e.g. where a high elevation species is dominant, or no interaction is present) because an upslope shift doesn't necessarily mean a dominant species is ‘pushing’ another upslope (e.g. Barve & Dhondt 2017, Boyce & Martin 2019).

Our study shows relatively weak asymmetric interspecific aggression between two tropical Nightingale-Thrushes with elevational ranges segregated by habitat type. Structural habitat differences at the contact zone suggest that divergent ecological niches rather than interference competition are the primary drivers of spatial segregation in this system. However, the aggressive defence of territories against heterospecifics also suggests that overt interactions may help to sharpen and maintain elevational range limits, particularly in areas where the habitat transition is less abrupt. Taken together, these findings support the view that biotic interactions work in conjunction with other factors to maintain the elevational ranges of species (Jankowski *et al.* 2013a, Srinivasan *et al.* 2018) and, further, that range limits between competing species may be set in ecological and evolutionary time because higher fitness within a given species niche prevents successful exploitation of one directly adjacent (Price & Kirkpatrick 2009). Further studies are needed to explore the relative roles of ecological niches, behavioural interference and competitive dominance in setting elevational range limits, and governing their response to climatic change.

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3 COMPARATIVE METABOLIC RATES AND HAEMOGLOBIN CONCENTRATIONS OF FIVE TROPICAL MONTANE SONGBIRDS; DIFFERENTIAL SEASONAL CHANGES AND COLD ADAPTION

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3.1 ABSTRACT

The physiology of tropical birds is poorly understood in how it relates to variable climates within sites and across seasons. This is particularly true of tropical montane species, which may have sensitive thermal tolerances to local microclimates. We studied metabolic rates, body mass and haemoglobin concentrations of five species of resident montane songbirds between the summer and winter at two elevations (1550 m and 1950 m, respectively) to ask whether there were uniform seasonal shifts in physiological traits across species, and whether there was evidence for cold tolerance in higher elevation species. We found shifts between season differed between species, where basal metabolic rates in one species- Black-headed Nightingale-Thrushes *Catharus mexicanus*- were up-regulated in summer (~19% increase of winter metabolism) but others (for which data existed) displayed no seasonal shifts. Haemoglobin concentrations were also uniform across season for all species, and body mass in two species was marginally higher in the summer. Physiological traits were distinctly different in the highest elevation species, Ruddy-capped Nightingale-Thrushes *Catharus frantzii* (although only summer data were available) indicative of cold tolerance. In this species, metabolism was constant across the range of temperatures tested (5-34ºC) and haemoglobin concentrations were higher than all other species. These results suggest that seasonal shifts in metabolic rates were highly variable between species and not related to environmental temperature changes. Further, while the higher elevation species displayed cold tolerance traits, it is unlikely this relates to present day temperatures. Taken together this offers intriguing insights into the variability of physiological shifts between species, and possible conserved physiological traits as a result of historic isolation.

3.2 INTRODUCTION

Physiological adaption, where the physiological characteristics of a species are shaped by local climate, is intrinsic to an ability to survive in changeable environments (Chown *et al.* 2004, Bozinovic *et al.* 2011). In birds, non-migratory species are frequently used to investigate such physiological adaptions, due to the temperature extremes they typically experience throughout the duration of the annual cycle (McKechnie 2007, Swanson 2010). Physiological responses to cold winter temperatures in particular have been displayed in a variety of metabolic traits in temperate species, such as winter increases in basal and peak metabolic rates (BMR/PMR; the lower and upper limits of metabolic power output, respectively) and body mass (M_b), reflecting the increased energetic demands of maintaining high internal body temperatures (McKechnie 2007, McKechnie & Swanson 2010, Smit & McKechnie 2010, McKechnie *et al.* 2015). However, while the patterns of physiological adaptions to cooler climates are relatively well established in high latitude birds (Swanson 2010), much less is known outside of these regions, particularly in tropical latitudes.

How avian physiology relates to the environmental conditions in tropical latitudes is poorly understood, and is largely restricted to studies in the warmer tropical lowlands (Pollock *et al.* 2019). Tropical birds have lower metabolic rates compared to temperate species (Wiersma *et al.* 2007, Londoño *et al.* 2015, Bushuev *et al.* 2017), suggested to be an ecophysiological adaption to the warmer and more seasonally stable climates across the tropics, in contrast to the cooler and more seasonally changeable conditions at temperate latitudes (White *et al.* 2007, Jetz *et al.* 2008, Khaliq *et al.* 2014). For example, tropical species typically have narrower thermo-neutral zones (TNZ; defined by the upper and lower temperature bounds at which a species begins thermoregulation), than temperate species as an adaption to warmer climates (e.g. Khaliq *et al.* 2015). Whether the physiological differences of tropical birds are a result of lower climatic variability has subsequently been challenged, however, when a recent study comparing species across elevation in the Peruvian Andes indicated that BMR of species inhabiting the highlands were comparable to lowland species (Londoño *et al.* 2015, 2017). This is despite the cooler and more variable environmental conditions at higher elevations (Ghalambor *et al.* 2006), suggesting that metabolic rates of highland species were not directly related to local temperatures.

That the physiology of tropical bird species is unrelated to environmental temperature has garnered further support from two recent studies investigating seasonal variation in the metabolic rates of lowland tropical species (McKechnie *et al.* 2015, Pollock *et al.* 2019). Both of these studies found that BMR, PMR, and thermoregulatory traits were considerably variable between the summer and winter months in lowland Panamanian rainforest species (Wells & Schaeffer 2012, Pollock *et al.* 2019). The direction of this variability was not consistent between species, however, with some species displaying no seasonal changes in BMR or thermoregulatory traits, while other species either increased or decreased these traits in the winter, despite the lack of seasonal temperature fluctuations (Pollock *et al.* 2019). Similarly, Wells & Schaeffer (2012) found tropical birds to decrease PMR in the winter months, the opposite compared to temperate species. Taken together, studies determining physiology across season and elevation suggest that the ecophysiology of tropical species is largely unrelated to ambient environmental temperatures. However, the ubiquity of this hypothesis remains unclear, due to the few comparative physiological studies undertaken on tropical montane species.

Tropical mountains offer valuable case studies to study the physiology of birds because they allow assessments of whether physiological traits of resident species relate to variable climates (Londoño *et al.* 2015). Increases in elevation along tropical mountains are typified by sharp changes in temperature isotherms and it has been historically hypothesised that tropical montane species have evolved particularly distinct physiological tolerances unique to their elevational distributions (Janzen 1967, Ghalambor *et al.* 2006). Thus, if tropical montane species are physiologically sensitive, the physiological traits of sedentary species may also reflect changes in seasonal conditions. To explore these notions further, we assessed evidence for whether cooler environmental temperatures at higher elevations in tropical mountains manifest in physiological cold-tolerance traits for resident species, and whether species displayed uniform shifts between season.

We addressed these hypotheses by comparing metabolic rates (both BMR and thermoregulation against manipulated temperatures), M_b, and total blood haemoglobin concentrations (H_b) of five sedentary songbird species in a cloud forest in Central America. BMR is a widely used measure in avian physiology, reflecting the lowest energetic requirements for homeostasis, while thermoregulation reflects the metabolic responses to changing temperatures (Lighton 2008). In addition, M_b and H_b reflect changes in condition related to both oxidative stress (H_b) and cold tolerance (Swanson 2010, Labocha & Hayes 2011, Minias 2015) allowing us to assess whether any observed changes in these parameters were also related to changes in condition. We asked whether physiological parameters changed in response to cooler winter conditions (non-breeding season) by increased BMR, M_b and H_b, and whether any changes were consistent between species. Additionally, we asked whether a higher elevation species displayed physiological differences attributable to colder higher elevation conditions (higher H_b content, increased BMR and lower temperature limits to the TNZ).

3.3 METHODS

3.3.1 *Study site and focal species*

We measured BMR, M_b, H_b and thermoregulation in Black-headed Nightingale-Thrushes *Catharus mexicanus,* Ruddy-capped Nightingale-Thrushes *Catharus frantzii,* Chestnut-capped Brushfinches *Arremon brunneinucha,* Grey-breasted Wood Wrens *Henicorhina leucophrys,* and H_b and M_b only in Common Bush Tanagers *Chlorospingus flavopectus* (hereafter, BHNT, RCNT, CCBF, GBWW and COBT, respectively) in Cusuco National Park, north-western Honduras (approximately N15.552, E - 88.296). Fieldwork was undertaken between June-August, 2017/18 and January 2018.

The majority of species breed during the warmer months of June-August (summer) at the field site (see Howell & Webb 1995), while January (winter) is typically cooler (**Figure 3.1).** We measured environmental temperature (T_e; ºC) at two research camps (1550m and 1950m, respectively- see below) using remote loggers (HOBO UA-001-64, Onset, USA, data pooled for 2-3 per camp) deployed for the entirety of the study period, in order to capture seasonal and elevational variation. Loggers were attached to trees ~1m off the forest floor to best represent the T_e experienced by the study species. Mean T_e (ºC ± SD) during the summer at 1550m was 17.4 ± 1.7 (max = 24.7, min = 12.2), and at 1950m was 15.5 ± 1.4 (max = 22, min = 11.2). During the winter, $T_{e,}$ at 1550m was 13.2 ± 2.2 (max = 21.2, min = 7.8), and at 1950m was 11.3 ± 2.11 (max = 16.3, min = 5.24) (**Figure 3.1**).

We studied birds at two research camps, at 1550 m and 1950 m, but because of logistical difficulties, all winter fieldwork was undertaken at the lower elevation camp. Each species, except RCNT (which occurs only at higher elevations – see **Chapter 2**), occur at both sites. Accordingly, we only obtained summer data on RCNT. None of the study species undertake any known seasonal movements across elevation at the study site (as determined by both consistent captures/re-sightings of banded birds at their site of original capture, and/or year-round territory occupation).

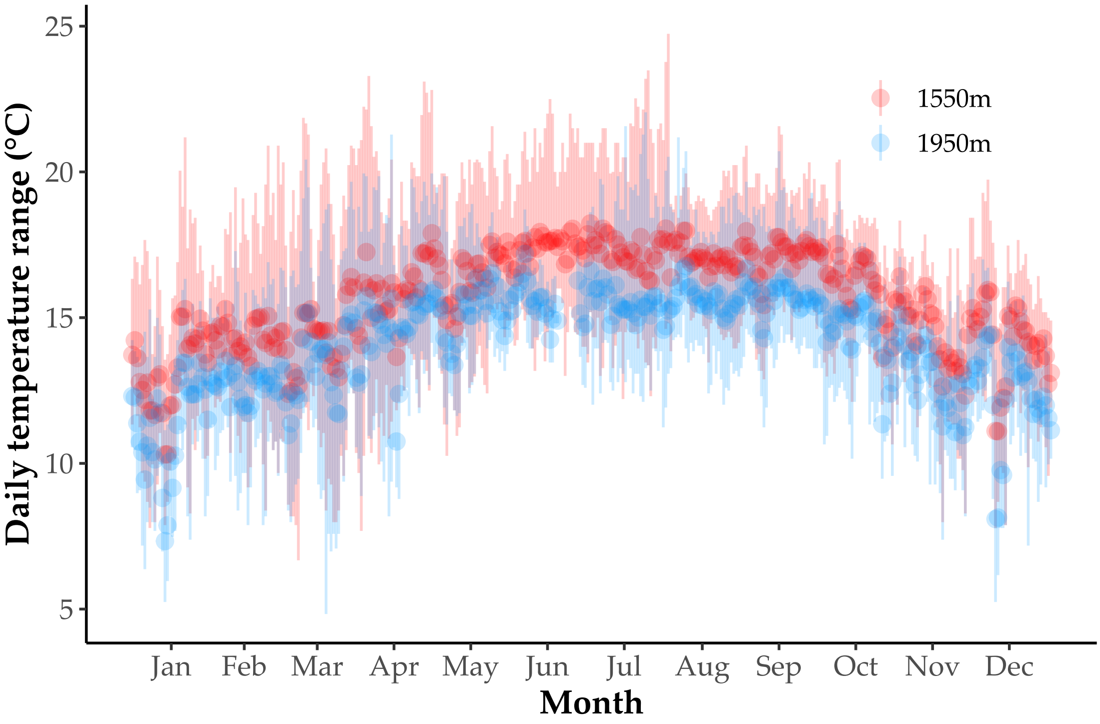

Figure 3.1 Daily mean (points) and minimum and maximum (lines) environmental temperature (ºC) variation across the year at two research camps in Cusuco National Park, Honduras between June 2017 to August 2018. Lower elevational data are denoted by red symbols, and higher elevational temperatures via blue symbols.

3.3.2 Capture and handling

We captured birds using 6 or 9 m mist-nets, generally lured into the nets by conspecific playback. This method inherently targeted territorial holders and as such our sample is biased towards male birds. After capture, each bird was weighed (M_b in g) to an accuracy of 0.1g using digital scales (SA-500, SATRUE, Taiwan) and banded with both a uniquely numbered aluminium ring (Aranea, Łódź, Poland), and a combination of field-identifiable coloured rings for future study.

Birds were aged and sexed, where possible, by a combination of plumage dimorphism, moult limits and checked for evidence of breeding condition (enlarged cloacal protuberance and brood patches). During the summer, most males of the study species (particularly BHNT, RCNT, CCBF) were in reproductive condition (enlarged cloacal protuberances). Breeding seasons for the study species are prolonged, and as such, it is generally not possible to sample birds in the summer season not in some state of reproductive condition. We did not include juvenile birds in any of our samples, although we did include first-cycle birds (i.e. born the previous breeding season), because they are functionally adult (e.g. Wolfe *et al*. 2010). Fat and muscle content was also assessed, but variance was virtually indistinguishable between season (where fat reserves were rarely present and muscle profiles were constant), and thus these data not suitable for statistical tests. Female birds with edematous brood patches were released after processing to allow them to return to incubating eggs and/or young. Birds that were transferred to the respirometry system (see below) for metabolic measurements were caught between 1500 and 1830 (dusk).

3.3.3 Blood sampling

We measured H_b concentration in grams per decilitre (g/dL) using a portable analyser (Prospect haemoglobin, prospect Diagnostics Ltd, UK). H_b concentrations reflect the ability of a bird to meet its oxygen requirements and as such it is a reliable measure of physiological condition and cold adaption (Swanson 2010, Dubay & Witt 2014, Minias 2015) that we used to compare between species and season. Blood was sampled shortly after capture from the alar vein and drained directly into a reagent-free cuvette (<8uL) specific to the analyser. After blood was drained into the cuvette, pressure was applied to the vein using cotton wool to stem the bleeding (see procedures described by Owen (2011)). Each blood sample was tested three times in the unit, with the resulting values averaged. The accuracy of the H_b analyser was measured every 14 days throughout fieldwork using standardised control measures (DiaSpect Control, DiaSpect Mecidal, Germany) at three concentrations: 8.0±0.4 g/dL, 12.6±0.6 g/dL and 16.0±0.8 g/dL.

3.3.4 Respirometry

We measured energy metabolism (as a rate of oxygen consumption $\dot{V}O_2$ ml/min[1]) using open flow respirometry with a portable gas analysis system (FoxBox, Sable Systems, USA; hereafter 'FoxBox'). Birds were placed in a custom-built $20cm^3$ Perspex chamber fitted with a perch into which a constant airflow was supplied. We experimentally manipulated temperatures by placing the respirometry chamber inside a modified cooler box fitted with a Peltier thermoelectric cooler module (AC-046, TE Technology, USA), capable of heating/cooling the interior of the cool box by a temperature controller (TC-48-20, TE Technology, USA). We checked temperatures inside the chamber with a logger (HOBO UA-001-64, Onset, USA) and offset slight differences between the temperature set inside the cooler box and inside the chamber using the relevant setting on the temperature controller. Temperature plates were powered by an analogue 24V power bench (ALF2412, ELC, UK), externally powered by a portable generator.

Ambient air was dried (self-indicating silica gel, GeeJay chemicals, UK) and pulled through the chamber at 1000±1 ml/min; a sufficient flow rate at which O_2 levels did not fall below 0.5% of natural levels, preventing hypoxia/hypercapnia. Air flow was measured and controlled by a linearized mass flow meter internal to the FoxBox, meaning temperature and barometric pressure compensation to STP were not required. Excurrent air from the chamber was dried again (silica gel) before entering the FoxBox where O_2 content, and CO_2 content were recorded at 1s intervals (Sable Systems ExpeData, Las Vegas, USA). A second air channel (outside ambient air, taken from directly adjacent to incurrent air to chamber) for use as reference ambient air was manually routed to the FoxBox following each temperature treatment (see below) to correct for drift in the analysers. Before sessions began, the O_2 analyser was spanned to

20.95% (environmental O_2 concentration) after a stabilisation period of ~45mins where the FoxBox measured the reference air channel only.

3.3.5 Experimental procedure and temperature manipulations

All respirometry sessions were undertaken at night to ensure birds were in their natural resting circadian phase. Following capture, we roosted all birds in cloth bags in a quiet room where they were fasted (without food). Birds were transferred to the respirometry system after dusk (1845 onwards) and left to acclimatise to the chamber for at least 45mins before temperature manipulations began. This acclimatisation period was sufficient washout time for residual chamber air to be replaced (see Lighton 2008).

Each bird was subjected to 1-4 temperature treatments per night. Temperature treatments for each bird were randomly chosen within five bands: 5-10ºC, 11-16ºC, 17-22ºC, 23-28ºC, and 29-34ºC, the treatment temperatures within which were systematically rotated (e.g. within 5-10ºC, 5 ºC, then 6ºC etc.). This semi-random approach was adopted to avoid potential problems with temperature acclimation associated with using a ramped profile of treatments. We did not exceed 34ºC during temperature manipulations as we were more interested in cold tolerance in the study species (the lower temperature limit of thermal-neutrality), and 34ºC represents temperatures that were almost certainly within thermal-neutrality (see Londoño *et al.* 2017). Once each temperature treatment was stable (±0.5ºC of target temperature), the bird was left for a further 45 minutes at temperature before data were accepted for metabolic measurements. We then recorded data for ~20 minutes before setting the next temperature treatment. Baselines of 7 minutes of ambient air were taken before each new temperature treatment was set (exact time intervals varied, but were typically every 1-1.5hrs). All metabolic data throughout the study were taken at least four hours after capture to ensure birds were post-absorptive (Karasov 1990). Respirometry sessions typically finished by 0300, and birds were released at the site of capture the following morning.

3.3.6 Data processing

Respirometry traces were baseline corrected and converted to $\dot{V}O_2$ (ml/min^{-1}) using equation 11.7 in Lighton (2008):

$$(1)\ \dot{V}O_2 = FR\ ((FiO_2 - FeO_2) - FiO_2\ (FeCO_2 - FiCO_2))\ /\ (1 - FiO_2)$$

Where, FR= Flow Rate and $FiO_2/CO_2/FeO_2/CO_2$= incurrent and excurrent fractional concentrations of O_2, and CO_2, respectively. Incurrent Oxygen (FiO_2) is at atmospheric levels (0.2095%). $\dot{V}O_2$ at each temperature trial was taken as the lowest continuous average over three minutes when the trace was low and stable (all values ≤0.05% of the mean), and where temperature had been constant for 45 minutes (see above). We subsequently converted $\dot{V}O_2$ to Watts (W) using a joule conversion of 20.1 J mL^{-1} (Gessaman & Nagy 2008).

3.3.7 Statistical analysis

All analyses were conducted in 'R' (R Core Team 2016). Values are presented as mean ± standard deviation (SD) or standard error (SEM) for β parameters, and statistically significance thresholds as *p* values <0.05. We used a mixed modelling approach for all analyses due to the repeated measures either inherent to the experimental design (for metabolic rates), or because of the repeated measures on individuals between seasons (i.e. summer and winter).

We analysed metabolic rates in a two-step process. Firstly we fitted linear mixed effects models for each species using the lme4 package (Bates *et al.* 2014). We modelled metabolic rate (W) as a function of season (summer/winter), M_b, experimental temperature treatment (ºC) and elevation (m) (of the captured bird) and a season × experimental temperature interaction as fixed effects, and included bird identity (individual) as a random effect. The factor 'season' was not included for RCNT models because no winter data were collected for this species. In incidences when M_b was identified as a significant effect in full models (BHNT and CCBF), we corrected for this effect by repeating the analyses using mass-specific metabolic rate as the response variable. All continuous explanatory variables were scaled to account for potential sensitivity in magnitudes of change within variables. We then selected best-fitting models for each species, where models were ranked using corrected Akaike Information Criterion (AICc) using the MuMIn package (Barton 2016), and examined models for significant parameters with Wald-Chi square tests using the *car* package (Fox et al. 2011). All models within 6Δ AICc of the best-fitting model, as well as the saturated and null models are presented in the supplementary materials. Hereafter, 'best-fitting model' refers to that with 0Δ AICc, while 'top model set' refers to all models within 6Δ AICc. When the best-fitting model had multiple parameters, parsimonious models for each significant effect in this model are also presented. This modelling approach broadly follows recommendations suggested by (Harrison et al. 2018).

Where treatment temperature was identified as a significant parameter in the top model set, we then fitted non-linear mixed models to the data in the nlme package (Pinheiro et al. 2017). These models estimated how metabolic rates related to temperature by estimating the inflection temperature (lower critical limit of thermoneutrality - T_{lc}) at which species began thermoregulation (i.e. an increase in metabolic rate), the slope of this relationship (minimum thermal conductance - C_{min}) and metabolic rate above T_{lc} (BMR). This method can underestimate minimum thermal conductance (see McNab 1980), but because we did not measure body temperatures, this allowed an approximation of energetic costs of thermoregulation below T_{lc}. Because of this, we elected not to statistically compare metabolic responses to temperature, instead qualitatively comparing values between species. In one species (BHNT), where we found an effect of season on metabolic rates during model selections, we added a two-way factor of 'season' (summer/winter) in order to assess specifically which parameters (BMR, C_{min} or T_{lc}) differed between season.

We analysed H_b using the same modelling process as described for the first step of metabolic data. We predicted H_b (g/dL) as a function of sex (male/female), season (summer/winter), elevation (m) and a sex × season interaction as fixed effects, with bird identity (individual) as a random effect. The factors 'sex' and 'season' for RCNT, and 'sex' for GBWW were not included for these models, as insufficient data were available. After testing the effects of these parameters we assessed intra-specific differences with Tukey post-hoc comparison tests (controlled for multiple testing) using the multcomp package (Bretz et al. 2010). We compared summer and winter datasets separately, however, to compare like for like data. Finally, we tested for seasonal differences in M_b using Wald-Chi square tests on linear mixed models of M_b as a function of season. For Black-headed Nightingale-Thrushes - the only species we were able to discern sex between season (because of sexually dimorphic plumage) - we also tested for sex specific season changes in M_b.

To place our results within the broader understanding of the physiological diversity of tropical birds, we compared BMR of species in our study to predicted values from mass-scaling exponents in the literature, using phylogenetically informed power equations in Londoño et al. (2015). Values are presented as percentages of predicted BMR, using coefficients presented for tropical species when those with ambiguous breeding distributions were excluded from the dataset (see Londoño *et al.* 2015). For species that displayed no seasonal changes in BMR, we pooled data across seasons. We considered values within 10% of those predicted as within the expected range broadly following similar studies (e.g. Smit & McKechnie 2010). We estimated BMR for each species in our study by taking the lowest measure of metabolic rate per individual above T_{lc}, comparable to similar studies (Londoño *et al.* 2015). Finally, for purposes of comparison, we present whole animal and mass-specific BMR, H_b concentrations, and M_b values between summer and winter for all species and calculate winter/summer ratios by dividing mean winter values by mean summer values (McKechnie 2007, Pollock *et al.* 2019).

3.4 RESULTS

3.4.1 Metabolism; responses to temperature and seasonal change

We found a strong effect of temperature on metabolic rates in best fitting models for three species (BHNT χ^2_1 = 58.708, *p* = <0.001; CCBF χ^2_1 = 29.19, *p* = <0.001; GBWW χ^2_1 = 19.72, *p* = <0.001) but not RCNT (**Figure 3.2; Table S3.1**). Metabolic rate (W) was best predicted by temperature alone for CCBF and GBWW (**Figure 3.2; Table S3.1**). For BHNT, however, season was also a significant covariate (χ^2_1 = 21.872, *p* = <0.001) in addition to temperature in the best-fitting model. For RCNT, the best-fitting model was one with the intercept alone (**Table S3.1**). We found no differences in metabolic rate dependent on elevation for any species, excepting RCNT where elevation was significant in the second ranked model (χ^2_1 = 4.056, *p* = <0.044, **Table S3.1**). M_b was a significant variable in full and top model sets for both BHNT and CCBF (**Table S3.1**), but when the same model selections were undertaken on mass-corrected values for these species, our results were comparable, in that temperature and season (BHNT) and temperature (CCBF) were the most important variables (**Table S3.1**). Because the results for these two species were comparable after controlling for the effects of M_b, we fitted subsequent models examining the specific effects of temperature using whole-animal values.

When we tested the specific influence of temperature on metabolic rate, we found that inflection temperatures (T_{lc} ºC ± SEM) were comparable between species (excluding RCNT); BHNT (winter=19.4 ± 2.7 ºC, summer=23.3 ± 3.14 ºC), CCBF (19.2 ± 2.46 ºC), and GBWW (22.3 ± 3.06 ºC) (see **Table 3.1/Figure 3.2**). Similarly, estimates of thermal conductance (W ºC ± SEM) were also comparable between species; BHNT (winter=0.0127 ± 0.004 ºC W, summer=0.0156 ± 0.005 ºC W), CCBF (0.0120 ± 0.004 ºC W) and GBWW (0.0097 ± 0.002 ºC W) (**Table 3.1**). No significant differences in either T_{lc} or C_{min} were detected between seasons for BHNT (T_{lc}: t=1.2141, df=57, *p*=0.229; C_{min}: t=0.6555, df = 57, *p* = 0.514), but BMR was significantly different between seasons (t = 2.3876, df = 57, *p* = 0.02) (**Table 3.1/Table 3.2**). In BHNT, summer BMR (0.43 ± 0.05 W) was 19.4% higher than winter BMR (0.36 ± 0.07 W). No seasonal differences in BMR were detected in CCBF (summer= 0.53 ± 0.04 W / winter= 0.52 ± 0.05 W) or GBWW (summer= 0.27 ± 0.04

W / winter= 0.31W), (**Table 3.2/Figure 3.2**), although we recognise the winter sample for the latter species is only from a single bird.

When compared to mass-scaling exponents for existing data on tropical birds, BMR in our species was generally greater than predicted (mean % difference ± SD). Winter BMR for BHNT (111.5 ± 3) was within the expected range, whereas summer BMR for BHNT (129.8 ± 3.5), summer RCNT (150.4 ±2.9), and pooled values across season for CCBF (132.1 ± 3.7) and GBWW (125 ± 1) were greater than expected (**Figure 3.3**).

Table 3.1 Non-linear mixed models (per species) on metabolic rate (W) as a function of temperature estimating lower critical temperatures (T_{lc}), minimum thermal conductance (C_{min}) and basal metabolic rate (BMR) above inflection temperature (see **Figure 3.2**) for all species except Ruddy-capped Nightingale-Thrushes *Catharus frantzii*. A multi-level non-linear mixed model with the two-way factor of 'Season' (summer or winter) was fitted for Black-headed Nightingale-Thrushes *Catharus mexicanus* because significant differences in metabolic rates were detected between seasons.

Black-headed Nightingale-Thrush *Catharus mexicanus*						
	Variable	Value	SE	df	t-value	*p* value
Winter *n* = 15	BMR (W)	0.391	0.015	57	25.291	**<0.001**
	C_{min} (W °C)	0.013	0.004	57	3.306	**0.002**
	T_{lc} (W °C)	19.479	2.772	57	7.026	**<0.001**
Summer *n* = 28	BMR (W)	0.441	0.022	57	2.388	**0.02**
	C_{min} (W °C)	0.016	0.005	57	0.656	0.514
	T_{lc} (°C)	23.303	3.149	57	1.214	0.229
Chestnut-capped Brushfinch *Arremon brunneinucha*						
n = 14	BMR (W)	0.534	0.012	17	43.400	**<0.001**
	C_{min} (W °C)	0.012	0.004	17	3.371	**0.004**
	T_{lc} (°C)	19.238	2.469	17	7.791	**<0.001**
Grey-breasted Wood Wren *Henicorhina leucophrys*						
n = 8	BMR (W)	0.291	0.016	11	18.023	**<0.001**
	C_{min} (W °C)	0.010	0.003	11	3.310	**0.007**
	T_{lc} (°C)	22.308	3.069	11	7.268	**<0.001**

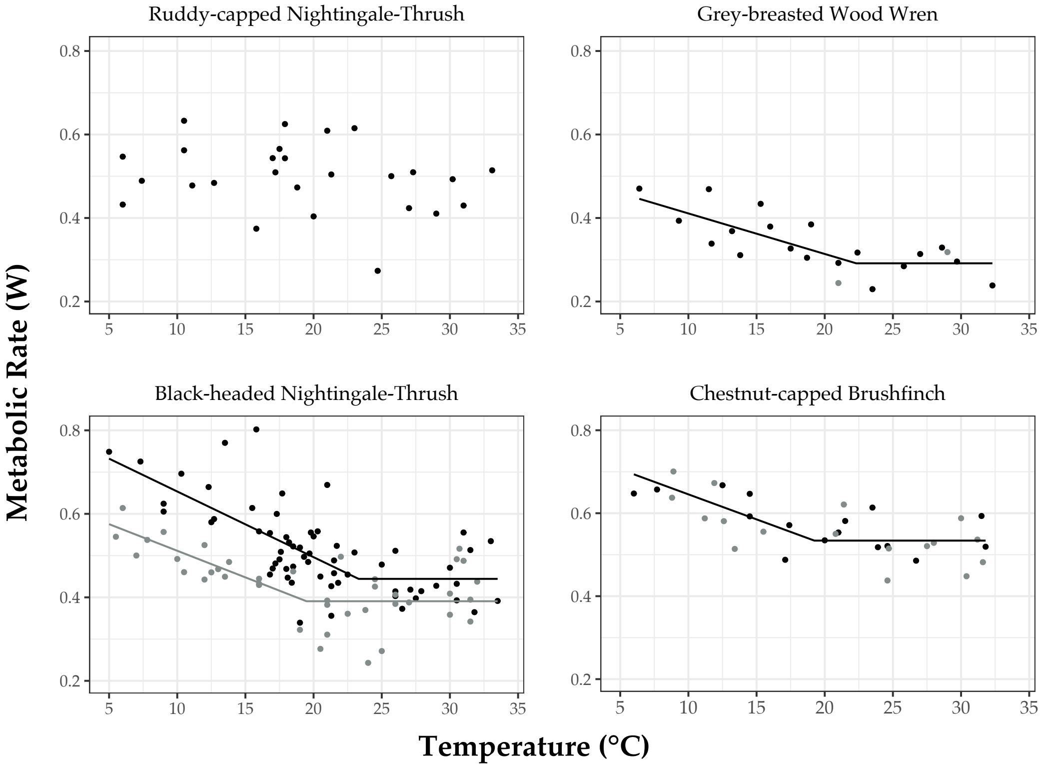

Figure 3.2 Metabolic rate (W) as a function of manipulated temperature (ºC) for four species of cloud-forest songbirds. Data points represent birds tested at temperature treatments (individuals where frequently tested at different temperatures- see sample sizes in **Table 3.1**) and fitted lines are non-linear mixed model fits estimating the inflection temperature at which thermoregulation began (T_{lc}), minimum thermal conductance (C_{min}) and metabolic rates above the inflection temperature (BMR). Summer (black) and winter (grey) data are plotted separately for each species, with summer and winter data separated for Black-headed Nightingale-Thrushes *Catharus mexicanus*. No effect of temperature was apparent for Ruddy-capped Nightingale-Thrushes *Catharus frantzii* (top left panel).

3.4.2 Body mass

Seasonal changes in M_b were apparent in two of the four species for which we had data (**Table 3.2**). Both BHNT and CCBF showed no seasonal changes in M_b (BHNT, $\chi^2_1 = 2.71$, $p = 0.09$; CCBF, $\chi^2_1 = 0.49$, $p = 0.48$), whereas GBWW and COBT had higher M_b in the breeding season (GBWW, $\chi^2_1 = 222.8$, $p = <0.01$; COBT, $\chi^2_1 = 17.15$, $p = <0.01$) (**Table 3.2**). In addition, for BHNT we found no seasonal changes in M_b dependent on sex ($\chi^2_1 = 1.71$, $p = 0.18$).

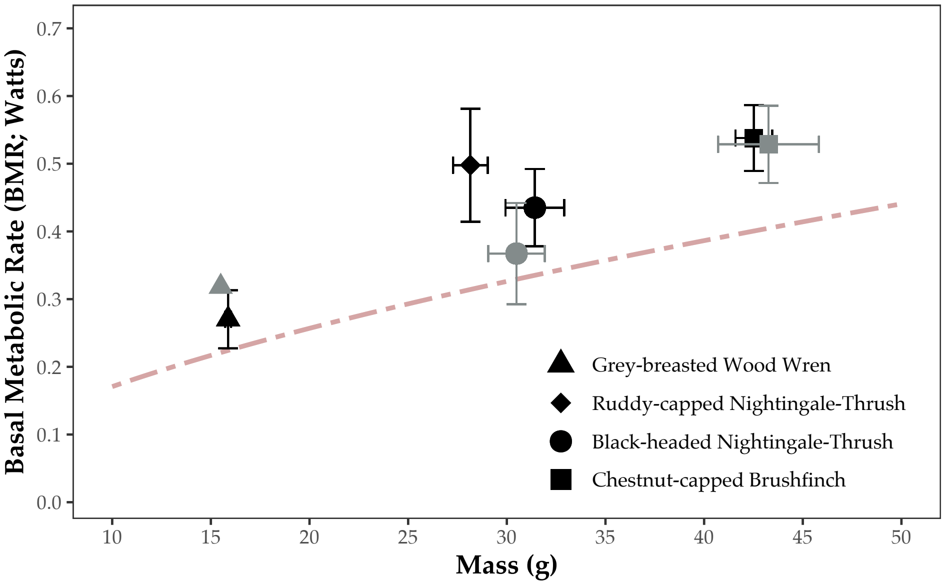

Figure 3.3 Mean (± SD) basal metabolic rate (BMR; W) and body mass (M_b; g) per species between summer (black) and winter (grey). Although the only significant difference in metabolic rates between season was in Black-headed Nightingale-Thrushes *Catharus mexicanus*, we plot all data separately for purposes of visual comparison. Expected allometric scaling relationship between basal metabolic rate and body mass for tropical birds (red dashed line) is taken from Londoño *et al*. (2015).

3.4.3 Haemoglobin concentrations

We found limited explanatory power of any of the variables tested for H_b content per species. For BHNT, however, the breeding season × sex interaction term was significant in the second ranked model ($\chi^2_1 = 4.663$, $p = 0.03$, see **Table S3.2**), where H_b may be slightly higher in summer, over winter males. For GBWW, elevation was also a significant effect in the second ranked model in the top model set ($\chi^2_1 = 5.074$, $p = 0.02$, see **Table S3.2**), indicating that H_b may be negatively associated with elevation (although the sample for this was small). For COBT, breeding season was also retained in a near identical second ranked model, but was marginally not statistically significant ($\chi^2_1 = 3.202$, $p = 0.07$), however it was also consistently present and marginally statistically significant in the three top ranked models (see **Table S3.2**). Generally though, H_b at the species level appeared to vary little with elevation, season and sex.

Finally, winter H_b concentrations were comparable between species (all species comparisons $p = \geq 0.55$; **Figure 3.4, Table 3.2/S3.3**). Summer H_b concentrations were also comparable between species ($p = \geq 0.13$; **Table 3.2/S3.3**) except for RCNT, which was distinctly higher than the other four species ($p = <0.001$ for all RCNT – other species comparisons; **Figure 3.4, Table 3.2/S3.3).**

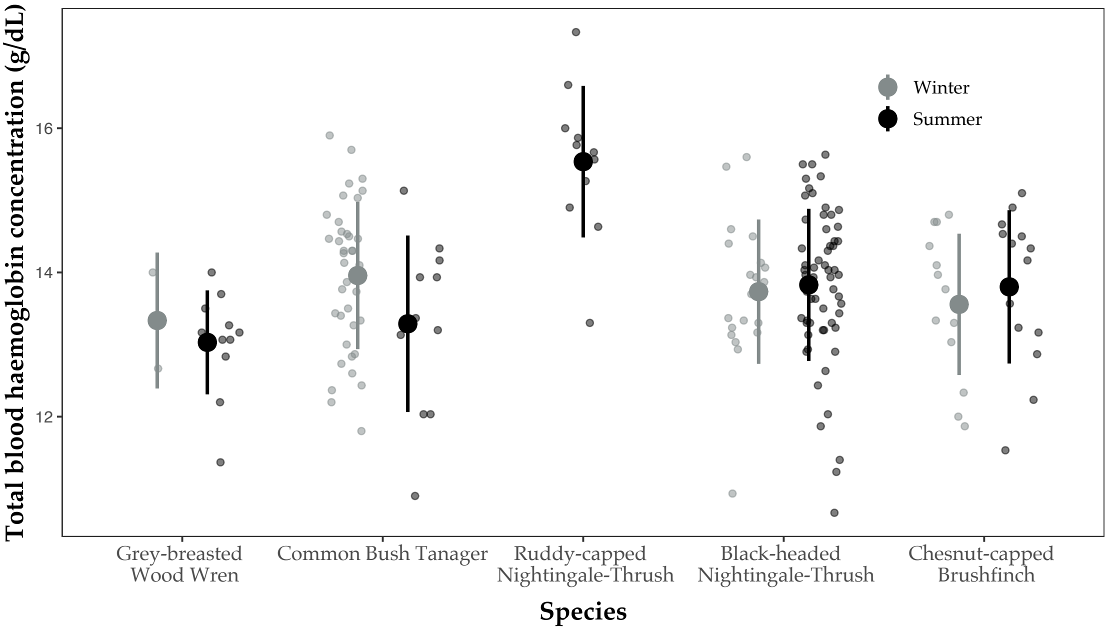

Figure 3.4 Haemoglobin concentrations (H_b g/dL, mean ± SD) per species/per season (ordered left to right by smallest to largest body mass). No winter data were available for Ruddy-capped Nightingale-Thrushes *Catharus frantzii* (see Methods).

Table 3.2 Seasonal comparisons of body mass (g), basal metabolic rate (BMR, whole animal (W) and mass-corrected (mW g^{-1})), and total blood haemoglobin content (g/dL) per species. Sample sizes are presented in parentheses. Statistically significant differences between seasons are denoted with an asterisk.

	Winter	**Summer**	**W / S ratio**
Black-headed Nightingale-Thrush *Catharus mexcanus*			
Body Mass (g)	30.4 ± 1.28 (20)	31.02 ± 1.54 (62)	0.98
Whole-animal BMR (W)	0.36 ± 0.07 (14) *	0.43 ± 0.05 (15) *	0.83
Mass-specific BMR (mW g^{-1})	12.02 ± 2.28 (14) *	13.88 ± 1.95 (15) *	0.87
Total blood haemoglobin (g/dL)	13.73 ± 1 (20)	13.82 ± 1.05 (60)	0.99
Ruddy-capped Nightingale-Thrush *Catharus frantzii*			
Body Mass (g)	-	28.22 ± 0.95 (11)	-
Whole-animal BMR (W)	-	0.47 ± 0.09 (11)	-
Mass-specific BMR (mW g^{-1})	-	16.77 ± 3.5 (11)	-
Total blood haemoglobin (g/dL)	-	15.53 ±1.05 (11)	-
Chestnut-capped Brushfinch *Arremon brunneinucha*			
Body Mass (g)	42.75 ± 2.17 (14)	42.32 ± 1.55 (13)	1.01
Whole-animal BMR (W)	0.52 ± 0.05 (8)	0.53 ± 0.04 (5)	0.98
Mass-specific BMR (mW g^{-1})	12.24 ± 1.46 (8)	12.64 ± 1.05 (5)	0.96
Total blood haemoglobin (g/dL)	13.55 ± 0.98 (14)	13.8 ± 1.06 (13)	0.99
Grey-breasted Wood Wren *Henicorhina leucophrys*			
Body Mass (g)	15.35 ± 0.21 (2) *	15.97 ± 0.6 (12) *	0.96
Whole-animal BMR (W)	0.31 (1)	0.27 ± 0.04 (4)	1.14
Mass- specific BMR (mW g^{-1})	20.53 (1)	17.03 ± 2.78 (4)	1.2
Total blood haemoglobin (g/dL)	13.33 ± 0.94 (2)	13.03 ± 0.72 (11)	1.02
Common Bush Tanager *Chlorospingus flavopectus*			
Body Mass (g)	15.8 ± 0.89 (38) *	16.95 ± 0.46 (11) *	0.93
Total blood haemoglobin (g/dL)	13.95 ±1.02 (38)	13.29 ± 1.22 (11)	1.05

3.5 DISCUSSION

The aims of our study were twofold. Firstly we wanted to determine whether the species in our study displayed uniform shifts in physiological traits between seasons, and secondly to ascertain whether there was evidence for a higher elevation species differing in its physiological traits. Contrary to our first hypothesis; for three species (BHNT, CCBF and GBWW) for which we had metabolic data between seasons, only BHNT changed metabolic rates with season, showing clear up-regulated BMR in the warmer summer, whereas the other two species showed no evidence for seasonal changes (although we recognise the sample size for winter GBWW is small and possibly inconclusive). We also found no evidence for seasonal shifts in H_b concentrations and variable seasonal change in M_b. In support of our second hypothesis, however, RCNT was a clear outlier in all measures, with evidence of cold tolerance traits (because RCNT sustained constant BMR throughout the entire temperature range tested), higher summer BMR relative to M_b, and higher H_b concentration than other species.

3.5.1 Physiological shifts with season

The variability in seasonal metabolic rates observed across species in our study is consistent with comparable studies on lowland tropical birds. For example, the interspecific variation in BMR in our study (W/S ratio range 0.83-1.14; **Table 3.2**) is within the range of that measured in Panama (W/S ratio range 0.71-1.33; Pollock *et al.* (2019)). Because our study was at higher elevation, and thus a cooler tropical environment, we hypothesised that seasonal changes in metabolic rates may reflect cooler local T_e and minor changes in season. Instead, our results appear unrelated to T_e because no shifts in metabolic rates were consistent with heat conservancy (i.e. increases in BMR and thermoregulatory traits in winter) as is common in temperate species (Swanson & Garland 2009, Swanson 2010, Smit & McKechnie 2010, McKechnie *et al.* 2015). This was also reflected in M_b between season, which was either static, or increased in summer (GBWW and COBT), the opposite of cold tolerance traits typical of temperate species. Pollock et al. (2019) did not detect any significant differences between M_b changes between temperate and tropical birds, although these authors also found substantial variation between species, similar to our results.

Our study is only the third we are aware of to compare seasonal metabolic rates in tropical species, but like other studies, we also show that variation in metabolic rates in tropical birds are apparently unrelated to T_e and is highly variable between species (Wells & Schaeffer 2012, Pollock *et al.* 2019). Although there are few studies from the tropics to which we can make direct comparison, this variation is also consistent with shifts in seasonal metabolic rates in subtropical birds. Substantial variation in BMR (the most widely measured trait) is present in subtropical species, ranging from considerable winter reductions, to considerable winter increases in BMR (W/S ratio range 0.66-1.63; McKechnie *et al.* 2015), a magnitude of shifts comparable to temperate species (Noakes *et al.* 2017).

Because seasonal changes in metabolic rates appears unrelated to T_e in our, and other tropical studies, it is intriguing to examine what might drive this relationship. One emerging explanation for this pattern is that 'metabolic niches' are greater at tropical latitudes (Anderson & Jetz 2005), where intrinsic relationships between a birds characteristics and metabolic rate reflect seasonal variation in physiological traits (McKechnie *et al.* 2015). For example, specific behavioural changes with season unique to a given species may manifest in changing maintenance costs of metabolically active tissues (Swanson 2010). In BHNT, BMR was up-regulated in summer (19.4% greater than winter), the opposite of patterns typically displayed in temperate species (e.g. Smit & McKechnie 2010). Thus, behavioural differences related to reproductive activity between season in our study species may covary with BMR because of the energetic costs associated with them (e.g. increases in male territorial activity). That physiological control mechanisms may shape the diversity of life history traits in tropical birds has previously been suggested (Ricklefs & Wikelski 2002, Williams *et al.* 2010). However, we are aware of only one study that has assessed the relationship between metabolic rate and behavioural energy usage in a tropical bird (Steiger *et al.* 2009), and none that have assessed seasonal differences in metabolic intensity with life-history traits experimentally or otherwise. Future species-specific studies are required to interrogate this hypothesis.

A shortfall in our interpretation of the potential processes underpinning seasonal changes, however, is that we did not measure body temperatures. Facultative hyperthermia, where short-term reductions in body temperature result in lower metabolic rates through reduced demand for metabolic heat production are thought to be common across birds (McKechnie & Lovegrove 2002) and have been found in various tropical species in cooler conditions in the short term (e.g. Bartholomew *et al.* 1983, Merola-Zwartjes 1998, Merola-Zwartjes & Ligon 2000, Steiger *et al.* 2009). As such, we cannot eliminate seasonal changes in body temperature as an alternative explanation for the interspecific differences we observe. Irrespective of the mechanism, however, our results still display species-specific differences in metabolic rate between seasons.

That we found no clear changes in H_b concentrations in our study species with season is intriguing, particularly when coupled with the variation observed in BMR. Firstly, thermogenic demands may result in elevated H_b concentrations (Swanson 1990, Powell *et al.* 2013), so our result that H_b concentrations appeared unchanged with season is consistent with the lack of other apparent physiological changes related to increased cold tolerance. Secondly, because H_b concentrations are considered a reliable marker of oxidative stress (Minias 2015), the lack of seasonal change in H_b concentrations in the species in our study are indicative of low levels of physiological stress between seasons. This may be particularly the case in BHNT, where seasonal changes in BMR did not appear to covary with H_b concentrations, suggesting that increased physiological stress during reproduction is not a sufficient explanation for elevated seasonal BMR in this species. We remain cautious in this conclusion, however, because there was some signal of a difference in male H_b concentrations between season, although there was much overlap and these quantities were similar (H_b g/dL content in winter/summer male BHNT; 13.5 ± 0.98 / 13.9 ± 1.04).

We are aware of no other studies on H_b concentrations in tropical forest birds from which to directly compare, but the general lack of seasonal changes across species in our study might be reflective of 'slow paced' life-histories of tropical birds, where longer lifespans are facilitated with greater investment in self-maintenance (Ricklefs & Wikelski 2002, Wiersma *et al.* 2007). Although, only on temperate species, evidence in support of a coevolution with slower paced life-history and decreased levels of oxidative stress has been displayed (Vágási *et al.* 2018). Reproductive periods in female birds may be particularly energetically demanding (Williams *et al.* 2004) but because of difficulties in determining sex in many of the species in our study in the winter season, we were not able to fully interrogate this hypothesis. Future studies could clarify this by comparing magnitudes of change between tropical and temperate species with the addition of new field data (which for H_b concentrations are cost-effective and relatively simple to collect).

3.5.2 Implications for elevational adaption

RCNT - the higher elevation species - was distinct among the species in our study. We found no low temperature limit at which this species began thermoregulation and higher BMR than predicted by mass-scaling exponents compared to the other species in our study (over greater 50% greater than that predicted for tropical birds), both traits consistent with cold tolerance (Pollock *et al.* 2019). Few studies on the physiology of tropical montane birds exist for which we are able to directly compare, although similar (albeit isolated) examples of cold tolerant birds in the Peruvian Andes (Londoño *et al.* 2017) and high elevation tropical bats and mice have also shown distinct cold tolerance traits (Soriano *et al.* 2002, Pasch *et al.* 2013).

The differences displayed in RCNT are particularly intriguing when considered against the backdrop of elevational range restriction in tropical montane species (e.g. McCain 2009). Realistically, it is unlikely that the traits we observe are a product of differences in present day T_e across elevation in our site; T_e did not strikingly differ across elevation, and that BHNT, a parapatric congener (see **Chapter 2**) displays divergent physiological traits. RCNT and BHNT are not sister species (Voelker *et al.* 2013), thus, one possibility is that the divergence in their physiological traits are conserved from historic isolation (Wiens *et al.* 2010) and that competitive interactions between the two species (**Chapter 2**) have developed upon secondary contact (Freeman 2015). This trait divergence (and competitive interactions) resembles that of morphological phenotypes between two parapatric Wood Wrens (*Henicorhina* sp.) in Colombia, the elevational ranges of which have converged through secondary contact (Caro *et al.* 2013).

A general lack of evidence for intraspecific variation in H_b concentrations or metabolic rates with increases in elevation is consistent with a lack of apparent elevational specialism in our study species (excepting RCNT). However, because we only tested for an intraspecific effect across a small elevational range we remain cautious in this conclusion as this may not be conclusive evidence that one does not exist. Intraspecific differences ascribed to elevational adaption have been displayed in Tyrannid Flycatchers (*Anairetes* sp.) across a larger elevational gradient in the Peruvian Andes (Dubay & Witt 2014) and for resident species in the subtropical Himalayas (Barve *et al.* 2016). Nonetheless, the lack of intraspecific signal in physiological traits across elevation is still consistent with an apparent lack of elevational specialism (excepting RCNT), although more comprehensive tests across a much broader elevational range at our study site would clarify this, particularly with the inclusion of more low elevation species.

The species in our study had generally higher BMR than predicted by M_b for tropical birds, with only winter BMR of BHNT falling within the expected values. To some extent this contrasts with Londoño *et al.* (2015) who, across a larger range of species displayed that higher elevation species in the tropics had comparable BMR to lowland tropical residents. The specific reasons our BMR measures were slightly higher than predicted is not clear and does not appear to be a result of intraspecific variation across elevation (e.g. Lindsay *et al.* 2012). One possibility is that species that do not undergo seasonal shifts in BMR maintain higher BMR throughout the year, although because little is known of the specific determinants of shifts in BMR between species at tropical latitudes rigorous testing is needed to determine this. Nonetheless, despite generally higher BMR than predicted by allometric scaling, our values are still well within the overall range previously measured for tropical birds (see **Figure S3.1**).

In conclusion, our results support two emergent schools of thought in the knowledge of the physiological diversity of tropical birds. Firstly, that shifts in BMR appear to be a flexible trait that may be species-specific, more broadly reflecting a growing appreciation for flexible phenotypic diversity in metabolic rates in birds (Piersma & Drent 2003, McKechnie *et al.* 2006, McKechnie 2007). Secondly, and generally speaking, the physiology of tropical birds appears unrelated to T_e as conventional hypotheses have suggested (see Chown *et al.* 2004, White *et al.* 2007, Londoño *et al.* 2015). However, we did find evidence of distinct interspecific differences in our study, the generalities of which require more comprehensive examination. Despite growing interest, substantial knowledge gaps remain in our understanding of the ecophysiology of tropical birds. This is particularly so for tropical montane species, that may be characterised by distinct interplays between physiological and life-history characteristics (Goymann *et al.* 2004, Scholer *et al.* 2019).

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3.7 SUPPLEMENTARY MATERIALS

Table S3.1 Linear mixed-effects model selections for metabolic rate (W) as a function of treatment temperature, breeding season, body mass and elevation for all species. Model fits are presented per species with full models (including all parameters), null models (intercept only) and best model fits as ranked by AICc scores. The best fitting model is denoted with Δ0, with all models within Δ6 AICc also presented. Parsimonious models of significant variables are also presented from model fits where best fitting models had multiple parameters. Statistically significant effects are assessed by Wald χ^2 tests. The 'BreedingY' parameter represents the summer breeding season.

Black-headed Nightingale-Thrush *Catharus mexicanus*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	-189.4	8	Intercept	0.4408	0.0138	31.88			
			Temperature	-0.0419	0.0100	-4.16	66.444	1	**<0.001**
			BreedingY	0.0697	0.0172	4.05	16.999	1	**<0.001**
			Mass	0.0235	0.0085	2.76	7.619	1	**<0.01**
			Elevation	0.0021	0.0082	0.25	0.064	1	0.798
			Temperature* Breeding	-0.0333	0.0143	-2.32	5.396	1	**<0.05**
Null Model	-165.6	3	Intercept	0.4832	0.0122	39.51			
Best model	-206.5 (Δ0)	5	Intercept	0.4324	0.0146	29.58			
			Temperature	-0.0564	0.0073	-7.66	58.708	1	**<0.001**
			BreedingY	0.0828	0.0177	4.68	21.872	1	**<0.001**
	-203.1 (Δ3.39)	6	Intercept	0.4412	0.0146	30.19			
			Temperature	-0.0587	0.0072	-8.13	66.065	1	**<0.001**
			BreedingY	0.0691	0.0180	3.84	14.707	1	**<0.001**
			Mass	0.0230	0.0089	2.57	6.618	1	**<0.05**
	-201.9 (Δ4.59)	6	Intercept	0.4324	0.0139	31.02			
			Temperature	-0.0408	0.0103	-3.95	59.448	1	**<0.001**
			BreedingY	0.0831	0.0170	4.87	23.978	1	**<0.001**
			Temperature* BreedingY	-0.0309	0.0146	-2.12	4.479	1	**<0.05**
Parsimonious models									
	196.2 (Δ10.24)	4	Intercept	-0.4857	0.0113	42.87			
			Temperature	-0.0567	0.0078	-7.24	52.352	1	**<0.001**
	-171.2 (Δ35.31)	4	Intercept	0.4317	0.0168	25.66			
			BreedingY	0.0816	0.0208	3.91	15.268	1	**<0.001**

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Mass-corrected values

Full Model	-864	7	Intercept	0.0508	0.0015	32.98			
			Temperature	-0.0049	0.0011	-4.24	66.698	1	**<0.001**
			BreedingY	0.0083	0.0018	4.39	19.510	1	**<0.001**
			Elevation	0.0003	0.0009	0.34	0.112	1	0.737
			Temperature* Breeding	-0.0035	0.0016	-2.17	4.691	1	**<0.05**
Null Model	-863.7	3	Intercept	0.0559	0.0013	40.99			
Best model	-893 (Δ0)	4	Intercept	0.0157	0.0003	45.76			
			Temperature	-0.0018	0.0002	-7.81	60.974	1	**<0.001**
	-892.5 (Δ0.47)	5	Intercept	0.0142	0.0005	31.38			
			Temperature	-0.0018	0.0002	-8.16	66.580	1	**<0.001**
			BreedingY	0.0022	0.0005	4.15	17.284	1	**<0.001**

Ruddy-capped Nightingale-Thrush *Catharus frantzii*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	-21.5	6	Intercept	0.4979	0.0150	33.09			
			Temperature	-0.0256	0.0171	-1.49	2.227	1	0.135
			Mass	0.0061	0.0169	0.36	0.130	1	0.717
			Elevation	-0.0366	0.0163	-2.25	5.044	1	**0.024**
Null Model	-42.9 (Δ0)	3	Intercept	0.4979	0.0163	30.42			
	-37.5 (Δ5.39)	4	Intercept	0.4979	0.0154	32.22			
			Elevation	-0.0317	0.0157	-2.01	4.056	1	**0.044**

Chestnut-capped Brushfinch *Arremon brunneinucha*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	-47.7	8	Intercept	0.5507	0.0158	34.68			
			Temperature	-0.0506	0.0114	-4.41	28.859	1	**<0.001**
			BreedingY	0.0313	0.0271	1.15	1.543	1	0.214
			Mass	0.0215	0.0089	2.40	5.781	1	**<0.05**
			Elevation	-0.0107	0.0146	-0.73	0.535	1	0.464
			Temperature* Breeding	0.0029	0.0187	0.16	0.024	1	0.876
Null Model	-72.2	3	Intercept	0.5656	0.0116	48.75			
Best model	-81.4 (Δ0)	4	Intercept	0.5688	0.0099	57.05			
			Temperature	-0.0466	0.0086	-5.40	29.19	1	**<0.001**
	-75.1 (Δ6.29)	5	Intercept	0.5663	0.0086	65.40			
			Temperature	-0.0477	0.0084	-5.66	32.034	1	**<0.001**
			Mass	0.019	0.0088	2.17	4.687	1	**<0.05**

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Mass-corrected values									
Full Model	-268.2	7	Intercept	0.0129	0.0003	35.62			
			Temperature	-0.0012	0.0002	-4.52	30.909	1	**<0.001**
			BreedingY	0.0007	0.0006	1.24	1.786	1	0.181
			Elevation	-0.0002	0.0003	-0.81	0.660	1	0.416
			Temperature* Breeding	5.939 x10^{-5}	0.0004	0.13	0.018	1	0.892
Null Model	-311.8 (Δ5.4)	3	Intercept	0.0132	0.0002	48.46			
Best model	-317.2 (Δ0)	4	Intercept	0.0133	0.0001	67.13			
			Temperature	-0.0011	0.0001	-5.79	33.548	1	**<0.001**

Grey-breasted Wood Wren *Henicorhina leucophrys*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	-9.2	8	Intercept	0.2386	0.0608	3.92			
			Temperature	0.0678	0.0616	1.10	16.739	1	**<0.001**
			BreedingY	0.0979	0.0628	1.55	0.1139	1	0.7357
			Mass	0.0108	0.0117	0.92	0.8599	1	0.3538
			Elevation	-0.0036	0.0122	-0.30	0.0909	1	0.763
			Temperature* Breeding	-0.1169	0.0626	-1.86	3.4804	1	0.062
Null Model	-40.4 (Δ3.93)	3	Intercept	0.3354	0.0148	22.62			
Best model	-44.3 (Δ0)	4	Intercept	0.3354	0.0106	31.48			
			Temperature	-0.0484	0.0109	-4.44	19.72	1	**<0.001**

Table S3.2 Linear mixed effect model selections for total blood haemoglobin content (g/dL) as a function of sex, season and elevation for each species as ranked by AICc scores. The best fitting model is denoted with Δ0, with all models within Δ6 AICc also presented. Statistically significant effects are assessed by Wald χ^2 tests. The 'BreedingY' parameter represents the summer breeding season, and 'SexM' as male birds. 'Sex' and 'Breeding' are not included as explanatory variables for Ruddy-capped Nightingale-Thrushes *Catharus frantzii*, because no data were available for this species. For the same reason, 'Sex' was not included in models for Grey-breasted Wood-wren *Henicorhina leucophrys*.

Black-headed Nightingale-Thrush *Catharus mexicanus*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	247.3	7	Intercept	14.4876	0.4604	31.47			
			Elevation	0.0456	0.1171	0.39	0.152	1	0.697
			SexM	-0.9839	0.5313	-1.85	0.042	1	0.837
			BreedingY	-1.0377	0.5846	-1.78	0.108	1	0.743
			SexM* BreedingY	1.4212	0.6562	2.17	4.689	1	**0.03**
Null Model	240.3 (Δ0)	3	Intercept	13.8116	0.1185	116.6			
	242.6 (Δ2.36)	6	Intercept	14.4828	0.4576	31.65			
			SexM	-0.9834	0.5284	-1.86	0.051	1	0.821
			BreedingY	-1.0183	0.5792	-1.76	0.125	1	0.724
			SexM* BreedingY	1.4069	0.6515	2.16	4.663	1	**0.03**
	242.9 (Δ2.61)	4	Intercept	13.8648	0.2952	46.97			
			SexM	-0.0627	0.3228	-0.19	0.038	1	0.846
	243.2 (Δ2.93)	4	Intercept	13.7484	0.2327	59.09			
			BreedingY	0.0836	0.2671	0.31	0.098	1	0.754
	244.8 (Δ4.57)	4	Intercept	13.8108	0.1192	115.9			
			Elevation	0.0356	0.1179	0.3	0.091	1	0.762
	245.8 (Δ5.56)	5	Intercept	13.8076	0.3398	40.64			
			SexM	-0.0759	0.3270	-0.23	0.054	1	0.817
			BreedingY	0.0904	0.2708	0.33	0.111	1	0.739

Ruddy-capped Nightingale-Thrush *Catharus frantzii*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	43.6	4	Intercept	15.4224	0.3667	42.05			
			Elevation	-0.1235	0.3610	-0.34	0.117	1	0.732
Null Model	38.2 (Δ0)	3	Intercept	15.4385	0.3429	45.02			

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Chestnut-capped Brushfinch *Arremon brunneinucha*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	99.7	9	Intercept	13.9593	0.5258	26.55			
			Elevation	-0.1634	0.2595	-0.63	0.396	1	0.529
			SexM	-0.6517	0.6522	-0.99	0.071	2	0.965
			SexU	-0.7622	0.7943	-0.96			
			BreedingY	-0.9408	0.8062	-1.17	0.246	1	0.620
			SexM* BreedingY	1.6674	0.9485	1.76	4.279	2	0.118
			SexU* BreedingY	2.7631	1.5421	1.79			
Null Model	87.5 (Δ0)	3	Intercept	13.8116	0.1185	116.6			
	90 (Δ2.41)	4	Intercept	13.7484	0.2327	59.09			
			Breeding	0.0836	0.2670	0.31	0.098	1	0.754
	91.6 (Δ4.06)	4	Intercept	13.8108	0.1192	115.9			
			Elevation	0.0356	0.1179	0.3	0.091	1	0.762
	92.2 (Δ4.64)	5	Intercept	13.8649	0.2952	46.97			
			SexM	-0.0628	0.3229	-0.19	0.038	1	0.846

Grey-breasted Wood Wren *Henicorhina leucophrys*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	43.7	5	Intercept	12.8907	0.5042	25.57			
			Elevation	-0.4421	0.2090	-2.12	4.472	1	**0.034**
			BreedingY	0.2201	0.5566	0.39	0.156	1	0.693
Null Model	37.4 (Δ0)	3	Intercept	13.0769	0.2001	65.36			
	38.9 (Δ1.42)	4	Intercept	13.0769	0.1729	75.64			
			Elevation	-0.4053	0.1799	-2.25	5.074	1	**0.024**
	40.8 (Δ3.35)	4	Intercept	13.3333	0.5261	25.34			
			BreedingY	-0.3030	0.5720	-0.53	0.281	1	0.596

Common Bush Tanager *Chlorospingus flavopectus*

	AICc	df	Variable	Estimate	SE	*t*-value	x^2	df	*p*-value
Full Model	157.2 (Δ2.84)	7	Intercept	14.1179	0.2955	47.78			
			SexM	-0.1215	0.3756	-0.32	2.218	2	0.330
			SexU	-0.9096	0.6091	-1.49			
			BreedingY	-1.3179	0.6823	-1.93	3.697	1	0.055
			SexM* BreedingY	0.8622	0.8087	1.07	1.136	2	0.286
Null Model	154.4 (Δ0)	3	Intercept	13.8245	0.1577	87.69			
	154.5 (Δ0.1)	4	Intercept	13.9568	0.1732	80.60			
			BreedingY	-0.6520	0.3644	-1.79	3.202	1	0.073
	157 (Δ2.6)	6	Intercept	14.0104	0.2751	50.93			
			BreedingY	-0.7445	0.3699	-2.01	4.052	1	**0.044**
			SexM	0.0557	0.3336	0.17	2.236	2	0.327
			SexU	-0.8021	0.5993	-1.34			
	157.5 (Δ3.1)	5	Intercept	13.8708	0.2754	50.37			
			SexM	0.0151	0.3449	0.04	1.368	2	0.505

Table S3.3 Tukey post-hoc comparison tests of blood haemoglobin concentrations (H_b; gd/L) between species per season from a linear mixed effect model of H_b as a function of species. Significant comparisons are emboldened and acronyms follow those presented in main text (Ruddy-capped Nightingale-Thrush *Catharus frantzii*, RCNT; Black-headed Nightingale-Thrush *Catharus mexicanus*, BHNT; Chestnut-capped Brushfinch *Arremon brunneinucha*, CCBF; Grey-breasted Wood Wren *Henicorhina leucophrys*, GBWW; Common Bush Tanager *Chlorospingus flavopectus*, COBT).

Species comparison	Estimate	SE	z-value	*p*-value
Summer (breeding season)				
RCNT - CCBF	1.7191	0.4258	4.038	**<0.001**
BHNT - CCBF	0.0322	0.3117	0.103	1.000
COBT - CCBF	-0.5121	0.4216	-1.215	0.733
GBWW - CCBF	-0.7697	0.4216	-1.826	0.347
BHNT - RCNT	-1.6869	0.3493	-4.829	**<0.001**
COBT - RCNT	-2.2312	0.4501	-4.957	**<0.001**
GBWW - RCNT	-2.4888	0.4501	-5.529	**<0.001**
COBT - BHNT	-0.5443	0.3442	-1.581	0.497
GBWW - BHNT	-0.8019	0.3442	-2.330	0.130
GBWW - COBT	-0.2576	0.4462	-0.577	0.977
Winter (non-breeding season)				
BHNT - CCBF	0.1762	0.3509	0.502	0.955
COBT - CCBF	0.3999	0.3149	1.270	0.559
GBWW - CCBF	-0.2238	0.7613	-0.294	0.990
COBT - BHNT	0.2237	0.2782	0.804	0.841
GBWW - BHNT	-0.4000	0.7469	-0.536	0.946
GBWW - COBT	-0.6237	0.7306	-0.854	0.816

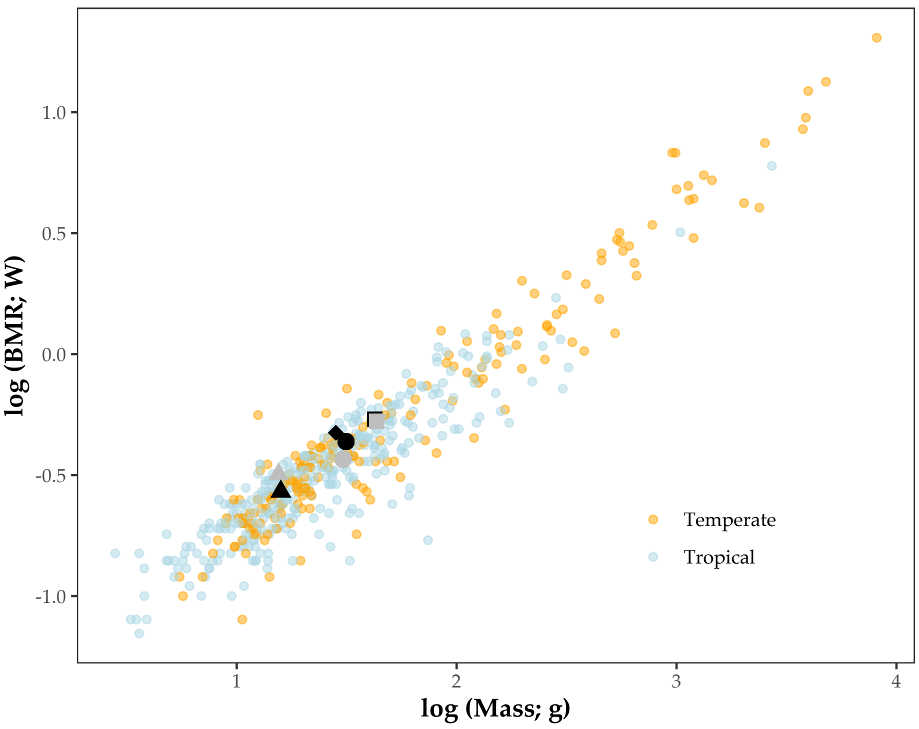

Figure S3.1 Basal metabolic rate (BMR; Watts) as a function of body mass (g) (both log-transformed) for tropical and temperate birds using data from Londoño et al. (2015a, 2015b). Species in this study (split for summer and winter for purposes of visual comparison) are presented as per **Figure 3.3** in main text for comparison to BMR data globally (using the same symbols per species and winter/summer shades). One extreme outlier was removed from this dataset (Common Ostrich *Struthio camelus*) for better visual comparison.

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4 PRIMED FOR PERFORMANCE? METABOLIC RESPONSES TO CONSPECIFIC SONG REFLECT SEASONALITY IN TERRITORIAL AGGRESSION IN A TROPICAL SONGBIRD

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4.1 ABSTRACT

Metabolic rates govern the energy expenditure of virtually all activities. In birds, much research has focused on how flexibility in metabolic rate covaries with energetically costly activities at different times of year, particularly in temperate species. In tropical species, however, annual cycles of energy expenditure are far less understood. We studied territorial defence behaviour across seasons in male Black-headed Nightingale-Thrushes *Catharus mexicanus,* a sedentary tropical montane songbird that upregulates basal metabolic rate (BMR) in the breeding season. We sought to identify if this shift in BMR was a product of the increased energetic demands of territoriality. To do this, we measured territorial defence behaviour in the field (through song rates and aggressive behavioural responses to playback experiments), and also the metabolic consequences of exposure to conspecific song in respirometry chambers between breeding and non-breeding seasons. Territorial defence behaviour was substantially higher in the breeding season. Concurrently, metabolic rates were also substantially higher during exposure to conspecific song in the breeding season indicating a functional link as to how birds engage in territorial aggression by audible recognition between season. Our results support a role of energetically costly territorial defence resulting in seasonal increases in BMR in the breeding season in order to meet the 'performance' demands of territorial defence. Taken together, this provides a rare insight into how territorial competition and metabolic rate may interact in tropical birds.

4.2 INTRODUCTION

Metabolic rate – the pace energy is allocated to competing functions – determines the pace at which virtually all biological activities occur (Brown *et al.* 2004). As such, life-history traits and physiology are inextricably related, where physiological control mechanisms link the fitness performance of an organism to its environment by allocating energy to competing activities generally linked to either reproduction or self-maintenance (Ricklefs & Wikelski 2002). Because the physiological state of an organism is rarely static, the phenotypic expression of metabolic rates can be highly variable (phenotypic flexibility) in order to meet the energetic requirements of different components of the annual lifecycle, particularly in variable environments (Jacobs & Wingfield 2000, Piersma & Drent 2003, McNamara & Houston 2007, Piersma & van Gils 2011). For example, in birds, energy may be managed such that increases in metabolic rate enhance metabolic endurance for long-distance migrations (Piersma *et al.* 1995, 1996, Lindström 1997), facilitate the energetic demands of rapid flight feather moult (Portugal *et al.* 2007, 2018), or increase heat conservancy in cold winters (O'Connor 1996, Swanson & Olmstead 1999, Swanson 2010, Smit & McKechnie 2010).

In songbirds, territorial defense – typically through signaling behaviour (song bouts) – represents an important tradeoff between the fitness benefits of holding a territory (e.g. increased pair bonds and provisioning for chicks), and the energetic costs incurred from defending territorial space (e.g. increased locomotor activity, reductions in foraging time and increased likelihood of physical conflicts) (Hinde 1956, Brown 1964, Davies 1980, Davies & Krebs 2009). A variety of studies have displayed metabolic costs associated with territory defense, such as reductions in condition following intense territorial singing and mate guarding (Thomas 2002, Thomas *et al.* 2003, Low 2005), physical conflicts (Briffa & Sneddon 2007, Viera *et al.* 2011), lower territorial aggression due to physiological state (Godfrey & Bryant 2000, Thomas & Cuthill 2002), and higher metabolic rates associated with aggressive dominance (Senar *et al.* 2000). However, despite evidence displaying the physiological implications of territorial defense, this has generally been considered over short time scales (e.g. instantaneously or within season; Wolf & Hainsworth 1971, Godfrey & Bryant 2000, Ward *et al.* 2004). Consequently, the relationship between territorial behaviour and seasonal flexibility in metabolic rates has received little attention.

The relationship between metabolic rates and territorial competition may be complex because increases in energy are required in order to meet the performance demands of maintaining territorial defense at the same time as costly energetic requirements for self-maintenance (Nilsson 2002, Gil & Gahr 2002, McNamara & Houston 2007, Mathot & Dingemanse 2015). As such, the longer-term energetic budgets of territorial behaviour may be diverse when considering the variation in territorial systems in birds across latitude (Goymann *et al.* 2004, Hau *et al.* 2010, Salisbury *et al.* 2012, Tobias *et al.* 2016). For northern latitude species, territorial activity is usually temporary, where territories are deserted after short breeding seasons, such that energy expended on territorial defense is likely to be short term. In tropical species, however, territorial occupation is frequently long term, often year round, posing an intriguing question as to how energy budgets are managed sustainably across the year in order to facilitate prolonged periods of territorial defense.

Potential links between metabolic rates and life-history traits in tropical birds have been hypothesized (Ricklefs & Wikelski 2002, Anderson & Jetz 2005, Williams *et al.* 2010). An increasing body of work has indicated that both peak, and basal metabolic rates (PMR/BMR; the lower and upper limits of metabolic power output, respectively) of tropical birds vary substantially across the year (Wells & Schaeffer 2012, Pollock *et al.* 2019), but that the directionality of this seasonal variation is inconsistent between species (McKechnie *et al.* 2015). This contrasts with resident temperate species, where colder seasonal conditions generally manifest in upregulation of metabolic rates as an adaptive response to maintain high body temperatures (Smit & McKechnie 2010, Swanson 2010, McKechnie *et al.* 2015). Anderson & Jetz (2005) postulated that tropical birds may have greater seasonal variation in metabolic energy expenditure because more seasonally benign conditions in the tropics place fewer constraints on energy allocation, where metabolic rates are instead driven by the intrinsic characteristics of a species. However, despite the suggestion of functional relationships between metabolic rates and life-history characteristics in tropical birds (e.g. Londoño *et al.* 2015, Scholer *et al.* 2019), direct links between the two have rarely been demonstrated.

Here, we test whether metabolic rate is related to territorial behaviour in a highly territorial songbird species of Central American montane forests; Black-headed Nightingale-Thrushes *Catharus mexicanus* (hereafter, BHNT). BHNT up-regulate their BMR in the summer breeding season (~19% higher than winter/non-breeding BMR), but display no distinct differences in physiological condition or body mass (**Chapter 3**). Thus, to investigate the relationship between metabolic rate and territoriality, we firstly assessed whether territorial aggression also increased with season, concurrent with the directionality of changes in BMR. Secondly, we tested whether metabolic rate is functionally related to territorial aggression by directly measuring the rate of oxygen consumption – a standard proxy of metabolic rate – of individuals during exposure to conspecific playback during both breeding and non-breeding seasons.

This approach allows us to test competing energy management strategies suggested by Mathot & Dingemanse (2015). On one hand, territorial aggression could represent an energetically costly activity, such that it covaries with BMR as a reflection of increased ability to mobilise energy; the *performance* model (e.g. Drent & Daan 1980, Hammond & Diamond 1997). On the other hand, territorial aggression could represent an energetic cost that requires re-allocation of energy within the overall energetic budget, subsequently conferring reductions in BMR because of lengthy periods of energetically costly activities; the *allocation* model (e.g. Wikelski *et al.* 1999, Vézina *et al.* 2006, Larivée *et al.* 2010). Therefore, we predicted that if BHNT increase BMR as a result of the performance requirements (enhanced metabolic endurance) required for long-term territorial defense, then territorial aggression should be lower in the non-breeding season. We also predicted that if there was a functional relationship between metabolic rate and territorial aggression, then BHNT should display metabolic responses to recognition of conspecific song that reflects levels of territorial aggression across the year (**Figure 4.1**).

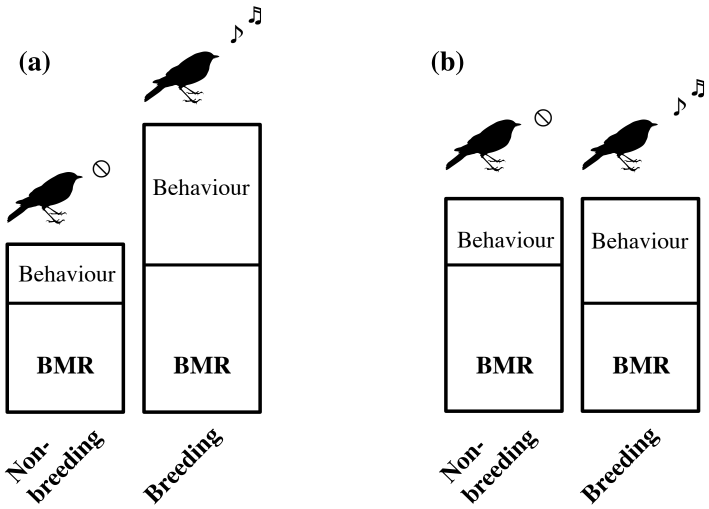

Figure 4.1 Theoretical energy allocation models for seasonal changes in basal metabolic rate (BMR) with territorial behaviour (see Mathot & Dingemanse 2015). In (a), the '*performance'* model, BMR covaries with territorial behaviour, in (b), the '*allocation'* model, energy is reallocated for territorial behaviour and BMR within a fixed budget.

4.3 METHODS

4.3.1 Field site and study species

We studied BHNT in Cusuco National Park, north-western Honduras (approximately 15.552ºN, -88.296ºW) between June-August 2017-2018, and January 2018. BHNT are a common, territorial and easily detectable understorey species with well-defined territories. They are resident year-round at the study site (**Chapter 3**) and occupy a relatively broad elevational range between ~900m-1850m in mixed broadleaf cloud forest (**Chapter 2**). BHNT display life-history traits common to tropical birds (e.g. high adult survival and low clutch sizes; Boyce & Martin 2017) and their breeding season is long, nesting from mid-April to at least late-August (Howell & Webb 1995). The moult cycles of BHNT are little known, but likely start in ~September (e.g. Taylor & Komar 2010) (no birds were found in moult throughout the course of the study). Pair bonds are little known, although individually marked males and females have been observed to occupy the same territories over several years and also associate in the non-breeding season (SEIJ pers obs). Males exclusively defend territories and frequently sing in territorial defence for large portions of the day. Accordingly, this study pertains to males only. All data on territorial behaviour was collected during peak activity periods in the first five hours from dawn (~0530/0630 dependent on season) and the last two and a half hours before dusk (~1530 onwards).

4.3.2 Territory mapping and individual marking

The locations of 28 territory holding males were mapped following methods in Gregory et al. (2004), where territorial singing birds were marked over the course of ~3 weeks and revisited on at least three occasions on separate days (see **Chapter 2** for details). If territorial birds were not present on each visit, they were removed from this sample. Because of the ease of detectability of BHNT during the breeding season, it is unlikely that territorial locations were removed as a result of this protocol. This mapping exercise was repeated throughout the study (both June-August 2017 and 2018), with no new territorial locations emerging in this time.

We captured territorial males by luring them into mist-nets using conspecific playback. Once the bird was captured, a standard set of biometrics (e.g. maximum wing chord, tail length and tarsus length) and body mass (using digital scales- SA-500, SATRUE, Taiwan) were taken and each bird fitted with a uniquely numbered metal band (Aranea, Łódź**,** Poland) and three coloured bands (Yellow, Blue, White or Red; A.C.Hughes, UK) creating a unique combination that was field-readable. The majority of territories were checked in the non-breeding season (n = 23), although logistical constraints meant it was not possible to check them all. Territories were checked in the non-breeding season by two observers systematically walking through the territory ~15m apart in order to observe the territory holding male (because birds were considerably less vocally active and were occasionally seen outside of the immediate territory boundaries). We were not always able to catch and individually mark each territory holder, and in these incidences were only able to confirm the territory as occupied, or not. We still undertook behavioural experiments on some unmarked birds, however. Throughout the course of the study, all territories remained occupied between the breeding and non-breeding season, but territory holders were replaced by new males on 42.8% of territories between seasons.

4.3.3 Vocalisation rates (song and call rates)

We conducted 85 focal watches (non-breeding n = 40, breeding n = 45) of territory holding males to quantify territorial defence activity in vocalisation rates (songs or calls per minute). Focal watches were undertaken on 18 different territories (non-breeding; n = 14, breeding; n = 16) for a comparable total time between seasons (total cumulative focal watch time; non-breeding, 15 hrs 3 mins; breeding 15 hrs 50 mins). The majority of territories were visited on at least 3 occasions on separate days (excepting 5/14 territories in the non-breeding season and 5/16 territories in the breeding season) and for a comparable time per territory between season (mean cumulative focal watch time per territory in minutes ± SD, non-breeding 64.65 ± 31.62; breeding 59.4 ± 30.1). Each focal watch lasted between 10 and 31 minutes (mean 22 ± 4.85 mins), with length occasionally depending on logistical constraints (e.g. sunset). During each focal watch, each vocalisation (song phrase or call) was tallied by hand on datasheets, and the total divided by the focal watch duration to give song/call rate per minute. We separated song and call rates (rather than a sum of total vocalisations) because song is more important in territory defence in the study species, whereas calling is used as a social signal or alarm (see Catchpole & Slater 2003). We continued focal watches irrespective of whether the territory holding bird was counter-singing with neighbouring males or not, but noted these incidences in order to compare song rates when and when not engaged in counter singing. We were not always able to visually observe focal birds during watches, but it is highly unlikely that the territorial holder was mistakenly identified because territory locations are well segregated (see **Chapter 2**).

4.3.4 Territorial aggression

We measured territorial aggression in the non-breeding (*n* = 17 experiments) and breeding seasons (*n* = 22 experiments) using playback experiments that simulated territorial invasions. Experiments were only conducted in the non-breeding season after the target territory had been confirmed as occupied. Experiments were not undertaken in rainy or windy conditions. If the territory holder was engaged in counter singing with a rival male upon arrival to the focal territory, the experiment was postponed by at least two hours. All playback experiments were undertaken on territory holders either immediately before they were captured for banding, or several months after capture to avoid pre-exposure effects that may influence behavioural responses (e.g. Budka *et al.* 2019).

Playback experiments began by placing a speaker (UE Roll; hereafter 'speaker') ~1m above the forest floor in the approximate centre of the territory (judged by movements of the territory holder during territory mapping). Each experiment consisted of two eight-minute treatments (timed with a handheld stopwatch) each consisting of three-minutes of song playback and five minutes of behavioural observation. Experiments began with a control treatment (to determine whether territory holders were simply responding to any novel sound in their territories), followed by a conspecific treatment of locally recorded song. For control lures, we broadcasted songs of Slate-throated Whitestarts *Myioborus miniatus*, a sympatric unrelated mid-storey foraging species that does not compete with BHNT (see **Chapter 2).** We broadcasted playback lures at an approximately natural volume (~78-84 decibels; adjudged by measuring song lures in the field compared to natural song).

Song lures were free from background song, but were occasionally amplified or filtered for clarity. To minimise the effects of pseudoreplication (see Kroodsma 1989), we used multiple song lures (control *n* = 6, conspecific *n* = 11) which were numbered arbitrarily and used in numerical sequence throughout the experiments. The majority of these playback lures (recorded by SEIJ) have been archived on the online avian sound repository xeno-canto (recording set: https://www.xeno-canto.org/set/5584).

We quantified territorial aggression by measuring the following behavioural responses to playback treatments; closest approach to speaker, latency to approach speaker, latency to first vocalisation, number of vocalisations, and time spent within 15 m of the speaker. Closest approach was measured between 0 m (a bird perched on top of the speaker) and bounded to 15 m (the distance generally at which it was not possible to observe behavioural responses through the forest understorey). Close approaches were first estimated by eye, and then checked for accuracy by pacing out the distance after the termination of experiments. Latency to approach and latency to vocalise were measured in seconds elapsed from the initiation of the playback treatment to approach to within 15 m of the speaker and first vocalise in response, respectively. Number of vocalisations was a tally of all individual call and song phrases within this 15 m radius, and time spent within 15 m radius of the speaker was total time elapsed from first approach, until leaving this area (we recorded no incidences of a focal bird leaving the 15 m radius and re-returning). Responses were observed from a concealed position roughly 10 m from the speaker.

4.3.5 Metabolic responses to playback

We undertook comparable playback experiments to simulated territorial intrusions in respirometry chambers (*n* = 10 non-breeding, *n* = 10 breeding, two of which where repeated between seasons) in order to measure metabolic responses in territorial males to competitor song. We measured energy metabolism as rates of oxygen (O_2) consumption ($\dot{V}O_2$ ml/min) using a field portable open-flow respirometry system (FoxBox, Sable Systems, USA). Air was pulled through a custom-built respirometry chamber (see below) at a rate of 1000 ml/min (controlled via linearised mass flow meter), a rate at which O_2 concentrations were maintained above 20.5%. Air was dried (Silica gel, GeeJay chemicals, UK) downstream of the chamber before entering the gas analysers (sampling at 1/sec). Baseline traces of ambient air (20.95% O_2) were taken at either end of the experiment to correct for drift in the analysers, and lag-corrected to match response time of the system. We calculated $\dot{V}O_2$ using equation 11.7 in (Lighton 2008), and corrected baselines using Sable Systems ExpeData. We then converting $\dot{V}O_2$ to Watts using a joule equivalent of 20.1 (Gessaman & Nagy 2008), (hereafter 'metabolic rate'). All birds used for these experiments were captured in the afternoon/evening and were roosted overnight in cloth bags before undertaking experiments on the following day. As such, all birds were in a post-absorptive state, meaning that the metabolic rates we measured could not be due to variation in digestive patterns arising from seasonal differences in food availability. We subsequently transferred birds to the respirometry system at, or before first light for experiments.

Birds were placed in a custom-built 20 x 20 cm^2 Perspex respirometry chamber (hereafter 'chamber') fitted with a dowel perch, and covered with a dark cloth during the experiment. The dark cloth did not completely exclude light to the chamber, but darkened its interior significantly. Temperature inside the chamber was not controlled during experiments, but was 17 °C ± 1 (measured by a remote logger; HOBO UA-001-64, Onset, USA), approximately matching daytime mean temperature at the study site (see **Chapter 3**). The chamber was placed inside a larger box lying on its side, with the box being open at one end in order to reduce ambient sound reaching the chamber. Experiments were undertaken in a quiet room in a small outbuilding at the field site to remove ambient noise. No movement or noise occurred in the room other than the playback treatment during experiments.

Playback experiments began after at least 20 minutes of acclimatisation after placing the subject bird in the chamber. If the bird was still not quiescent (judged by stable $\dot{V}O_2$ with minimal fluctuations) after 45 minutes, the experiment was abandoned. Each bird was then subjected to two experimental playback treatments, one of a control species (Slate-throated Whitestarts) and one of conspecific song, the order of which was alternated between experiments. Song playback was broadcast to birds from the speaker placed 1.5 m from the chamber. The chamber marginally affected broadcast volume, although this was only slightly lower than in the field (between 70-75 decibels). We broadcast playback treatments for five minutes, followed by five minutes of ‘response’ time after termination of the playback treatment. We then allowed a further 5+ minutes of acclimatisation before the next playback treatment was initiated. Comparable to playback experiments in the field, multiple song lures were used for each treatment in order to minimise the effects of pseudoreplication (Kroodsma 1989). We compared metabolic rates during the experiment to a mean three-minute baseline measure of resting metabolic rate (RMR) in the chamber before any experimental playback trials began (RMR is used here because birds were outside of thermalneutrality and during their active circadian phase).

4.3.6 Statistical analysis

All analyses were undertaken in ‘R’ (R Core Team 2016). Values presented are mean ± SD unless otherwise stated. We assessed seasonal differences in vocalisation rates, territorial aggression, metabolic responses to playback and RMR (before playback) with linear mixed effects models fitted using the *lme4* package (Bates *et al.* 2014), where focal individual was fitted as a random effect throughout different models because of repeated measures in each dataset. Significance of the fixed effects were assessed through Wald-Chi square tests conducted using the *car* package (Fox *et al.* 2013), and seasonal effects between experimental treatments were compared by Tukey post-hoc comparison tests (controlled for multiple testing) using the *emmeans* package (Lenth 2016).

For vocalisation rates, song and call rate per focal observation was modelled as a function of season (a two-way factor of breeding/non-breeding) and length of the focal watch (in minutes) as fixed effects, and bird identity (the territory holder) as a random effect. We detected no effect of focal watch duration on song or call rates measured in these models (song rate; $\chi^2_1 = 0.50$, $p = 0.48$, call rate; $\chi^2_1 = 0.53$, $p = 0.47$), but still retained this term as a nuisance variable when examining seasonal effects on vocalisation rates.

For territorial aggression (playback experiments in the field), we first collapsed the five behavioural responses to playback treatments into a single composite aggression score via a principal component analysis (PCA). Because different measurement units were used to measure behavioural responses (m and s, respectively) we scaled variables within the PCA. The first axis (PC1) explained the majority of variance (83%) (PC1 loadings: close approach to speaker -0.45, latency to approach -0.46, latency to vocalise -0.44, number of vocalisations 0.42, and time in proximity to the speaker 0.46) with higher PC1 scores reflecting closer and faster approaches with birds staying in proximity to the speaker for longer, and singing in response. Accordingly, we took this composite score (PC1) as an index of territorial aggression. We modelled these PC1 values as a function of an experimental trial type (control or conspecific) × season (a two-way factor of breeding/non-breeding) interaction term, bird identity (the territory holder) as a random effect.

For metabolic responses to playback, in order to standardise measures between birds we first converted metabolic rates during playback experiments to relative delta values (in Watts) as a fraction of pre-playback RMR (delta 1). Delta values (where increases were multiples of RMR) were then averaged within each trial; five minutes during playback (control or conspecific, respectively) and the immediate five minutes after playback was terminated ('response'). We then modelled these delta values as a function of playback treatment (a five-way factor comprising the baseline metabolic rate before playback, metabolic rates during conspecific/control playback and metabolic rates immediately following conspecific/control playback, respectively), breeding season (non breeding or breeding), a treatment × breeding season interaction term, and the trial order (whether control or conspecific playback was played first). We included individual bird identity as a random effect. After constructing this model, we first assessed the effect of trial order on metabolic responses to playback (i.e. if playing the conspecific or control trial first rendered them more likely to respond to the subsequent treatment). We then compared metabolic rates between baselines and playback treatments within season, and then between the two seasons.

To better visualise the predicted trajectories of metabolic rate for breeding and non-breeding birds in response to playback treatments, we then fitted a general additive mixed model with Gaussian error in the mgcv package (Wood 2017). This model was fitted with a status-specific smoother to allow for breeding/non-breeding specific trajectories across the experiment. Breeding status was also specified as a categorical (non-smoothed) term in the fixed effects to allow for differences in intercept between the breeding and non-breeding season. To fit these models, the data were re-ordered, such that control trials were followed by conspecific trials.

We noted that metabolic rates during chamber playback experiments sometimes drifted below pre-playback RMR (giving negative delta values). Because this may influence the magnitude of metabolic responses (because the ‘starting point’ for the second treatment is already negative), we repeated the described analysis, but this time calculating delta metabolic rate for each playback treatment from separate baseline RMR measures – one before playback began, and a second between the playback treatments – where delta metabolic rates for the playback treatment was calculated against its preceding RMR baseline. RMR taken between playback treatments was calculated in the same way as pre-playback RMR, and was taken at least 10 minutes after the termination of the preceding playback treatment. In this analysis, we detected an effect of treatment order ($\chi^2_1 = 6.34$, $p = 0.01$), indicating that baseline RMR between playback treatments was slightly higher than baseline RMR before any playback was broadcasted. Our results from this analysis were comparable to the original analytical approach, however, we present results from our initial analysis as the most conservative estimate, but provide this repeated analysis in the supplementary material.

4.4 RESULTS

4.4.1 Vocalisation rates (song and call rates)

Singing in territorial defence (song phrases per minute; SPM) was considerably higher during the breeding season (4.24 ± 2.84 SPM) than in the non-breeding seasons (0.08 ± 0.32 SPM) ($\chi^2_1 = 80.19$, $p = <0.001$; **Figure 4.2**(a)). In the breeding season, song rates appeared marginally higher when birds were engaged in counter-singing bouts with neighbours (counter-singing, 5.14 ± 2.83 SPM; not counter-singing, 3.83 ± 1.86 SPM), but were not significantly different ($\chi^2_1 = 1.92$, $p = 0.17$). By contrast, we found no difference in call rates (calls per minute; CPM) of territory holders in the breeding (0.28 ± 0.8 CPM) and non-breeding seasons (0.18 ± 0.57 CPM) ($\chi^2_1 = 0.22$, $p = 0.64$). Together this indicates that call rates (serving a social function) remain similar year-round, whereas song rates (territorial defence function) differ with respect to breeding season.

4.4.2 Territorial aggression

Territorial aggression directed towards conspecific playback trials was lower in the non-breeding season, than in the breeding season (t-ratio = -11.65, $p = <0.001$), but aggressive responses to control trials were comparable between seasons (t-ratio = 0.48, $p = 0.64$) (**Figure 4.2(b)**). Aggression was still greater in response to conspecifics than control trials in both seasons (non-breeding, t-ratio = 2.25, $p = 0.03$; breeding, t-ratio = 15.9, $p = <0.001$). Taken together, birds were substantially more aggressive during the breeding season, but still displayed some territorial aggression in the non-breeding season.

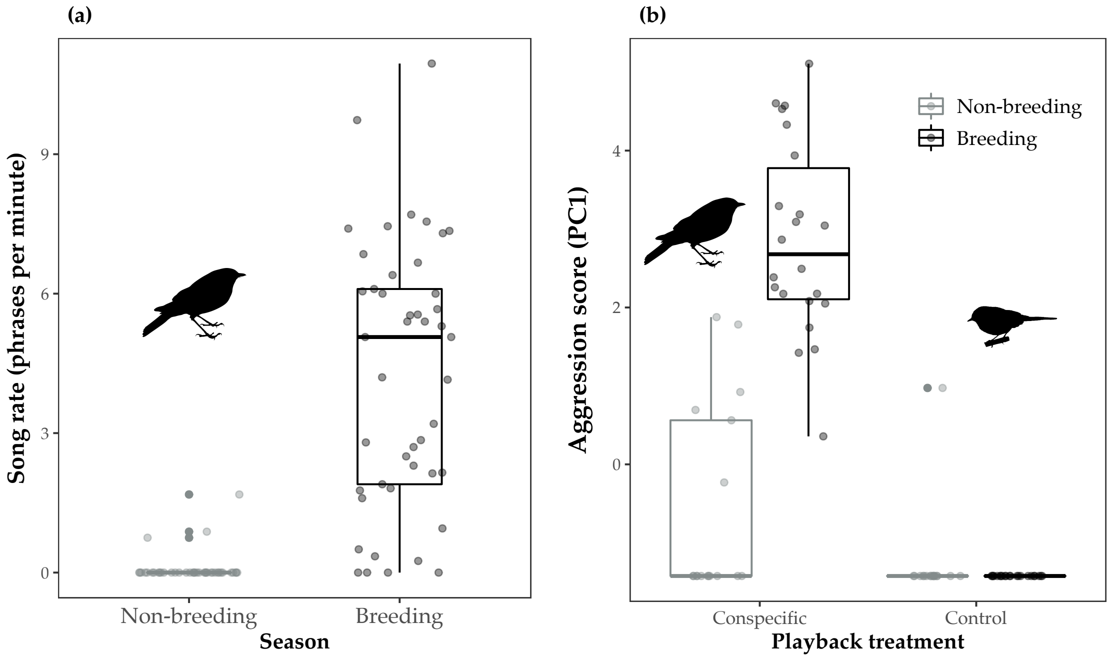

Figure 4.2 Territorial behaviour between seasons in male Black-headed Nightingale-Thrushes *Catharus mexicanus*. Song rates (a) represent the mean number of song phrases per minute from each focal watch in the non-breeding (*n* = 40 focal watches for 15 hrs 3 mins observation time across 14 territories) and breeding seasons (*n* = 45 focal watches for 15 hrs 50 mins observation time across 16 territories). Territorial aggression (b) represents responses to playback experiments between the non-breeding (*n* = 17 experiments) and breeding season (*n* = 22 experiments), where aggression scores (PC1) are an index of behavioural responses to playback treatments, with higher scores denoting higher levels of territorial aggression. Boxplots in both panels display the median and interquartile range.

4.4.3 Playbacks in chambers

We found no effect of playback order on metabolic responses to playback ($\chi^2_1 = 2.25$, $p = 0.13$) (i.e. the order of conspecific or control treatment did not pre-dispose the bird to greater metabolic responses to either playback trial) but we adopted a conservative approach and retained this term in the model for subsequent analysis.

i) RMR pre-playback

We found no difference in RMR (before playback treatments began) between the non-breeding season (0.56 W ± 0.17) and breeding season (0.65 W ± 0.13) (**Table 4.1)** ($\chi^2_1 = 2.02$, $p = 0.15$) (**Figure S4.1**).

ii) Comparisons of metabolic rate in playback treatments between season

Metabolic rates (delta Watts; dW) during conspecific playback trials (i.e. the five minutes while conspecific playback was being broadcasted) were significantly lower in the non-breeding season (1 ± 0.16 dW), compared to the breeding season (1.13 ± 0.13 dW) ($p = <0.01$; **Table 4.1**). For all other trials, however (after conspecific playback was terminated, and for the whole duration of the control trial), metabolic rates were comparable between seasons (all $p = \geq 0.18$, **Table 4.1**; **Figure 4.3/4.4**).

Table 4.1 Metabolic rates during playback experiments in the non-breeding and breeding seasons (mean values over five minutes of each respective trial). Both raw values (Watts), and 'Delta Watts' (difference from resting metabolic rates pre-playback; RMR) are presented. Statistical test results are pairwise least squares post-hoc comparison tests on linear mixed effects models of metabolic rates (Delta Watts) in response to playback treatments between seasons. 'During' playback treatment represents metabolic rate for five minutes while playback was being broadcast, and 'post' playback treatment represents five minutes immediately following termination of playback. Statistically significant results are emboldened. Test statistics for comparisons 'Pre-playback RMR' are not presented here for clarity (because they are from raw values), but are presented in text. Post-hoc comparison tests comparing delta watts metabolic rates between each of the treatments are presented in **Table S4.1.**

Trial	Non-breeding		Breeding			
	Watts	**Delta Watts**	**Watts**	**Delta Watts**	**t-ratio**	***p***
Pre-playback RMR	0.56 ± 0.17	1.00	0.65 ± 0.13	1.00	-	-
During control	0.58 ± 0.17	1.04 ± 0.21	0.66 ± 0.19	1.02 ± 0.08	0.07	0.95
Post control	0.57 ± 0.20	1.02 ± 0.20	0.62 ± 0.13	0.95 ± 0.08	0.73	0.47
During conspecific	0.55 ± 0.13	1.00 ± 0.16	0.73 ± 0.12	1.13 ± 0.13	**-3.11**	**<0.01**
Post conspecific	0.54 ± 0.13	0.99 ± 0.13	0.67 ± 0.13	1.03 ± 0.10	-1.36	0.18

iii) Comparisons of metabolic rates in playback treatments within season

In the non-breeding season, metabolic rates during playback trials (the five minutes both during playback and after playback was terminated) did not differ from resting baseline values (0.98 - 1.04 dW, **Table 4.1/Figure 4.3**; all *p* = ≥0.83) or when compared between trials (all *p* = ≥0.64).

In the breeding season, however, metabolic rate during the conspecific playback trial (i.e. metabolic rates during the five minute treatment of conspecific song) was significantly higher than resting baseline values (1.13 dW, **Table 4.1**; *p* = 0.01), but in all other trials (after playback was terminated in the conspecific trial and during/after playback in the control trial) where similar to baselines (0.96 - 1.03 dW **Table 4.1**; all *p* = ≥0.85; **Figure 4.3).** Metabolic rates between trials in the breeding season were largely comparable (all *p* = ≥0.12), except during control trials and during conspecific trials (*p* = 0.06) and after the termination of control playback and during conspecific playback (*p* = <0.01). For full results of all contrasts between treatments see **Table S4.1**.

Taken together, BHNT showed clear spikes in metabolic rate in recognition to competitor male song in the breeding season, but not in the non-breeding season (**Figure 4.3/4.4** also see **Figure S4.2**). These results were comparable, but with slightly greater magnitudes of change when we ran analyses using a second baseline between treatments (see chapter supplements for replicated results) indicating that drifting delta values (i.e. drifting into negative dW) did not significantly influence our results.

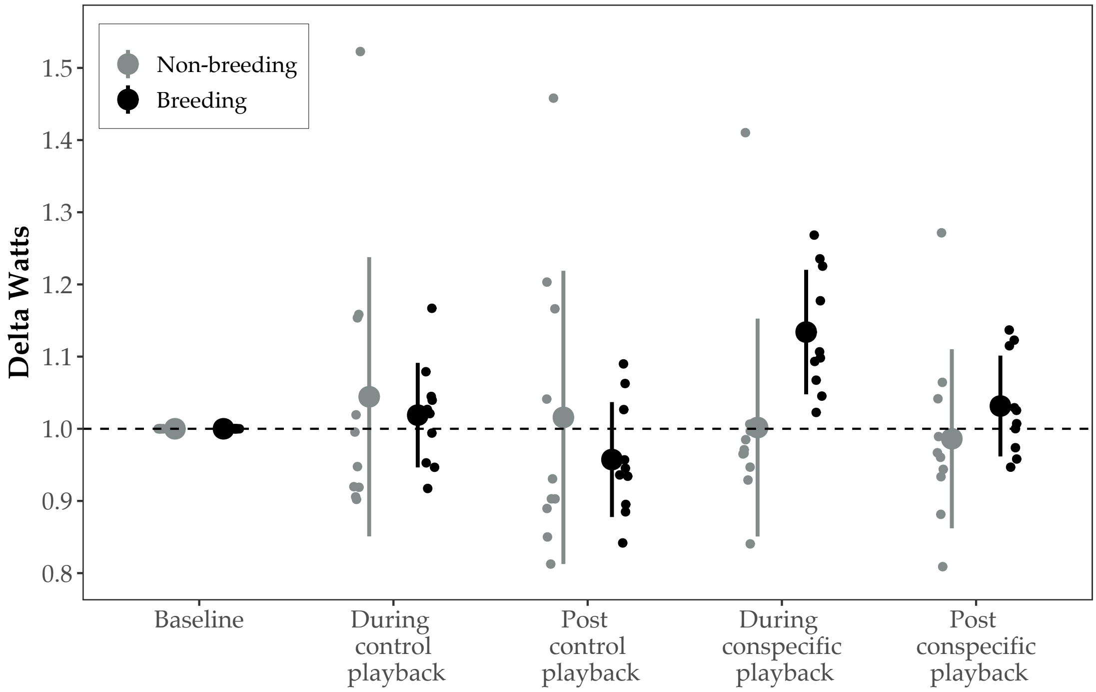

Figure 4.3 Delta metabolic rates during experimental playback treatments in male Black-headed Nightingale-thrushes *Catharus mexicanus* (*n* = 10 each for breeding/non-breeding season). Values are presented as mean (± SD) delta metabolic rate (in Watts) over five minutes either during (while playback was being broadcast) or post (the immediate five minutes of 'response' following termination of the respective playback trial). Delta metabolic rates are calculated per bird from values before any playback was broadcast.

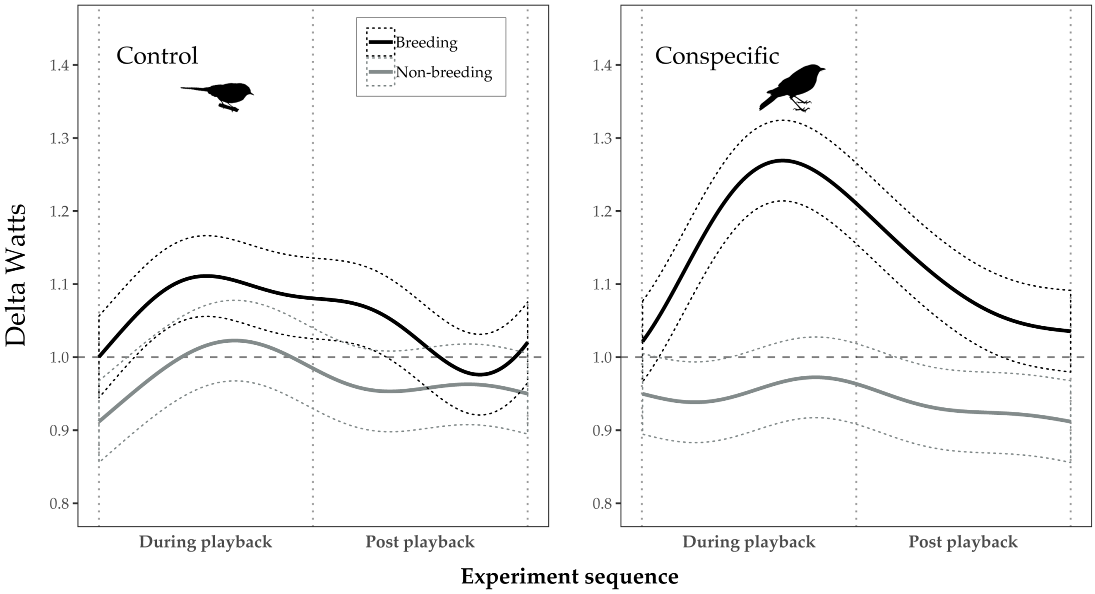

Figure 4.4 Predicted responses in metabolic rate to control and conspecific playback in male Black-headed Nightingale-Thrushes *Catharus mexicanus* (*n* = 10 non-breeding, 10 breeding). Delta metabolic rate (Watts) is calculated as difference from baseline values (delta 1) before any playback began where increases represent multiples in resting metabolism (RMR) before any playback began. Predicted fits (± SEM) are from general additive mixed models with a status specific smoother (non-breeding/breeding season, respectively). Each playback trial (during and post playback, respectively) represents five-minute intervals of second by second measurements of O_2 consumption ($\dot{V}O_2$).

4.5 DISCUSSION

The central aim of this study was to assess whether seasonal changes in BMR are related to territorial activity in male BHNT, and whether these differences could be functionally linked to territorial defence. We found support for both our hypotheses. Firstly, despite year-round territorial occupation, territorial defence was considerably higher in the breeding season compared to the non-breeding season, in line with the directionality of changes in BMR (**Chapter 3**). Secondly, we displayed a mechanistic link between recognition of conspecific song and metabolic rate, where recognition of conspecifics caused increases in metabolic rate in the breeding season, but not the non-breeding season. Taken together, this suggests that territorial defence may be the basis of seasonal increases in BMR as a product of the increased energetic requirements for 'performance', where BMR positively covaries with energetically demanding behaviours (Biro & Stamps 2010, Piersma & van Gils 2011, Mathot & Dingemanse 2015).

While our results are supportive of a functional link between metabolic rates and territorial behaviour, it is important firstly to explore exactly the nature of this link. Our finding that the seasonal differences we observed in territorial responses to playback experiments were also mirrored in metabolic responses to recognition to conspecific song is important, because it identifies how BHNT might engage in territorial defence across the year. That signalling behaviour is a fundamental component in avian territorial defence is well established (e.g. Read & Weary 1992), but we are aware of no other studies that have identified the metabolic consequences of recognition to conspecific song. However, recognition of conspecific song has been shown in numerous studies on songbirds to consistently cause neural activity in the auditory forebrain (see Louder *et al.* 2019), in our case manifesting in an instantaneous metabolic response of recognition.

Neural recognition can be adaptive, however, where neural selectivity (recognition of conspecific song) is determined by physiological state (see Phillmore *et al.* 2011, Louder *et al.* 2019). This is consistent with the decreases in territorial aggression and lack of metabolic responses to conspecific song that we observed in the non-breeding season. Although the physiological state of BHNT changes in the breeding season (BMR is upregulated by ~19%), consistent with energy allocation for 'performance', it is probably unlikely that increases in BMR alone cause this increase in territorial aggression. Perhaps the most likely concurrent physiological state shift are increases in androgen levels, particularly testosterone, subsequently changing the extent to which conspecific song evoke responses to playback (**Figures 4.2/4.3/4.4**) (possibly via transcription regulation see Louder *et al.* 2019). During our study, BHNT had enlarged cloacal protuberances in breeding seasons, generally indicative of increased testosterone profiles (e.g. Rödl *et al.* 2004). However, while a relationship between testosterone and territorial aggression is well established in birds (Hau *et al.* 2010, Goymann & Landys 2011), evidence for consistent covariance between BMR and testosterone concentrations is inconclusive (e.g. Wikelski *et al.* 1999, Buchanan *et al.* 2001, Holtmann *et al.* 2016). It is also worthy of note that call rates did not differ between season in our study, indicative of different social and defence functions of these vocalisation types (e.g. Phillmore *et al.* 2011). Taken together, the exact relationship between BMR, androgen profiles, territorial defence, and their environmental triggers (e.g. changes in photoperiod Wikelski *et al.* 2000, Hau 2001), require further experimental investigation, but this level of intricacy was beyond the scope of the current study.

Regardless of the exact mechanism that causes seasonal increases in territorial defence, our results are supportive of the performance model (**Figure 4.2(a)**) where BMR increases with the requirements necessary to maintain metabolic endurance for territorial defence. This result prompts an important question; how might BHNT obtain sufficient energy during the breeding season in order to sustain increased energetic expenditure? One possible explanation for this is the increased intake hypothesis (Nilsson 2002), where a bird is able to ingest and process more food due to increases in the alimentary tract (Piersma & Lindström 1997, Chappell *et al.* 1999, Hammond *et al.* 2000). In support of this theory, the initiation of breeding (~mid April) in BHNT corresponds with the initiation of the wet season, when prey is more available (e.g. Wolda 1989), similar to many other Central American forest species (e.g. Wikelski *et al.* 2000, 2003). Alternatively (although not necessarily mutually exclusive) is that territorial behaviour may vary over short time periods (e.g. day to day), where foraging is prioritised over other activities in order to increase energy intake (Godfrey & Bryant

2000, Spencer & Bryant 2002). While our methods were not at sufficient resolution to offer insight into these possibilities, both merit further investigation.

We suggest that seasonal changes in BMR are a result of increased territorial activity in male BHNT. The instantaneous metabolic cost of song production in birds have not been convincingly displayed to be energetically demanding (e.g. Ward *et al.* 2003, 2004), so it is unlikely that singing itself manifests in increased BMR. However, the intensity of song output and associated locomotor in territorial defense activity may drive increases in BMR (Koteja 2000). Little is known about locomotor activity in tropical forest birds, but one previous study found very low locomotor activity and low BMR in a lowland tracheophone songbird (Spotted Antbirds *Hylophylax naevioides*) (Steiger *et al.* 2009), with year round territorial aggression (Hau *et al.* 2000, 2004). These authors suggested that very low locomotor activity was key in maintaining low energetic expenditure, although whether this is comparable to our study is debatable. Tracheophone songbirds generally have much lower song rates (see Fedy & Stutchbury 2004, Tobias *et al.* 2011) compared to BHNT in the breeding season in our study. BHNT undertake frequent short flights to different singing locations while defending territories (Howell & Webb 1995) and while this has not been quantified, it is likely that their locomotor activity is higher than that by Steiger *et al.* (2009). More fine scale work would clarify this hypothesis, although such data can be challenging to obtain in a fieldwork environment (e.g. heart rate telemetry; Steiger *et al.* 2009).

Our study offers an intriguing insight into the energetic budgets of territorial defense based upon proximity to congeners. Mean metabolic rate during exposure to conspecific song during the breeding season was 0.73 ± 0.12 W, translating to 1.7 × BMR (where breeding season/summer BMR = 0.43 W; see **Chapter 3**). Because we measured these metabolic costs instantaneously, it is challenging to directly translate these into daily energetic budgets, however, sustainable daily energy use for tropical birds has been suggested as between 2-3 × BMR (Jankowski *et al.* 2013). Considering that BHNT occur in close territorial proximity (**Chapter 2**) it is likely that this continual ‘cost’ of exposure to congeners manifests in relatively high proportions of energy use in their daily energetic budgets, particularly when combined with the increased activity costs of territorial defense. Accordingly, one possibility is that this continual energetic impact of territorial defense is traded off against potentially losing territorial space (48% of territorial holding birds were replaced with new birds between breeding season), consistent with the principles of territorial behaviour, where the benefits of holding territorial space outweigh the costs of holding defending territories (Davies & Krebs 2009).

Our study suggests a link between territorial behaviour and seasonal changes in metabolism, but this suggestion must be taken with some caveats. Firstly, in comparing seasonal regulation of BMR in BHNT, we did not compare body temperature (see **Chapter 3**). Reductions in body temperature (shallow hypothermia) is linked to changes in metabolic rate by reducing metabolic heat loss to the environment (McKechnie & Lovegrove 2002); an energy saving strategy that has been suggested to play an role in maintaining low energy expenditure in some tropical species (Steiger *et al.* 2009). We thus cannot rule this out as a possibility also influencing seasonal changes in BMR. This is not mutually exclusive to our hypothesis, but could represent an additional strategy for energy saving. Our finding that RMR was not significantly different between seasons supports this, because birds may gradually change body temperatures at dawn, manifesting in similar daytime RMR (McKechnie & Lovegrove 2002).

A second caveat is that we also cannot account for metabolic costs that might be associated with some territorial holding birds feeding chicks during our study. Increases in activity during chick rearing may result in energetic demands due to workload (Nilsson 2002, Swanson 2010). While male BHNT defend territories for lengthy periods that extend beyond breeding attempts (Howell & Webb 1995), to quantify the exact nesting status of each territorial bird and potential fluxes in their territorial behaviour in relation to breeding was beyond the scope of this study. However, even with some increased energetic cost caused by provisioning for chicks, this does not contrast with the performance hypothesis that our results support. Considering this, it is also important to reiterate that our study pertains only to male birds. Thus, how female BHNT may shift metabolic rates in the breeding season is unknown. The energetic demands of reproduction are likely to be different for female birds (e.g. egg production; Vézina *et al.* 2006, Swanson 2010) and further study would establish a more holistic understanding of seasonal energetic expenditure in the species as a whole (possibly also employing other methods of energy expenditure such as doubly-labelled water to measure field metabolic rates).

The results of our study are particularly intriguing when applied to the broader understand of the physiology of tropical birds. It is well established that tropical birds have lower metabolic rates in comparison to temperate species (Wiersma *et al*. 2007a, b, Londoño *et al*. 2015, Bushuev *et al*. 2017), and life-history traits generally prioritising self-maintenance over higher reproductive output (Ricklefs 1980, Ghalambor & Martin 2001, Jetz *et al*. 2008b, Martin 2015). This 'slower paced' trait divergence has previously been interpreted as phenotypic adaption to the more stable environmental conditions in the tropics (White *et al*. 2007, Jetz *et al*. 2008a), but recent studies have suggested that metabolic rates of tropical species are unrelated to environmental conditions and possibly linked to intrinsic species characteristics (Londoño *et al*. 2015, 2017, Scholer *et al*. 2019). Our results offer support for this, because we link seasonal changes in BMR to life-history characteristics independent of temperature (see **Chapter 3**). This result is consistent with suggestions that the seasonal variation in metabolic traits in tropical birds are a result of species-specific life-history characteristics (Wells & Schaeffer 2012, McKechnie *et al*. 2015, Pollock *et al*. 2019).

In conclusion, our study is the first we are aware of to make a direct link between metabolic rates and territorial activity in a tropical bird and one of few (e.g. Steiger *et al*. 2009) to investigate the metabolic interplay with life-history characteristics in tropical birds. Our result that metabolic rates were related to recognition to congeners in different seasons also provides a novel insight into the metabolic consequences of signalling behaviour. Together, these results provide functional evidence for suggestions that life-history characteristics are linked to changes in metabolic rates (BMR and PMR) with season in tropical species (Wells & Schaeffer 2012, McKechnie *et al*. 2015, Pollock *et al*. 2019). Moreover, our study supports suggestions that life-history characteristics of 'slow paced' tropical species may be intrinsically linked to their physiological characteristics (Ricklefs & Wikelski 2002, Williams *et al*. 2010, Londoño *et al*. 2015).

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4.7 SUPPLEMENTARY MATERIALS

Table S4.1 Pairwise post-hoc comparison tests of metabolic responses to playback treatments for breeding (n = 10) and non-breeding males (n = 10) from a linear-mixed model including the trial order term. Delta metabolic rate (in Watts) is calculated as difference in metabolic rate from baseline value (delta 1) per individual before being subjected to any experimental playback. 'During' playback treatment represents delta metabolic rate for five minutes while playback was being played, and 'post' playback treatment represents five minutes immediately following termination of playback. Statistically significant results are emboldened.

	Contrast	**Estimate**	**SE**	**d.f**	**t ratio**	***p* value**
Non-Breeding	Baseline – during control	-0.0444	0.0424	72.9	-1.047	0.832
	Baseline – post control	-0.0158	0.0424	72.9	-0.372	0.996
	Baseline – during conspecific	-0.0017	0.0424	72.9	-0.040	1.00
	Baseline – post conspecific	0.0138	0.0424	72.9	0.326	0.998
	During control – post control	0.0286	0.0424	72.9	0.675	0.961
	During control – during conspecific	0.0427	0.0424	72.9	1.007	0.852
	During control – post conspecific	0.0582	0.0424	72.9	1.373	0.647
	Post control – during conspecific	0.0141	0.0424	72.9	0.332	0.997
	Post control – post conspecific	0.0296	0.0424	72.9	0.698	0.956
	During conspecific – post conspecific	0.0155	0.0424	72.9	0.366	0.996
Breeding	Baseline - during control	-0.0189	0.0424	72.9	-0.446	0.992
	Baseline - post control	0.0426	0.0424	72.9	1.006	0.852
	Baseline - during conspecific	**-0.1340**	**0.0424**	**72.9**	**-3.161**	**0.019**
	Baseline - post conspecific	-0.0315	0.0424	72.9	-0.744	0.945
	During control - post control	0.0615	0.0424	72.9	1.452	0.597
	During control - during conspecific	-0.1151	0.0424	72.9	-2.715	0.061
	During control – post conspecific	-0.0126	0.0424	72.9	-0.298	0.998
	Post control – during conspecific	**-0.1766**	**0.0424**	**72.9**	**-4.167**	**<0.001**
	Post control – post conspecific	-0.0742	0.0424	72.9	-1.749	0.411
	During conspecific – post conspecific	0.1025	0.0424	72.9	2.417	0.122

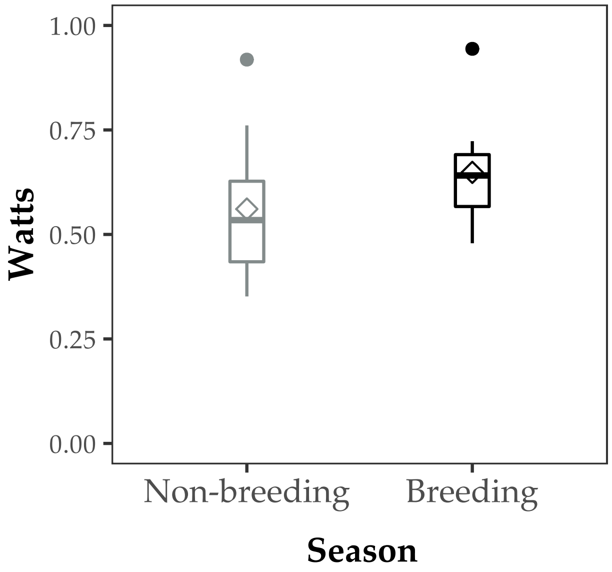

Figure S4.1 Resting metabolic rate (in Watts) pre-playback between non-breeding season (n = 10) and breeding season (n = 10) in male Black-headed Nightingale-Thrushes *Catharus mexicanus*. Diamonds represent mean values and boxplots represent median and interquartile range. Values were comparable between season (no statistical difference was determined).

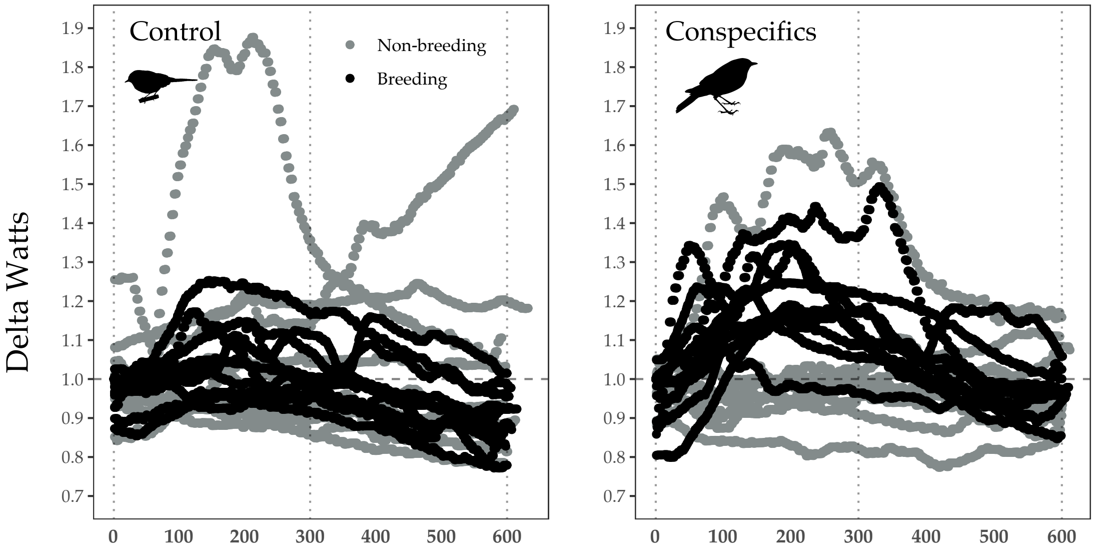

Figure S4.2 Raw data for instantaneous $\dot{V}O_2$ traces (in delta Watts- see Methods) during control and conspecific playback experiments in respirometry chambers between seasons. Traces are presented per individual experiment with dashed lines at (0 – 300 seconds) representing the 5 minutes during playback, and 5 minutes immediately following playback (300 - 600 seconds).

Table S4.2 Metabolic rates during playback experiments in the non-breeding and breeding seasons (mean values over five minutes of each respective trial). Both raw values (Watts), and 'Delta Watts' (difference from resting metabolic rates pre-playback; RMR) are presented. Statistical test results are from pairwise least squares post-hoc comparison tests on linear mixed effects models of metabolic rates (Delta Watts) in response to playback treatments between non-breeding and breeding seasons where the Delta value was taken from the baselines preceding the respective treatment. As such, raw values are the same as **Table 4.1** (main text), but both Delta Watts, and test statistics differ.

Trial	Non-breeding		Breeding			
	Watts	**Delta Watts**	**Watts**	**Delta Watts**	**t ratio**	***p***
During control	0.58 ± 0.17	1.05 ± 0.20	0.66 ± 0.19	1.04 ± 0.10	0.09	0.92
Post control	0.57 ± 0.20	1.02 ± 0.16	0.62 ± 0.13	0.98 ± 0.08	0.71	0.48
During conspecific	0.55 ± 0.13	1.04 ± 0.13	0.73 ± 0.12	1.21 ± 0.18	**-3.70**	**<0.01**
Post conspecific	0.54 ± 0.13	1.03 ± 0.12	0.67 ± 0.13	1.10 ± 0.14	-1.67	0.09

Table S4.3 Pairwise post-hoc comparison tests of metabolic responses to playback treatments for breeding (*n* = 10) and non-breeding males (*n* = 10) from a linear-mixed model including the trial order term. Delta metabolic rate (in Watts) is calculated as difference from baselines (delta 1) preceding the playback treatment (i.e. two baseline values were used per experiment). 'During' playback treatment represents delta metabolic rate for five minutes while playback was being played, and 'post' playback treatment represents five minutes immediately following termination of playback. Statistically significant results are emboldened.

	Contrast	**Estimate**	**SE**	**d.f**	**t ratio**	***p* value**
Non-Breeding	Baseline – during control	0.04796	0.0453	73.5	-1.060	0.826
	Baseline – post control	-0.01475	0.0453	73.5	-0.326	0.998
	Baseline – during conspecific	-0.03712	0.0453	73.5	-0.820	0.924
	Baseline – post conspecific	-0.02345	0.0453	73.5	-0.518	0.985
	During control – post control	0.03321	0.0453	73.5	0.734	0.948
	During control – during conspecific	0.01084	0.0453	73.5	0.239	0.999
	During control – post conspecific	0.02452	0.0453	73.5	0.542	0.983
	Post control – during conspecific	-0.02237	0.0453	73.5	-0.494	0.988
	Post control – post conspecific	-0.00869	0.0453	73.5	-0.192	0.999
	During conspecific – post conspecific	0.01368	0.0453	73.5	0.302	0.998
Breeding	Baseline - during control	-0.04466	0.0424	72.9	-0.987	0.861
	Baseline - post control	0.01803	0.0424	72.9	0.398	0.995
	Baseline - during conspecific	**0.21585**	**0.0424**	**72.9**	**-4.769**	**<0.001**
	Baseline - post conspecific	-0.10457	0.0424	72.9	-2.310	0.153
	During control - post control	0.06269	0.0424	72.9	1.385	0.639
	During control - during conspecific	**-0.17119**	**0.0424**	**72.9**	**-3.782**	**0.003**
	During control – post conspecific	-0.05991	0.0424	72.9	-1.324	0.6775
	Post control – during conspecific	**-0.23389**	**0.0424**	**72.9**	**-5.168**	**<0.001**
	Post control – post conspecific	-0.12260	0.0424	72.9	-2.709	0.062
	During conspecific – post conspecific	0.11128	0.0424	72.9	2.459	0.112

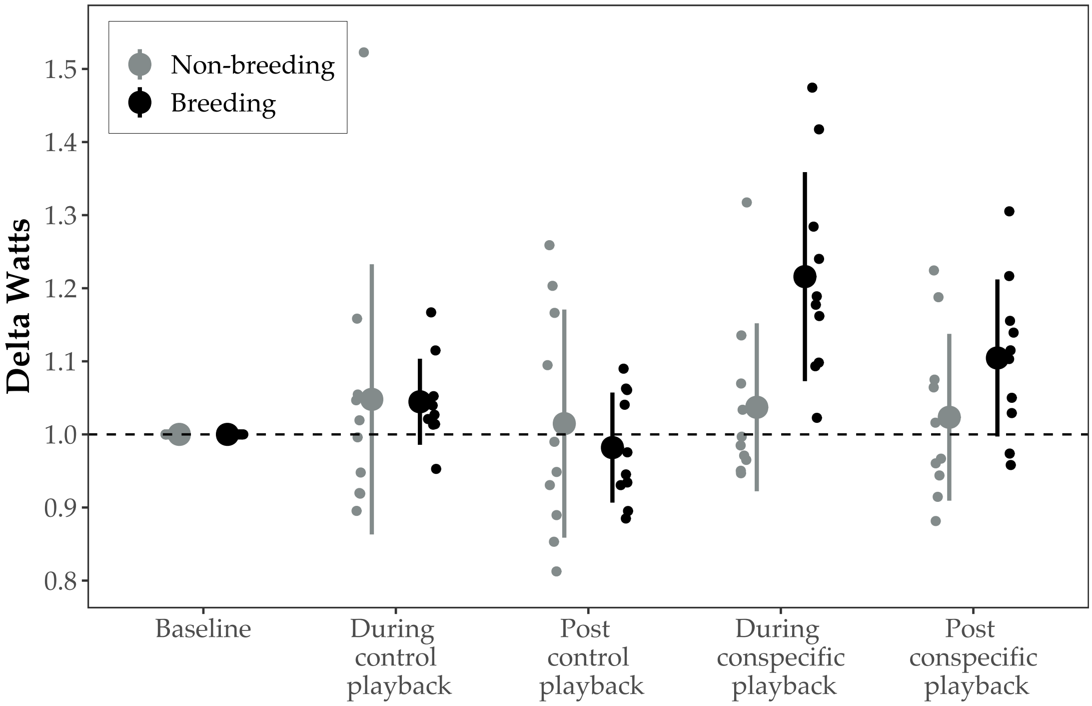

Figure S4.3 Delta metabolic rates during experimental playback treatments in male Black-headed Nightingale-thrushes *Catharus mexicanus* (*n* = 10 each for breeding/non-breeding season), where delta values are calculated from baselines preceding the respective treatment (as opposed to baseline values taken before any playback began). Values are presented as mean (± SD) delta metabolic rate (in Watts) over five minutes either during (while playback was being broadcast) or post (the immediate five minutes of 'response' following termination of the respective playback trial).

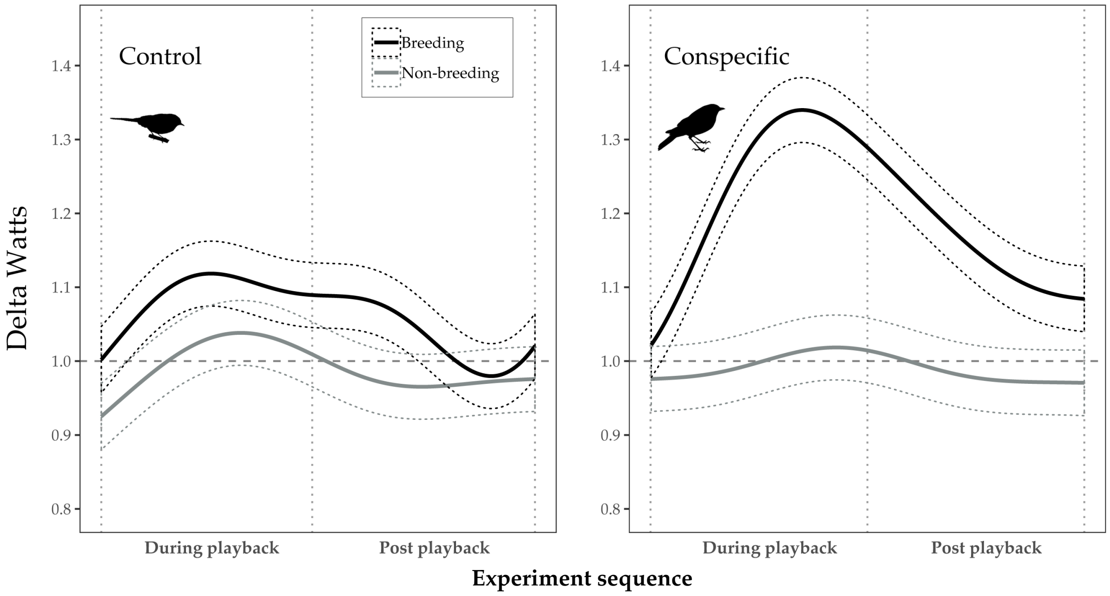

Figure S4.4 Predicted metabolic responses to control and conspecific playback in male Black-headed Nightingale-Thrushes *Catharus mexicanus* (*n* = 10 non-breeding, 10 breeding) using baselines pre-treatment. Predicted fits (± SEM) are from general additive mixed models with a status specific smoother (non-breeding/breeding season, respectively). Each playback trial (during and post playback) represents five-minute intervals.

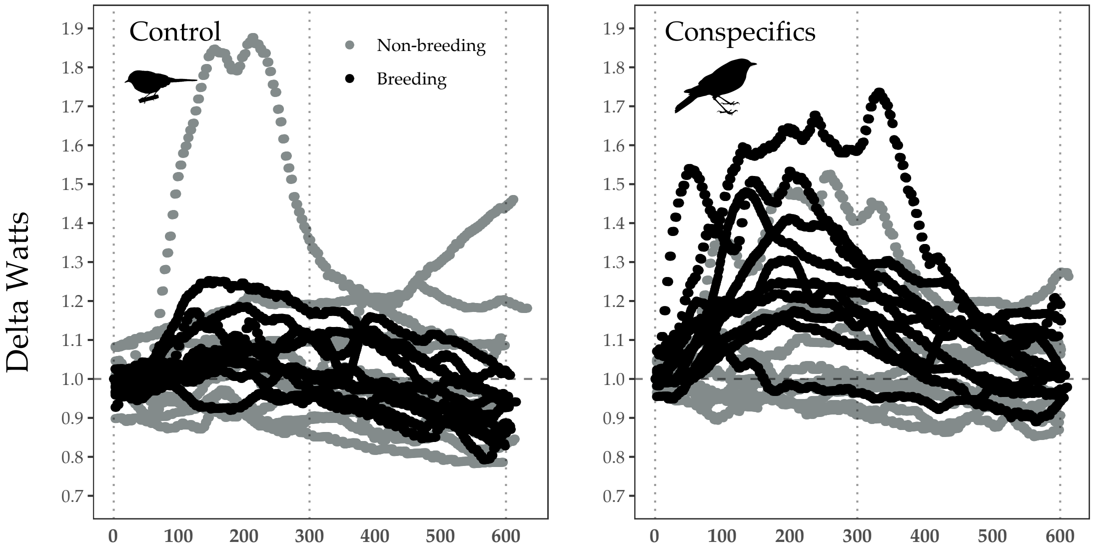

Figure S4.5 Raw data as described in **Figure S4.2**, except delta Watts is calculated from two baselines (from metabolic rate preceding the respective playback treatment).

5 THE ROLES OF THERMAL PHYSIOLOGY, MICRO-HABITAT AND COMPETITION IN DETERMINING THE ELEVATIONAL DISTRIBUTIONS OF MESOAMERICAN CLOUD FOREST SONGBIRDS

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5.1 ABSTRACT

Restricted elevational ranges are common across tropical montane species, however, despite much interest in these systems, the mechanisms generating and maintaining these elevational range patterns remain poorly resolved. We test evidence for three longstanding hypotheses – (*i*) habitat specialism, (*ii*) interspecific competition and (*iii*) thermal physiology – in determining the elevational distributions of four Mesoamerican cloud forest songbirds. Two species (Ruddy-capped and Black-headed Nightingale-Thrushes *Catharus frantzii/mexicanus*; RCNT/BHNT) are parapatric, with the former restricted to mountaintops and the latter occurring over a broader elevational range, another species (Grey-breasted Wood Wren *Henicorhina leucophrys;* GBWW) is restricted to mountain tops with no lower elevation congener, and a final species (Chestnut-capped Brushfinch *Arremon brunneinucha,* CCBF) occurs over the entire elevational range. We predicted site level abundance against habitat variables and experimentally informed covariates specific to testing contrasting hypotheses. Contrary to physiological hypothesis (*iii*), we found no evidence to suggest that thermoregulatory costs impose constraints on species distributions, and that the entire elevational range was comfortably within sustainable physiological limits for each species. We found mixed support for the biotic factors we tested, however. For the two species of Nightingale-Thrushes (*Catharus* sp.), habitat preference was most important in setting the elevational ranges of the lower elevation species, and competitive exclusion was more important in setting the elevational range of the higher elevation species. For GBWW, we found no explanatory power of habitat preference, contrary to our expectations. For CCBF we found no explanatory power of any habitat covariates, in line with our expectations. Collectively, our findings suggest that while biotic factors may be more important in setting the elevational distributions of these species, the exact mechanisms may be complex, species-specific and that different hypotheses are not mutually exclusive. Our findings also concur with recent literature, suggesting that thermal physiology is an inadequate explanation for elevational range limits of tropical birds.

5.2 INTRODUCTION

Understanding the distributional patterns of species is a fundamental question in ecology (Gaston 2009). Species are inextricably linked to environmental gradients, along which they are exposed to a variety of abiotic (e.g. changes in temperature, humidity or soil) and biotic factors (e.g. changes in vegetation, disease prevalence or proximity to competitors) that subsequently influence the extent of their distribution (e.g. Bull 1991, Guisan & Thuiller 2005). The importance of different factors influencing range patterns, however, remains disputed and often incomplete (Sexton *et al.* 2009, Gaston 2009).

Tropical elevation gradients provide intriguing natural systems on which to examine the determinants that potentially drive species range occupation. Tropical mountains contain large environmental variation over comparatively small spatial scales (see Rahbek *et al.* 2019), in turn limiting the influence of dispersal constraints as an explanation for species distributions (see Gaston 2009). Consequently, distinct patterns of elevational zonation ('species replacements') are characteristic among tropical montane species, where rapid transitions between the ranges of related taxa occur with increases in elevation (e.g. Terborgh 1971, Navas 2002, McCain 2004, Brehm *et al.* 2007, McCain 2009, Pyrcz *et al.* 2009). These elevational patterns on tropical mountains have attracted generations of ecologists (Darwin 1839, Humboldt 1849, Wallace 1876, Diamond 1973, Terborgh 1977, McCain 2009, Jankowski *et al.* 2013a, Pigot *et al.* 2016, Freeman 2018) but despite longstanding interest, the mechanisms generating and maintaining their species distributions remain poorly resolved.

Empirical explanations for species replacement patterns on tropical mountains have broadly converged on three hypotheses. Firstly, the ecotone hypothesis suggests that transitions between habitat boundaries dictate elevational ranges because of species-specific ecological specialism (Terborgh 1971, Jankowski *et al.* 2013b). For example, specific nesting requirements or foraging habitats may render an adjacent habitat unsuitable from that occupied (e.g. Freeman & Mason 2015). Secondly, the competition hypothesis suggests that competition between species truncates their elevational ranges (Diamond 1973). This hypothesis suggests that elevational ranges are limited by behavioural interactions between species (e.g. interference competition- see Grether *et al.* 2017) because one, or both, species are excluded by the presence of a related species that occupies a similar ecological niche (Grether *et al.* 2017). Thirdly, the physiology hypothesis suggests that species are limited by physiological constraints that set elevational ranges (Janzen 1967). This hypothesis suggests that tropical species have evolved specific thermal tolerances to their elevational ranges because of low seasonal temperature fluctuation, and that microclimates outside of this pose physiological barriers that prevent dispersal (see Ghalambor *et al.* 2006).

Of these three primary hypotheses, habitat specialisation (the ecotone hypothesis) is arguably the 'default' explanation for elevational range segregation (Wiens 1989, Jankowski *et al.* 2013a). In support of this, strong correlations with changing habitat types and bird communities have been found along elevation gradients (Terborgh 1985, Jankowski *et al.* 2009, 2013b, Freeman & Mason 2015). Support for the competition hypothesis has also been offered through a number of experimental case studies between replacement species at their elevational range limits (Jankowski *et al.* 2010, Pasch *et al.* 2013, Freeman *et al.* 2016, Boyce & Martin 2019), further supported by suggestions that aggressive territorial behaviour may influence range patterns at community scales (Freeman *et al.* 2019a). Finally, while Janzen's influential physiological hypothesis (Janzen 1967) has been central to explanations of elevational range restriction (Ghalambor *et al.* 2006), studies that have tested this hypothesis have found little evidence for physiological tolerance alone limiting elevational ranges (Freeman 2015, Londoño *et al.* 2017, Jiménez Robles & la Riva 2019). While it is unlikely that species are acutely limited by temperature exposure, some high elevation species have shown physiological traits related to cold environments (Soriano *et al.* 2002, Dubay & Witt 2014) and it may be that energetic costs expended on thermoregulation at non-native altitudes place fitness limitations on species (Jankowski *et al.* 2013a).

A shortfall in our understanding of elevational range determinants is that these competing hypotheses have largely been assessed in isolation. It is more likely, however, that combinations of these factors describe spatial distributions (Gaston 2009). Following the concept of ecological niches (Hutchinson 1957, Holt 2009), Jankowski et al. (2013a) suggested that elevational ranges of tropical species may be determined by how the entire physiologically suitable extent of a species range, is constrained by biotic factors. There is good premise from which to expect factors to interact dependent on species or local conditions; while the limits of species elevational ranges frequently end abruptly, abiotic conditions along elevation gradients do not. Indeed, patterns of elevational zonation can also vary between species or regions (e.g. Cadena & Loiselle 2007), suggesting that different factors influence different patterns of elevational ranges. For example, competition between replacement species on tropical mountains is typically asymmetric (Freeman 2019), suggesting that dominant species select not to, or are unable to, occupy the range of a subordinate. As such, it may be that competitive dominance limits the subordinate species to only a specific portion of its physiologically suitable range (e.g. Pasch *et al.* 2013), or that habitat limits a dominant species from encroaching the range of the subordinate. Similarly, a mountaintop species may be physiologically capable of living across a broader spatial range but is limited to high elevations because of the small spatial extent of another important biotic factor such as elevation-specific habitat. Indeed, for species restricted to lower elevations, the colder ‘leading edge’ (upper limit) of their elevational range is more likely to be a physiological barrier, whereas warmer ‘trailing edges’ (lower limit) are more likely to be limited by biotic factors (MacArthur 1972).

Despite important theoretical advancements in this field, we are aware of no studies to date that have assessed the relative contributions of biotic and physiological mechanisms driving elevational ranges of species in one tropical system. The importance of such mechanistic assessments has gained increasing relevance, however, as upslope movements and local extirpations in montane species have been documented throughout the tropics (Pounds *et al.* 1999, Chen *et al.* 2009, Forero-Medina *et al.* 2010, Freeman & Class Freeman 2014, Rosselli *et al.* 2017, Freeman *et al.* 2018, Neate-Clegg *et al.* 2018), in line-with projections that tropical montane climates may be severely altered by environmental change within a century (Williams *et al.* 2007). Without mechanistic assessments of species range occupation we lack fundamental data from which to make informed predictions of how species will respond against scenarios of environmental change.

To explore the roles of thermal physiology, habitat specialisation and interspecific competition influencing different elevational range patterns, we studied the elevational ranges of four species of understorey songbirds along a closed-canopy elevational gradient in the Sierra del Merendón, Honduras. Specifically, we study three patterns of elevational ranges; (i) an example of species turnover, where two species abruptly stop at a parapatric range boundary with no spatial overlap (Black-headed Nightingale-Thrushes *Catharus mexicanus* and Ruddy-capped Nightingale-Thrushes *C.frantzii*; BHNT/RCNT, respectively), (ii) where a species is confined to high elevations only (Grey-breasted Wood Wrens *Henicorhina leucophrys*; GBWW) and (iii) where one species occupies the complete elevational range (Chestnut-capped Brushfinches *Arremon brunneinucha*; CCBF) (**Figure 5.1**).

We predicted species abundances against habitat covariates, energetic costs of thermoregulation, and (in the case of (i)) the presence of a competitive congener. In (i), if the ‘physiological’ niche was broad, we expected either habitat or the presence of a dominant congener to constrain the distributions of both species. Alternatively, if physiological costs were important for range delimitation then conditions at high elevations should be above the sustainable limits of thermoregulatory energy expenditure for the lower elevation species (BHNT), physiologically restricting them from living at higher elevations. In (ii), because GBWW are restricted entirely to high elevations we expected that their elevational range would be constrained by habitat type and that their physiologically suitable range would extend to lower elevations. In (iii) because of its broad elevational range we expected that no variables would explain the range of CCBF (i.e. that the species would not be affected by habitat variables and live well within sustainable thermoregulatory limits).

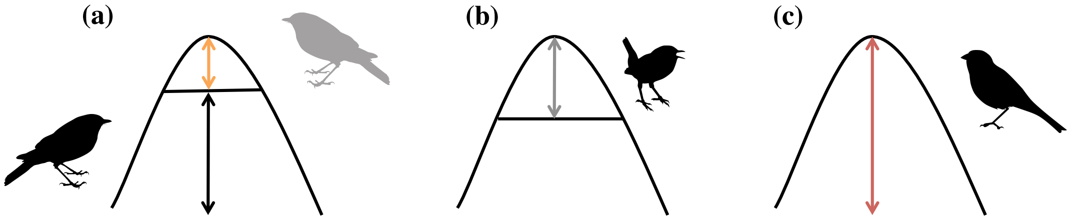

Figure 5.1 Elevational ranges of the four study species; (a) is an example of elevational turnover where (lower elevation) Black-headed Nightingale-Thrushes *Catharus mexicanus* are 'replaced' by Ruddy-capped Nightingale-Thrushes *Catharus frantzii* at higher elevations, (b) Grey-breasted Wood Wrens *Henicorhina leucophrys* limited to mountaintops with no lower elevation congener, and (c) Chestnut-capped Brushfinches *Arremon brunneinucha* distributed across the elevational range. The elevational range in this study was 1197-2183 m, although the higher elevation species occur up to the mountaintop at the field site at ~2290 m.

5.3 METHODS

5.3.1 Study site

We surveyed the cloud-forest bird community of Cusuco National Park in the Sierra del Merendon, Honduras (approximately 15.552ºN, -88.296ºW) from June - August 2016 along a continuously forested gradient from 1197-2183 m. Below the lower limit of this elevational range, varying degrees of deforestation affect much of the study site and as such, all transects were located in primary or disturbed primary forest (where some selective logging was undertaken in the 1960s). Approximate habitat types are broadleaf and mixed broadleaf and pine at elevations up to ~1900 m, with stunted elfin forest dominated by ferns at higher elevations (**Chapter 2**, Batke *et al.* 2014). Fieldwork was undertaken during the breeding season for the majority of the species at the field site, and when the study species are at their most vocally active. We recorded all bird species during surveys, and then limited our dataset to the study species of interest during the analysis phase.

5.3.2 Bird surveys

We undertook point counts (see Sutherland 2004) at 83 survey localities situated along 15 transects (see Introduction; **Figure 1.1**) uniformly covering the elevational gradient between 1197-2183 m. Each survey location was separated by at least 200m (although typically 300+ m) to ensure sampling localities were discrete, following recommendations in Sutherland (2004). We undertook 351 independent surveys with sites generally receiving between 2-5 replicates (with a range between 1 - 7) over the 6-week study period. All surveys were undertaken during peak vocal activity between 0515 and 0900 and avoided heavy wind or rain that might significantly affect avian activity or detection. Because all transects were situated radially from remote research camps (where the start of each transect began at the camp) we surveyed each transect at least once in reverse order (i.e. the most distant points were visited first) to account for temporal biases in survey effort. Data were primarily collected by SEIJ but were augmented by surveys from other ornithologists with similar experience on the sites avifauna (Martin *et al.* 2016, Neate-Clegg *et al.* 2018). As such, we are confident that the study species were identified accurately.

Surveys began at each point with a one minute settle period following recommendations in (Sutherland 2004). All species of birds were then recorded visually and audibly for 10-minutes, and distance to each detection was estimated with careful observation of singing individuals to eliminate double counting. There is no consensus for optimum count length for tropical bird surveys and longer counts may increase the chances of birds moving into the detection area (Robinson *et al.* 2018). However, each of the study species are territorial and are not expected to undertake wide-ranging movements (e.g. **Chapters 3/4**). It is thus unlikely that abundances estimates are inflated as a result of lengthier surveys.

That detections decrease with distance is common in avian vantage surveys, but we truncated all survey data to a 70 m radius during data sorting. All observers undertook a series of initial counts together, in order to calibrate as best as possible individual distance estimates and to ensure inter-observer reliability. While fitting species-specific detection functions is a common way of accounting for declining detection probability with perpendicular distance from the observer (e.g. Buckland *et al.* 2008), we wanted to bound all our data to habitat attributes surrounding survey points (see below) so adopted to use this universal cut off distance.

5.3.3 Habitat surveys

Habitat types were quantified through surveys at each survey point in a 20 m^2 habitat plot surrounding each point, following methods described in **Chapter 2**. The majority of habitat plots were surveyed the same year as the bird surveys (2016), although for sites not surveyed in 2016 we used habitat data collected the previous year. Because survey points were in undisturbed habitats (where no loss of habitat has occurred via human activities), however, it is unlikely that occupancy of species will have changed between years. We quantified the following attributes of the habitat; percentage density of broadleaf, fern, or palm trees, understorey density and leaf litter depth/soil density. We selected these variables in order to typify different parts of the forest strata relevant to the habitat occupied by the study species.

Percentage densities of each tree type were taken as a proportion of cumulative diameter of all trees in each plot. We took this approach in order to account for size differences between trees (mean diameter at breast height, cm ± SD), because broadleaf trees averaged slightly larger (16.8 ± 3.69) than ferns (9.82 ± 2.16) or palms (8.09 ± 3.41) (mean diameter per plot; ANOVA $F_{2, 194} = 146.5$, $p = <0.001$), thus better reflecting the structural complexity relative to tree type in the microhabitat. Mean understorey density was measured within 3m of the forest floor using the 'touch pole' technique described in **Chapter 2**, where higher scores represented thicker understorey and lower scores more open understorey. Finally, to quantify the forest floor, we grouped soil depth and leaf litter depth together using a principal component analysis. Both soil depth and leaf-litter depth loaded positively (soil depth= 0.71, leaf-litter depth= 0.71) onto the first axis, explaining 66% of overall variance. We thus took this as a 'forest floor' index, where higher scores represented thicker/deeper forest floors and lower scores as less dense.

This approach to habitat variables, means changes in each variable are not mutually exclusive, better reflecting gradual changes in habitat with elevation, rather than assuming immediate shifts at ecotones. While our 20 m^2 habitat plots did not extend to the entire radius of the area surveyed for birds (70 m radius), we took them as representative of the general habitat types present in the extended area. We adopted this approach instead of truncating distances of recorded birds to the immediate area surrounding survey points (e.g. within 20 m) because this would likely severely underestimate both species present and their abundance predictions (for discussion see Robinson *et al.* 2018).

5.3.4 Interspecific competition

We included interspecific competition (by proxy of interference competition (see Grether *et al.* 2017) informed by behavioural experiments at the field site between both Nightingale-Thrush species (**Chapter 2**). In our system, BHNT are aggressively dominant over RCNT, and we thus incorporated this directionality in this explanatory variable. For each survey site, we calculated distance from contact for RCNT to BHNT. Distance from contact was measured to the closest known territory (which were mapped at the spatial limits of elevational turnover – see **Chapter 2**), or the closest survey site at which BHNT were present. While the latter method may occasionally underestimate distance to contact, such inaccuracy is unlikely to influence this variable because competition between the species is most important within immediate proximity (within ~150 m, see **Chapter 2**).

5.3.5 Predicting sustainable thermal limits

i) Environmental temperature

We measured air temperature (environmental temperature; T_e (ºC)) with remote loggers (*n* = 31) (HOBO Onset Pedant UA002-64 or EasyLog, EL-USB-2) situated at ~80m increases in elevation (subject to slight GPS inaccuracies) across the entire elevational range surveyed, as well as each aspect of the mountain slope (facing east, north-west, and south-west, respectively). Loggers were attached to the trunk of small trees or ferns ~1m above the forest floor. While our temperature measurements are not technically a standard operative temperature (independent of clear skies at night, sunlight and wind), the understorey of the forest is effectively independent of these factors, and thus logger values represent the microclimates understorey species experience (Jankowski *et al*. 2013a). Loggers recorded T_e at 30-minute intervals for 24hrs a day throughout the entire survey period.

From these data, we calculated mean T_e, mean max T_e and mean min T_e between sunrise (~0530) and sunset (~1830) (and vice-versa) per calendar date (as taken from official sunrise and sunset times- see www.timeanddate.com). We found little evidence for a strong influence of slope aspect on nighttime or daytime T_e (OLS ANCOVA, mean T_e- time of day × aspect $F_{2,51} = 2.09$, $p = 0.13$, aspect × elevation $F_{2,51} = 0.05$, $p = 0.61$; mean max T_{e} - time of day × aspect $F_{2,51} = 3.18$, $p = 0.05$, aspect × elevation $F_{2, 51} = 0.46$, $p = 0.63$; mean min T_e- time of day × aspect $F_{2, 51} = 0.08$, $p = 0.93$, aspect × elevation $F_{2, 47} = 1.37$, $p = 0.27$- see supplementary materials) and as such, lumped all data across different slope aspects. We then fitted linear models to this data to predict T_e regimes at each survey site, dependent on its elevation. T_e decreased linearly with elevation (all r^2 between- 0.84 - 0.96- see **Table S5.1/5.2**). The highest elevation logger failed during the study, but we fitted temperature data up to this elevation because of the consistency of lapse rates.

ii) Physiological limits imposed by T_e

We predicted the thermoregulatory costs associated with living at different elevations by using modeled values of estimated minimum thermal conductance (C_{min}), lower critical temperature (T_{lc}) and basal metabolic rates (BMR) previously collected for each of the study species at the field site (**Chapter 3**) For example, if T_{lc} was 18ºC, then temperatures greater than this would incur no energetic cost of thermoregulation, but temperatures below it would incur costs dependent on the slope of C_{min}. Using these thermoregulation models (see **Figure S5.3**), we predicted metabolic rate for each species in multiples of BMR (see below) at the location of each temperature logger for the full time between sunset and sunrise per calendar day. We then averaged this value for the entire field season at the elevation of each temperature logger, and predicted it across the complete elevational range by fitting a linear model in the same manner as T_e (see above). We thus ignored potential influences of slope aspect because nighttime temperatures were comparable irrespective of aspect (see **Table S5.1/Figure S5.1).**

Using the framework presented by Jankowski *et al.* (2013a), we estimated the fundamental physiological niche as elevations at which thermoregulatory costs consistently breached the upper limits of sustainable energy use (where energy was expended on the costs of thermoregulation at temperatures below T_{lc}). We defined the upper limit of sustainable energy use expended on thermoregulation as 2 × BMR, following Jankowski *et al.* (2013a). This is lower than temperate species (where sustained periods of energetic expenditure in excess of 4 × BMR are suggested as above sustainable limits (Peterson *et al.* 1990)), however BMR is lower in tropical species in comparison to temperate species (Wiersma *et al.* 2007, Londoño *et al.* 2015) so we assume this reflects in their respective energetic 'ceilings' (Jankowski *et al.* 2013a). We predicted thermoregulatory costs only for night hours for two reasons. Firstly, our physiological data were only available from birds measured at nighttime, and thus are directly comparable to field data. Secondly, because body temperatures were not measured in our physiological study, it was not possible to accurately predict daytime costs using standard conversions of daytime body temperatures (e.g. Londoño *et al.* 2017). Because nighttime T_e invariably represents the lowest T_e, this method thus models the most energetically demanding conditions for thermoregulation.

Because we found seasonal differences in the metabolic rates of BHNT (**Chapter 3**), we took the summer thermoregulation models for this species (taken at the same time of year as our surveys). The thermal physiology of RCNT was different to that of the other three species in that our physiological experiments did not detect any thermoregulatory costs across all temperatures tested (**Chapter 3**). Thus, there were effectively no costs to thermoregulation for this species across the temperature range studied, and we assigned a value of '1' (i.e. BMR) for all modeled temperatures across elevation. Our thermoregulation models may slightly underestimate C_{min} because we were not able to measure body temperature (Mcnab 1980). They are, however, direct measurements of nightly energetic costs against temperature at the field site, and thus applicable to energetic costs associated with different T_e.

5.3.6 Statistical analysis

i) Modelling bird abundances

We modelled site level abundances for each species in 'R' (R Core Team 2016) using *N*-mixture models in the *unmarked* package (Fiske & Chandler 2011). *N*-mixture models are a class of hierarchical model that correct for detection imperfections using repeated count data (Kéry & Royle 2015). As such, our data include both true and false absences of detection, where a species was genuinely not present (e.g. above its elevational range limits), or was present but not detected (e.g. a non-singing bird). We thus used zero-inflated Poisson (ZIP) *N*-mixture models to model both of these detection disparities. Because *N*-mixture models are hierarchical, they simultaneously model Poisson distributed abundance of a species at a survey site corrected for zero inflation, and the probability of detecting the species given its true abundance.

ZIP *N*-mixture models are used with repeated measures of site level data (repeated visits per survey site) to model the number of individuals based upon the numbers observed during counts over repeated sampling periods (e.g. at site y, individual counts of a species… 1, 0, 2). Because each sampling site was discrete, we were able to match species abundance estimations with site level covariates to test our hypotheses.

For the detectability aspect of the model, we included both minutes since midnight (of the survey visit) and ordinal day (days since 1st January) for each species model. These two variables represent possible differences in detection probability associated with song timing at dawn, or variation in singing intensity in the breeding season.

We fitted these models in a two-step process comparable to similar recent studies (e.g. Rodríguez *et al.* 2019), where we first determined the importance of detection covariates for each species before modelling the abundance covariates. To do this we fitted the detection probability covariates against an intercept only model for abundance covariates, and then used the highest-ranking model with top detection covariate(s) from this phase for posterior modelling (**Table S5.3**). To determine the best detection covariates, models were ranked based on the Akaike Information Criteria corrected for small samples (AICc) using the *AICc modavg* package (Mazerolle 2019).

ii) Assessing evidence for factors influencing elevational ranges

After establishing the best fitting detection covariates per species (see **Table S5.3**), we then included abundance covariates to test our hypotheses. For each species we fitted habitat variables and thermoregulatory costs, with the exception of RCNT, where it was not possible to include thermoregulatory cost as the lack of variance led to model convergence issues (i.e. because nightly thermoregulatory costs were ‘1’). However, because there were no thermoregulatory costs for this species, this variable was already effectively redundant. In addition, to determine the competition aspect for RCNT, we also included distance to BHNT.

We scaled all abundance variables so that magnitudes of change were comparable, and $\log_{10}$ transformed understorey density so that it was normally distributed. For RCNT, we included a quadratic polynomial term for distance to contact with BHNT in order to best model a threshold in abundance, consistent with competitive exclusion. Saturated models for each species were checked for over-dispersion and goodness of fit using Chi-square (χ^2) and sums of squares (SSE) statistics from 10,000 bootstrap samples (see Kéry & Royle 2015). All species models were within acceptable levels of dispersion and had good fit (χ^2 and SSE all ≥ 0.35), except for RCNT which were marginally over-dispersed (c-hat – 2.5), however the models still had good fit. This over dispersion is small, however, and unlikely to influence the parameter estimates, but may overestimate precision (e.g. smaller SEM) (see Kéry & Royle 2015).

We assessed evidence for our hypotheses by examining model parameters for significant effects. For RCNT, in addition to assessing the parameters we also assessed the strength of interspecific competition by assessing two model iterations. Firstly a saturated model with all abundance covariates, and then a reduced model with all covariates except distance to BHNT, which we then compared by their respective AICc weights.

Our models estimate abundance only and we do not attempt to convert values to species densities. Converting abundance to density is problematic because of the difficulties of truly limiting sampling areas (see Kéry & Royle 2015). Density estimates require much finer scale/time intensive studies (e.g. territory mapping) which are generally not possible across the scale we were interested in and we were more interested in interpreting the broader spatial patterns along the elevational gradients. Finally, owing to the methods employed, we cannot entirely eliminate non-territorial 'satellite' birds occurring outside of their typical range limits, however, these incidences are likely to be random (e.g. see **Chapter 2**) and as such will not significantly influence abundance parameters.

5.4 RESULTS

Across all surveys (n = 351 total surveys), we documented 310 records of BHNT, 63 RCNT, 379 GBWW, and 203 CCBF. BHNT were recorded between 1234-2053 m, although all but 5/310 records were below 1960 m. We recorded RCNT between 1823-2183 m, with no apparent extralimital records. We recorded GBWW between 1315-2183 m, although all but 2/379 records (both at one site at 1315 m) were above 1587 m. CCBF were recorded across almost the entire elevational range from 1234-2183 m. CCBF are frequently observed below this range, however, so the lower limit observed is likely to be an artefact of birds not being present at the specific survey site.

5.4.1 Predictions of abundance

i) Black-headed Nightingale-Thrushes

Consistent with habitat specialism, we found that BHNT favoured specific habitat over others (**Table 5.1**). Abundance was negatively related to fern-dominated forests found at higher elevations (z = -2.345, p = 0.02) but positively related to proportions of palm in survey plots (z = 3.979, p = <0.01) (**Figure 5.2**). We found no evidence in BHNT to suggest that T_e at high elevations (i.e. above their elevational range limits) represented conditions at which thermoregulatory costs were unsustainable. Even at the highest, elevations surveyed (unoccupied by BHNT), the maximum predicted mean nightly energetic cost of thermoregulation was 1.34 × BMR (**Figure 5.3**).

ii) Ruddy-capped Nightingale-Thrush

Consistent with an influence of competition, we found little influence of habitat preferences on abundance (all habitat variables p = ≥0.1; **Table 5.1**). Instead, abundance increased non-linearly with increasing distance from BHNT (1^{st} order polynomial term z = 3.808, p = <0.01; 2^{nd} order term z = -0.568, p = 0.57) (**Table 5.1/Figure 5.2**). When we compared between models, a model including the competition term performed better (AICc = 262.24) than one without it (AICc =

273.19), suggesting that competitive exclusion is the most important factor driving their elevational segregation. Because we were not able to establish any thermoregulatory threshold temperatures in physiological tests of RCNT (**Chapter 3**), all elevations/T_e appear physiologically suitable for this species (**Figure 5.3).**

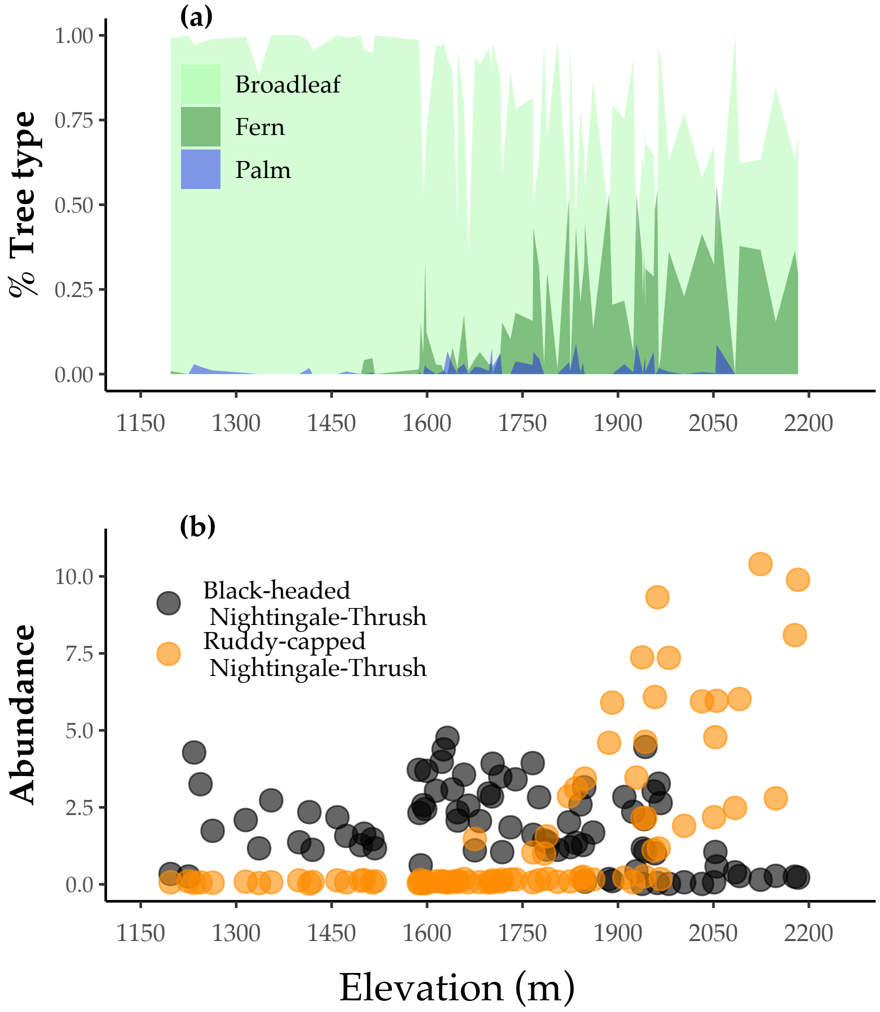

Figure 5.2 Relationships between proportional changes in tree type at survey sites and elevation (a), and abundance relationships at each survey point across elevation in both species of Nightingale-Thrush (*Catharus* sp.) (b). Abundance of Black-headed Nightingale-Thrushes *Catharus mexicanus* is negatively related to prevalence of ferns, typical of higher elevations, consistent with ecological specialism, whereas abundance of Ruddy-capped Nightingale-Thrushes *Catharus frantzii* was more strongly influenced by competition with Black-headed Nightingale-Thrushes, consistent with competitive exclusion.

iii) Grey-breasted Wood Wrens

Contrary to our hypotheses, we found no evidence that specific habitat types influenced abundance of GBWW (all habitat variables $p = \geq 0.16$; **Table 5.1**) and subsequently could not explain their elevational range restriction. We found that abundance was significant and positively related to thermoregulatory costs (z = 3.581, $p = <0.01$; **Table 5.1**), however, in that conditions that incurred higher thermoregulatory costs were associated with greater abundance. However, this is likely to be an artefact of the linear increase in T_e and elevation, and that GBWW occur over a sufficient elevational range that a signal of abundance would covary positively with thermoregulatory cost. We found no evidence to suggest that GBWW approached unsustainable thermoregulatory costs, where even at the highest elevations the maximum predicted mean nightly energetic cost of thermoregulation was 1.29 × BMR (**Figure 5.3**).

iv) Chestnut-capped Brushfinches

Consistent with our hypothesis, we found no evidence that either specific habitat or the costs of thermoregulation influenced abundance of CCBF; their elevational distribution appeared independent of these factors. Abundance estimates were not related to habitat variables (all habitat variables $p = \geq 0.15$; **Table 5.1**) and there was no relationship between thermoregulatory costs and abundance (z = 0.181, $p = 0.86$; **Table 5.1**). We found no evidence to suggest that CCBF approached unsustainable thermoregulatory costs across the elevational range surveyed. Even at the highest elevations (where they were present) the maximum predicted nightime thermoregulatory cost was 1.13 × BMR (**Figure 5.3**).

Table 5.1 Abundance (λ) and detection (p) covariates from saturated models for each study species. Detection covariates differ per species dependent on the best fitting detection model (see **Table S5.3**) and abundance covariates differ dependent on hypotheses being tested. Statistically significant terms are emboldened.

Species		**Estimate (SE)**	**z**	***p***
Black-headed Nightingale-Thrush *Catharus mexicanus*				
λ (log)	**Intercept**	**0.464 (0.16)**	**2.916**	**<0.01**
	Broadleaf %	-0.033 (0.18)	-0.18	0.86
	Fern %	**-0.489 (0.21)**	**-2.345**	**0.02**
	Palm %	**0.378 (0.1)**	**3.979**	**<0.01**
	Understorey density	-0.225 (0.13)	-1.743	0.08
	Forest floor index	-0.091 (0.11)	-0.848	0.4
	Thermoregulatory cost	0.064 (0.14)	0.457	0.65
p (logit)	Intercept	0.961 (0.99)	0.969	0.33
	Time	<0.01 (<0.01)	-1.421	0.16
Ruddy-capped Nightingale-Thrush *Catharus frantzii*				
λ (log)	Intercept	-0.735 (0.59)	-1.245	0.21
	Broadleaf %	0.001 (1.12)	<0.001	0.99
	Fern %	0.359(1.03)	0.347	0.73
	Palm %	-0.063(0.19)	-0.338	0.74
	Understorey density	0.134(0.22)	0.618	0.54
	Forest floor index	-0.299 (0.18)	-1.642	0.1
	Distance from congener (1)	**11.044(2.90)**	**3.808**	**<0.01**
	Distance from congener (2)	-0.648(1.14)	-0.568	0.57
p (logit)	Intercept	-2.80(2.14)	-1.31	0.19
	Ordinal day	**0.03(0.01)**	**2.84**	**<0.01**
	Time	**-0.013(<0.01)**	**-3.17**	**<0.01**
Grey-breasted Wood Wren *Henicorhina leucophrys*				
λ (log)	**Intercept**	**0.945 (0.14)**	**6.647**	**<0.01**
	Broadleaf %	-0.130 (0.17)	-0.740	0.46
	Fern %	-0.075 (0.18)	-0.417	0.68
	Palm %	0.102 (0.07)	1.394	0.16
	Understorey density	0.014 (0.09)	0.146	0.88
	Forest floor index	-0.007 (0.08)	-0.091	0.93
	Thermoregulatory cost	**0.454 (0.13)**	**3.581**	**<0.01**
p (logit)	Intercept	-0.612 (0.93)	-0.656	0.51
	Ordinal day	<0.001 (<0.01)	0.090	0.93
Chestnut-capped Brushfinch *Arremon brunneinucha*				
λ (log)	**Intercept**	**1.380 (0.58)**	**2.397**	**0.02**
	Broadleaf %	0.285 (0.27)	1.044	0.3
	Fern %	0.282 (0.27)	1.034	0.3
	Palm %	0.132 (0.09)	1.408	0.16
	Understorey density	-0.182 (0.13)	-1.454	0.15
	Forest floor index	-0.001 (0.10)	-0.014	0.99
	Thermoregulatory cost	0.024 (0.13)	0.181	0.86
p (logit)	Intercept	-0.428 (1.19)	-0.359	0.72
	Ordinal day	**0.012 (0.01)**	**2.124**	**0.03**
	Time	**-0.01 (<0.01)**	**-4.937**	**<0.01**

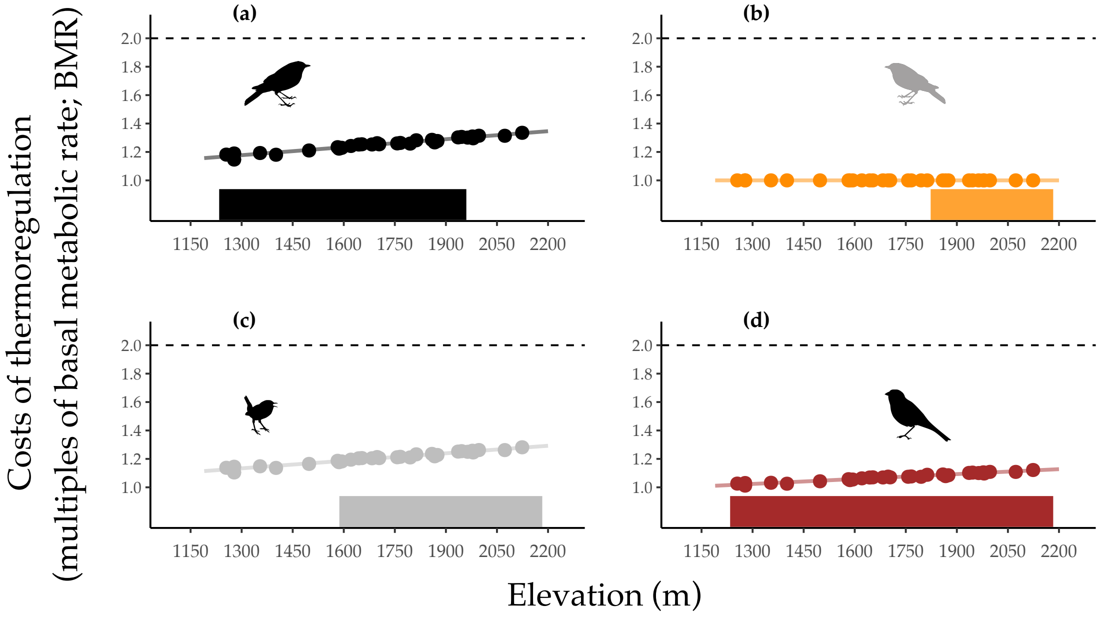

Figure 5.3 Energetic costs of metabolic heat production (in multiples of basal metabolic rate; BMR) against elevation, and elevational range occupation in the four study species; (a) Black-headed Nightingale-Thrushes *Catharus mexicanus,* (b) Ruddy-capped Nightingale-Thrushes *Catharus frantzii*, (c) Grey-breasted Wood Wrens *Henicorhina leucophrys*, and (d) Chestnut-capped Brushfinches *Arremon brunneinucha*. Solid bars display the typical elevational range of each species (excluding extralimital records – see Results), and points represent the mean nightly thermoregulatory costs at each temperature logger, from which data were predicted across all point count locations (fitted line). The dashed line at 2 × BMR displays the hypothesised threshold above which the energetic costs of metabolic heat production are unsustainable (see Jankowski *et al*. 2013a). No species approaches conditions exceeding unsustainable limits to nightly thermoregulation either within, or outside, of their native elevational ranges.

5.5 DISCUSSION

The central aim of our study was to better understand the determinants of elevational ranges by assessing the importance of habitat specialisation, interspecific competition and physiological tolerance across three case studies with different range patterns (**Figure 5.1**). We found mixed support for these hypotheses. Only one of the four species showed strong preference for habitat type (BHNT). This species also limited the elevational range of RCNT, but we did not find the latter to display any habitat specialism, suggesting that competition was the key determinant in defining the elevational range of this species. For the remaining two species (GBWW/CCBF), we found no effect of habitat on their elevational distributions. A commonality across all species, however, was that thermal limits did not pose dispersal barriers, contrary to the hypothesis proposed by (Janzen 1967).

This latter point - that for each species the entire elevational range represents a physiologically suitable environment - represents an important part of the conceptual framework of our study; whether biotic factors place limitations to the fundamental niche (Hutchinson 1957). Taking the physiologically tolerable range as that within sustainable limits of energetic costs expended on thermoregulation (Jankowski *et al.* 2013a), all species in our study appear physiologically capable of living at all elevations across those included in study site (**Figure 5.3**). This suggests that biotic factors are more important in setting elevational range limits for our species, a result comparable to similar studies in the tropics on lizards in the Bolivian Andes (Jiménez Robles & la Riva 2019) and New Guinean montane birds (Freeman 2015). How different species related to biotic factors varied between species and patterns of turnover, however.

5.5.1 Biotic drivers of elevational ranges

That BHNT were related to specific habitat preferences is consistent with previous studies at the field site, where the contact zone between the two Nightingale-Thrushes was located at divergences in habitat (**Chapter 2**). The specifics of this habitat preference are notable, in that BHNT selectively did not occupy fern dominated forest common to higher elevations, while RCNT displayed no specific relationship to the habitats measured. This is consistent with competitive exclusion by interference competition, where an aggressively dominant species limits the presence of another to a habitat it selectively does not occupy (Grether *et al.* 2017).

Whether competitive interactions are a sufficiently strong ecological force to define elevational ranges has been questioned (Cadena & Loiselle 2007), and indeed may not occur without some prior ecological segregation (see Freeman *et al.* 2019). Our results broadly support both conclusions; that a degree of ecological separation is necessary to also structure elevational range divergence. Despite this, quantification of ecological segregation (e.g. habitat) is lacking in numerous studies investigating competition dynamics in the literature (e.g. Freeman *et al.* 2016, Boyce & Martin 2019, Freeman *et al.* 2019), leading to suggestions that competition represents a dominant role in elevational segregation throughout the tropics (e.g. Elsen *et al.* 2016). This suggestion is problematic, however, because the mechanistic strength of competitive exclusion alone limiting elevational ranges is difficult to validate without widespread removal experiments (Martin & Martin 2001, Pasch *et al.* 2013). Further, the prevalence of asymmetry in interspecific interactions between replacement species strongly suggests it cannot act alone (**Chapter 2**, Martin *et al.* 2017a, Freeman 2019).

What is notable about these habitat attributes that are important for one Nightingale-Thrush, but not the other? BHNT avoid fern dominated forest and may favour habitat with more open understorey (although this was not significant $p = 0.08$). As such it seems that BHNT selectively avoid habitats, perhaps for resource availability or nesting habitat. For example, BHNT showed a strong relationship with palms; in our survey plots these are generally small (~1.5m in height) and we located multiple BHNT nests in their crowns. Another possible explanation for an aspect of habitat preference is sensory drive (the “acoustic adaption hypothesis”), where the signalling behaviour of a species is specifically related to its habitat preference (see- Wilkins *et al.* 2013). Strong territoriality and extended periods song defence are particularly important for BHNT (**Chapter 2/4**, Howell & Webb 1995). Consistent with this, the songs of the two species are distinctly different, where RCNT sing at lower frequencies and lower amplitude than BHNT (see- Jones 2014, 2015), an acoustic trait that both aids signal transmission through denser habitats and has been shown in divergent signalling behaviour with habitat between congeners in the tropics

(Tobias *et al*. 2010). Although the results from this chapter are not completely consistent with this suggestion, BHNT appear to live in a lower diversity of habitats than RCNT a factor that may relate to territorial behaviour. Further experimental tests and finer scale ecological studies are required to validate these suggestions, however.

That the abundance of RCNT was not explicitly related to any habitat types is also notable. This is broadly consistent with (Rangel-Salazar *et al*. 2008), who found this species across a variety of habitat types. Whether this means that RCNT are less specialised, however, remains unclear. The habitats occupied by both species appear more heterogeneous when measured across a broader elevational scale than at their contact zone (**Chapter 2**), suggesting that some habitats at higher elevations had similar attributes to those at lower elevations. As such, our results support previous conclusions that suggested a combination of interference competition and habitat selection drove their elevational segregation. We advance our understanding of this relationship here, however, because asymmetry in both competition and habitat selection combine to segregate the elevational divergence between the two Nightingale-Thrush species, resigning RCNT to higher elevations, where BHNT are physiologically and competitively capable of living.

Contrary to our predictions, we did not find evidence for habitat-mediated range restriction in our mountaintop species, GBWW. At our field site we often observed this species utilising microhabitats such as bamboo stands and edges of tree fall gaps and vine tangles. From an observational perspective, these habitats exist continuously across the elevational range so a lack of an observable habitat driver within the variables that we collected is probably robust. It is unlikely that we overlooked competition as a potential mediator of this range restriction. A closely related congener, White-breasted Wood Wrens *Henicorhina leucosticta* (WBWW), also occurs at the field site but their elevational distributions are separated by ~300 m (the upper elevational limit for WBWW recorded during surveys was 1336 m). Intriguingly, this also contrasts with elevational gradients elsewhere, where the two species come into contact and display strong interspecific interactions at their elevational range limits (Jankowski *et al*. 2010).

Thus, what limits GBWW specifically to high elevations is unclear. A possible explanation is that the habitat attributes we measured were not at sufficient resolution to determine site level abundances of GBWW. Previous studies on WBWW, in different forest types found their abundance to be positively related to higher arthropod abundances and possibly aerial leaf litter depth (leaf litter collecting above the forest floor) (Sánchez *et al*. 2014). Because GBWW (and indeed each of the species in our study) forage both terrestrially and in the understorey strata (see Howell & Webb 1995), aerial leaf litter or bromeliad density are both potentially important habitat attributes for which the functional importance is ignored by our methods. Bromeliads are an abundant microhabitat feature at our field site, within which the arthropod community change with elevation (Jocque & Field 2014). Variation in different habitat features with elevation may influence important factors such as prey availability (or even nesting materials) which in turn affect species densities (Cintra & Cancelli 2008, Vargas *et al*. 2010). Our habitat data were not sufficient to elucidate such effects, and future studies coupling behavioural data on habitat usage with these finer-scale attributes would offer better insight into this relationship.

Consistent with our expectations, we found no influence of habitat specialism on the elevational range of CCBF. To some extent, all species have some degree of ecological requirements, although these appear to be met in this species irrespective of elevation. This is broadly consistent with Cadena & Loiselle (2007) who suggested that the elevational ranges of CCBF were broad, where their ecological requirements are met. One argument explaining the broader elevational ranges of Brushfinches in Mesoamerica is that they are released from competitor pressure in the absence of other competing Brushfinch species (Grether *et al*. 2017), where in South America their elevational ranges covary with putative competitors (Remsen & Graves 1995). However, that competition plays an important role in the distributional limits of this species group has been strongly questioned and more likely to reflect ecological differences as a result of biogeographic history (Cadena & Loiselle 2007, Cadena 2007).

One further possible factor influencing each of these results is that the area surveyed for birds (70m from the centre of the survey point) extended beyond the immediate area surrounding the point for which we quantified habitat (20m^2 around the point). Because each of our study species is territorial, we did not necessarily measure the exact habitat used, but assume that the immediate area surrounding survey points is representative of the wider area. While we cannot rule out the fact that our survey data and habitat data might not directly match, the fact that BHNT displayed a similar relationship to habitat types more directly measured in the proximity of territories (**Chapter 2**) indicates that the relationship to habitat variables in some species is consistent. Moreover, this method of pairing habitat at survey sites to avian surveys is comparable to similar studies (e.g. Elsen *et al.* 2016).

It is also important to note that we only assessed two biotic variables in our study, where in practice other biotic factors may also influence the elevational ranges of species. For example, host parasite relationships may be an important determinant in defining the warmer 'trailing edge' of high elevation species, if the ranges of parasites/their vectors themselves correspond to temperature gradients (e.g. van Riper *et al.* 1986). One intriguing possibility in moisture rich highland forests is the role of rainfall shadows and the presence of water holding vegetation (e.g. tank bromeliads) that may be important in limiting the influence of mobile parasites such as mosquitos (Armbruster *et al.* 2002, Jocque & Field 2014). Similarly, strong selective pressures such as nests predation rates could also be important as a limiting factor on local distributions (e.g. Martin 1988), but the degree to which nest-predation rates change with elevational is poorly known (Jankowski *et al.* 2013a), although presumably decreases owing to fewer overall predators (Boyle 2008a). However, while we recognise the potential role of these, and other biotic factors, in setting elevational range limits, the data from which to assess their importance in tropical montane systems often do not exist although represent a clear research gap.

5.5.2 Physiological determinants of elevational ranges

Our finding that each species in our study appeared to be physiologically capable of living across the extent of the elevational gradient is consistent with the results of Londoño *et al.* (2017) and Freeman (2015). Collectively, these results strongly suggest that critical thermoregulatory limits do not preclude elevational dispersal in birds as suggested by Janzen (1967). As such, while broad scale studies have found correlational support for this theory (e.g. McCain 2009), its mechanistic relevance, at least in birds, appears limited (see Ghalambor *et al.* 2006).

While we found no evidence to suggest that our species experienced unsustainable thermoregulatory costs outside of their elevational ranges, some important considerations are worth noting. The full energetic costs of avian thermoregulation are a function of complex relationships between the environment (e.g. exposure to sunlight and wind), physiological attributes of a species (BMR, body temperature and thermal conductance) and other elements of behaviour and condition (e.g. use of microclimate refugia, activity patterns and plumage condition) (Cunningham *et al.* 2015, van de Ven *et al.* 2016, Ryeland *et al.* 2017, Thompson *et al.* 2018). Much of these data are not available for the species in our study, however, we consider our conclusion that the physiological costs of thermoregulation have no influence on the elevational ranges of these species robust for the following reasons.

Firstly, T_e in closed canopy forest is generally 'stable' (i.e. low wind and little influence of insolation (Jankowski *et al.* 2013a)) and are thus unlikely to affect thermal budgets for the understorey forest species in our study. Secondly, our study was undertaken using thermal budgets measured on birds at the same time of year as our surveys and thus included seasonal metabolic regulation in one species (BHNT). Even though temperature changes do occur at the field site during the non-breeding season, we are aware of no elevational movements in each study species (see **Chapter 3**), implying that even during cooler seasonal conditions, each species still lives within sustainable thermal limits. Thirdly, because nighttime hours represent the greatest thermal challenges (i.e. cooler temperatures- see **Figure S5.2)** it is unlikely that our results would change if we also measured thermoregulatory costs during the daytime. Daytime thermal conductance and body temperatures are generally greater than at night (Aschoff 1981, Prinzinger *et al.* 1991, Schleucher & Withers 2001, Steiger *et al.* 2009), but still incur less energetic demands (Londoño *et al.* 2017) because of reduced heat loss to the environment during the day.

Finally, the unsustainable threshold of energy expended on thermoregulation that we used (2 × BMR) is the most conservative estimate suggested by (Jankowski *et al.* 2013a) and birds may be able to sustain energetic costs in the short or median term of up to 4-7 × BMR (Drent & Daan 1980, McKechnie & Swanson 2010). Our values all fell considerably below this figure, suggesting that each species was well within its metabolic scope. Indeed, while we calculated mean values, the greatest single predicted nighttime thermoregulatory cost incurred by any species across all temperatures measured at the field site (BHNT; 1.52 × BMR) was still substantially below suggested unsustainable limits.

It is important to note, however, that we draw these conclusions with two caveats. Firstly, we only measured thermal tolerances on adult birds, but throughout much of the nestling stage, chicks are ectothermic. We are aware of no studies that have investigated physiological differences in nestling stages along tropical mountains, but it is possible that thermal sensitivity of nestlings could influence elevational range limits, or alternatively be counter-acted by specific nest structures that confer thermal 'refugia' for sensitive nestlings (Martin *et al.* 2017b). Secondly, we only measured physiological limits against one abiotic variable (T_e) but other abiotic factors such as rainfall and humidity can also influence physiological condition at range edges or high elevations (e.g. Boyle 2008b, Busch *et al.* 2011). However, previous studies found no strong evidence of physiological condition differences with altitude in our study species (**Chapter 3**), suggesting that species are equally physiologically fit across their elevational range (e.g. Dallas *et al.* 2017, Freeman & Beehler 2018). This said, a recent study indicated that survival of tropical birds decreased with increasing elevation and although the reasons for this remain unclear these authors suggested that harsher conditions at higher elevations may explain this observation (Scholer *et al.* 2019).

5.5.3 Conclusions

Taken together, we conclude that biotic factors are more likely to limit the elevational ranges of species in our study than physiological determinants. Given the consistency of our physiological results to other studies globally (the Peruvian Andes and New Guinea) it seems unlikely that Janzen's (1967) predictions that thermoregulatory barriers place limits on elevational dispersal are correct for birds, excepting a few extreme examples (see Londoño *et al.* 2017). However, exactly how, or what, biotic factors influence different species is probably site and/or species dependent. Accordingly, we echo previous authors (e.g. Jankowski *et al.* 2013a, Londoño *et al*. 2017) in emphasising the importance of site/species-specific studies to better interrogate the importance of different elevational range determinants across these complex montane systems.

Finally, our results are particularly important when placed in the context of climate driven elevational range shifts. At our field site, both BHNT and GBWW have experienced upslope movements over the last decade, at the same time as CCBF have not (Neate-Clegg *et al*. 2018) (data were not sufficient to assess elevational shifts in RCNT in this study). Our results do not necessarily offer a mechanistic explanation for these shifts, but they do offer three important conclusions for this system. First, that direct influences of temperature on thermal limits of birds are unlikely to independently drive upslope shifts on tropical mountains because species live considerably within their sustainable thermal limits. Second, that the specific mechanisms driving elevational ranges - and thus shifts in elevational ranges - are complex and possibly species-specific. Finally (albeit intuitively) that species with broader elevational ranges may be less susceptible to changing climate because of less specific ecological requirements. Taken together, this study underlies the importance of understanding the mechanisms defining species distributions, ideally using species-specific, site level data.

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5.7 SUPPLEMENTARY MATERIALS

Table S5.1 Analysis of variance tables for environmental temperature (T_e) assessing the effect of slope aspect on per-diel T_e between 1197-2183 m. The 'TimeDay' variable represents hours between sunrise and sunset, and vice-versa ('day' and 'night', respectively) and 'Aspect' represents loggers facing east, north-west and south-west, respectively. Significant terms are emboldened. Because there was little evidence for differences in temperatures regimes dependent on slope aspect, all temperatures were lumped for subsequent analyses.

	df	F-value	*p*
Daily mean temperature			
TimeDay	1	221.124	**<0.001**
Aspect	2	138.112	**<0.001**
Elevation	1	526.051	**<0.001**
TimeDay*Aspect	2	2.095	0.134
Aspect*Elevation	2	0.496	0.612
Daily max temperature			
TimeDay	1	247.828	**<0.001**
Aspect	2	96.31	**<0.001**
Elevation	1	322.055	**<0.001**
TimeDay*Aspect	2	3.175	0.05
Aspect*Elevation	2	0.463	0.632
Daily min temperature			
TimeDay	1	0.943	0.336
Aspect	2	128.002	**<0.001**
Elevation	1	595.546	**<0.001**
TimeDay*Aspect	2	0.078	0.925
Aspect*Elevation	2	1.361	0.266

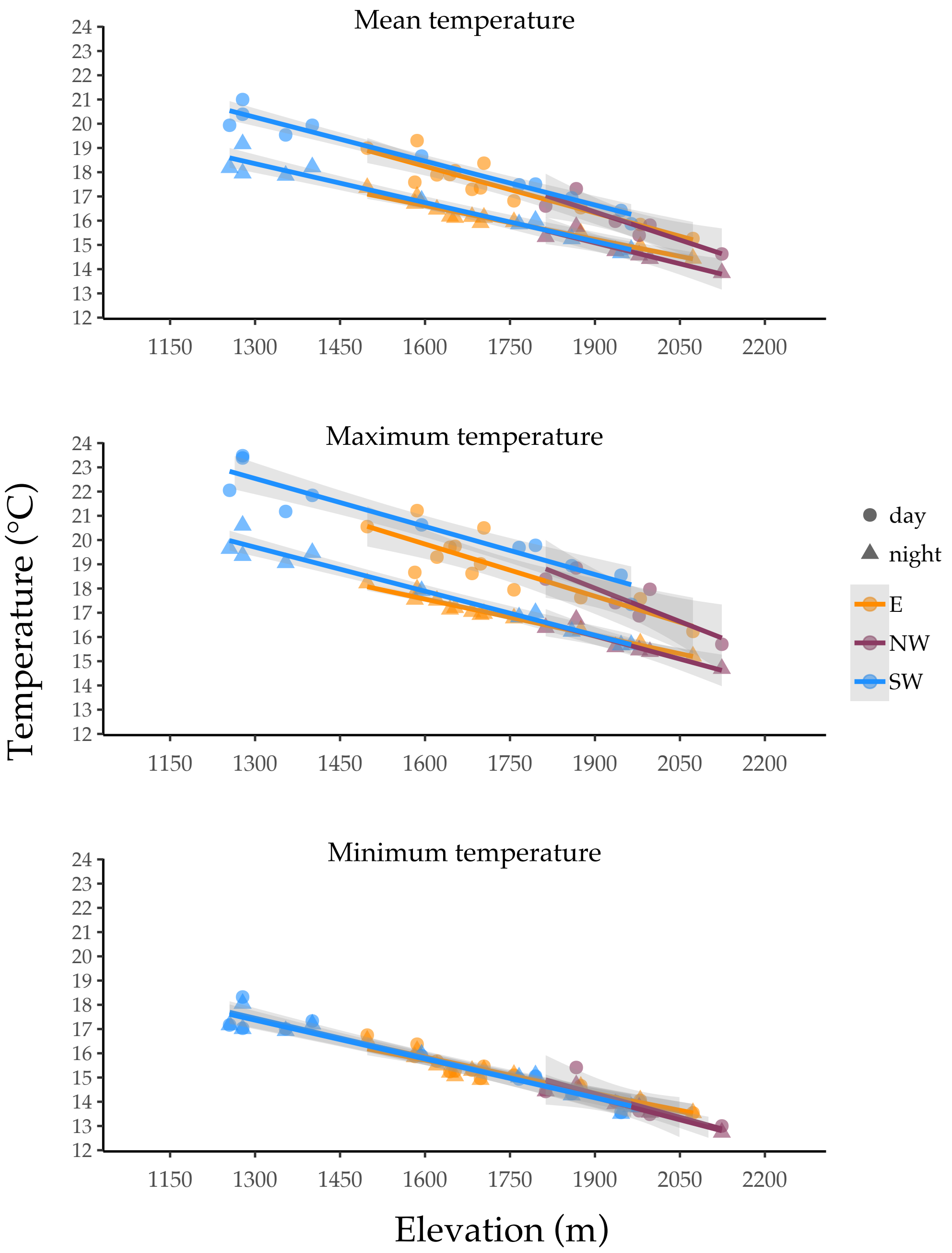

Figure S5.1 Environmental temperature (T_e) lapse rate with increasing elevation at the field site per slope aspect. Plots display mean temperature, mean maximum temperature and mean minimum temperature, per calendar day, between sunrise and sunset and vice-versa ('day' and 'night', respectively). Daily and nightly T_e lapse rates are comparable with increasing elevation, irrespective of slope aspect (see **Table S5.1**).

Table S5.2 Linear models of environmental temperature (T_e), as a function of elevation, from which we predicted T_e regimes at each survey site.

	Estimate	SE	t-value	*p*	r^2
Daytime mean temperatures					
Intercept	28.681	0.577	49.73	<0.001	
Elevation	-0.006	0.3×10^{-3}	-19.48	<0.001	0.93
Nightime mean temperatures					
Intercept	25.178	0.37	68.01	<0.001	
Elevation	-0.005	0.2×10^{-3}	-24.81	<0.001	0.95
Daytime max temperatures					
Intercept	31.974	0.996	32.1	<0.001	
Elevation	-0.007	0.6×10^{-3}	-12.86	<0.001	0.85
Nightime max temperatures					
Intercept	27.231	0.394	69.03	<0.001	
Elevation	-0.006	0.2×10^{-3}	-25.98	<0.001	0.96
Daytime min temperatures					
Intercept	24.354	0.482	50.49	<0.001	
Elevation	-0.005	0.2×10^{-3}	-19.11	<0.001	0.93
Nightime min temperatures					
Intercept	24.166	0.386	62.55	<0.001	
Elevation	-0.0052	0.2×10^{-3}	-23.58	<0.001	0.95

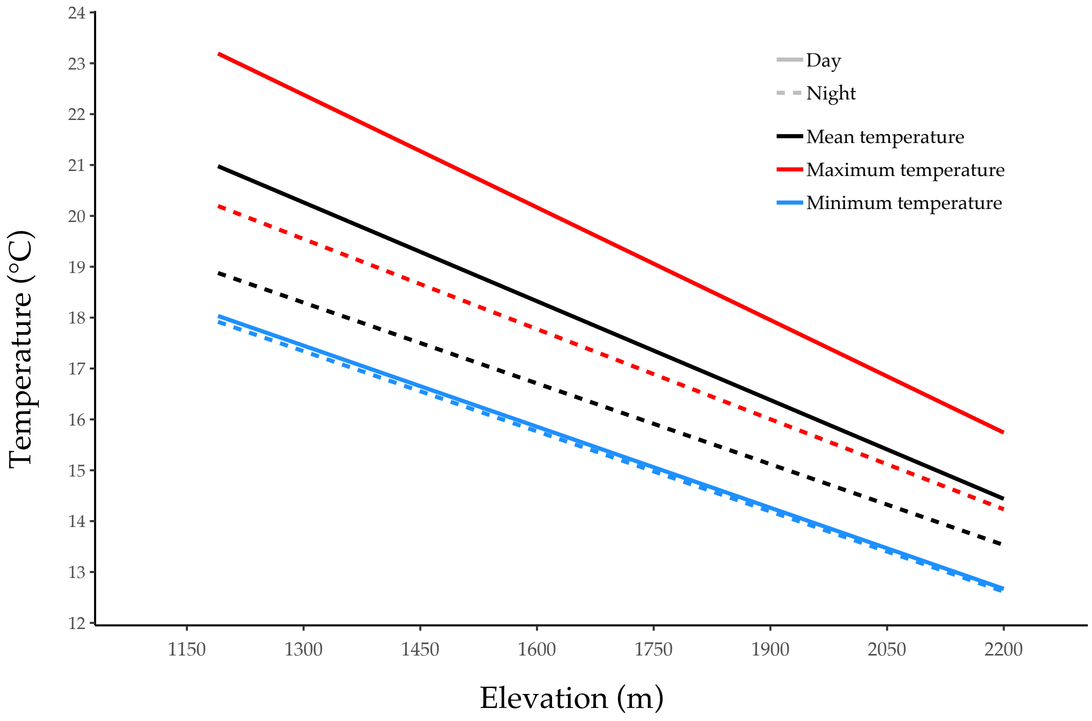

Figure S5.2 Modelled environmental temperature (T_e) lapse rates as a function of elevation for mean, maximum and minimum T_e between sunset and sunrise (‘night) and sunrise and sunset (‘day’). These fits were subsequently used to predict T_e regimes at each survey site.

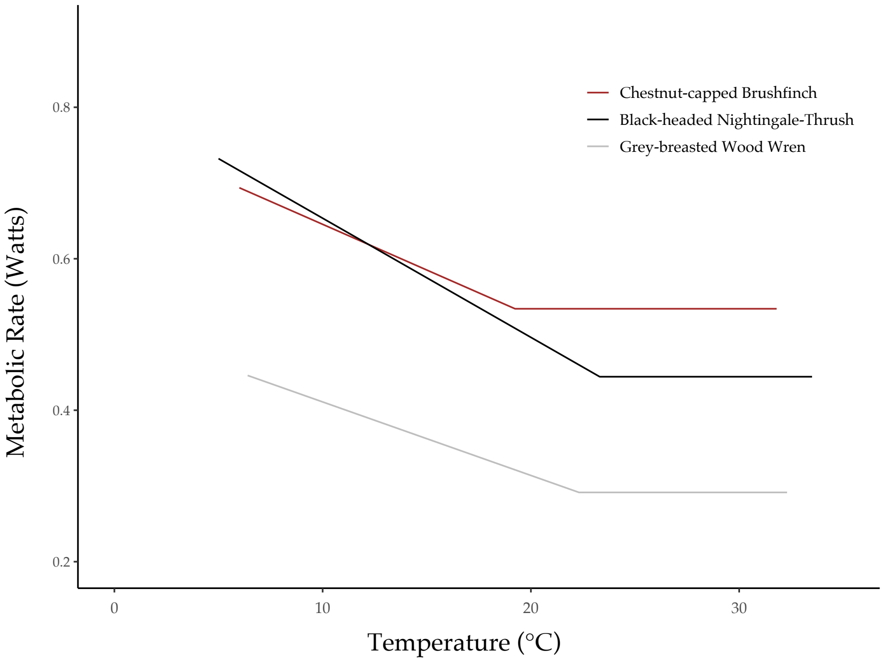

Figure S5.3 Thermal physiology models from which we predicted thermoregulatory costs at different environmental temperatures across elevation (for full methods from which these data were obtained see **Chapter 3).** No thermal model is presented for Ruddy-capped Nightingale-Thrushes *Catharus frantzii* (RCNT) because for this species we did not detect any changes in metabolic rate as a function of temperature across the whole range tested (5-34ºC). As such, thermal costs (multiples of BMR in abundance model covariates) were all ‘1’ for RCNT, denoting no change in thermoregulatory cost across different altitudes. Because metabolic rates were higher in the summer for Black-headed Nightingale-Thrushes *Catharus mexicanus*, the thermal model presented is that of summer metabolism in order to match survey data.

Table S5.3 Fitted models of detection covariates (*p)* against intercept only abundance for *N*-mixture models per species. The top ranking detection model was subsequently used for posterior modelling of abundance.

p	**K**	**AICc**	**Delta AICc**	**AICc Weight**	**Cumulative Weight**	**LL**
Black-headed Nightingale-Thrush *Catharus mexicanus*						
Time	3	738.9	0	0.5	0.5	-365.19
Day + Time	5	739.25	0.36	0.42	0.93	-364.24
Day	4	742.72	3.83	0.07	1	-367.11
Ruddy-capped Nightingale-Thrush *Catharus frantzii*						
Day + Time	5	287.55	0	0.99	0.99	-138.39
Day	4	298.62	11.07	0	1	-145.05
Time	4	298.87	11.32	0	1	-145.18
Grey-breasted Wood Wren *Henicorhina leucophrys*						
Day	4	849.21	0	0.57	0.57	-420.35
Time	4	850.29	1.08	0.33	0.9	-420.89
Day + Time	5	852.79	3.55	0.1	1	-420.99
Chestnut-capped Brushfinch *Arremon brunneinucha*						
Day + Time	5	655.11	0	0.77	0.77	-322.17
Time	4	657.57	2.46	0.23	1	-324.53
Day	4	684.47	29.36	0	1	-337.98

6 DISCUSSION

The central aims of this thesis were twofold. Firstly, I aimed to interpret the elevational range determinants of tropical songbirds by testing three prominent hypotheses of spatial range restriction. Secondly, I aimed to assess how physiology changes with season in montane songbirds, with a particular focus on territorial behaviour in one species, Black-headed Nightingale-Thrushes *Catharus mexicanus* (hereafter BHNT). **Chapters 2, 3** and **5** dealt with the former, investigating behavioural, physiological and ecological specialism in different elevational ranges. **Chapters 3** and **4** dealt with the latter, investigating the physiological state/metabolic cost basis for seasonal differences in territorial aggression in BHNT and comparing physiological attributes between several species. At its broadest scale, this project aimed to understand the ecology of tropical montane species by interpreting ecological processes of ecology and behaviour through prominent behavioural and physiological mechanisms.

6.1 DETERMINANTS OF ELEVATIONAL RANGES

The overarching finding from the three chapters that addressed different elements of elevational range specialisation was that range determinants were caused by a combination of factors, and were complex and possibly species-specific. In **Chapter 2**, I found an asymmetric interaction between two parapatric Nightingale-Thrushes (BHNT and Ruddy-capped Nightingale-Thrushes *Catharus frantzii* - hereafter RCNT), where the lower elevation BHNT was aggressively dominant over its high elevation congener. There was strong evidence to suggest that interspecific competition alone was not a sufficient force to segregate the elevational ranges of the two species, however: their elevational contact zone was also located in accordance to habitat preferences.

In **Chapter 3,** the hypothesis that physiological specialisation may influence elevational ranges was addressed. My results suggested that the study species from **Chapter 2** (BHNT and RCNT) in addition to three other species (Grey-breasted Wood Wrens *Henicorhina leucophrys*, Chestnut-capped Brushfinches *Arremon brunneinucha* and Common Bush Tanagers *Chlorospingus flavopectus,* hereafter GBWW, CCBF and

COBT, respectively*)* did not appear to exhibit physiological specialism that was directly related to the environmental temperatures. Physiological differences were notable between the two species of Nightingale-Thrushes, however, with the higher elevation RCNT (and subordinate – see **Chapter 2**), characterised by physiological traits related to cold tolerance (e.g. considerable broader thermal tolerance and higher haemoglobin concentrations). It appears unlikely that this cold tolerance is an adaption to current climate, however, but instead probably represents conserved traits as a result of historic isolation.

In **Chapter 5**, when a combination of factors predicting species ranges was collated into a single analysis to test the role of different abiotic and biotic factors on elevational range restriction, the drivers of elevational ranges were unclear. I found varying support for the biotic factors tested and found no evidence to suggest that the energetic costs expended on thermoregulation limited elevational ranges. Specifically, for the two species of Nightingale-Thrushes, habitat selection and interspecific competition were most important in driving their range restriction in combination. This extends the results of **Chapter 2** in suggesting that habitat differences were more important for limiting the range of BHNT, rather than RCNT. However, for a mountaintop-restricted species (GBWW), habitat selection (at least within the measured variables) was not the principal factor driving its elevational range contrary to expectations. Finally, for a wide-ranging species (CCBF) there were no obvious factors limiting elevational distribution in accordance with my hypotheses.

6.1.1 Do thermal limits set elevational ranges in tropical birds?

In alignment with Janzen's theory (Janzen 1967), elevational range restrictions of tropical montane species generally corresponding with thermal isotherms have been observed (McCain 2009, Cadena *et al.* 2011). However, despite generating much scholarship (Ghalambor *et al.* 2006), direct evidence for thermal range restrictions have only been tested on tropical mountains in **Chapter 5** and three other studies (Freeman 2015a, Londoño *et al.* 2017, Polato *et al.* 2018). While one of these studies on freshwater ectotherms found support for thermal restriction (Polato *et al.* 2018), the other two (on birds) both concluded that thermal limits did not appear to limit elevational dispersal (Freeman 2015a, Londoño *et al.* 2017). In many respects, the results of these studies are intuitive; the physiology of ectotherms is directly related to local climate, whereas birds have well designed physiological systems to cope in changing climates (e.g. cold tolerance and hypoxia) (e.g. Tucker 1968, Porter & Gates 1969). The findings of my study were thus consistent with those on other tropical montane birds suggesting that thermal barriers do not directly limit dispersal (Londoño *et al.* 2017). Indeed, even with rapid changes between isotherms along tropical mountains, the general abundance patterns of tropical montane birds (typically sharply truncated at range edges, rather than a gradual decline; e.g. **Chapter 2**) suggests that species elevational distributions do not have abundant centres (e.g. Freeman & Beehler 2018), where climatic effects set increasing physiologically, less optimal, limits towards range edges (Sagarin & Gaines 2002).

My results, combined with the two previous studies on thermal barriers to elevational ranges in tropical birds, display a lack of support for Janzen's predictions across disparate tropical regions (Freeman 2015a, Londoño *et al.* 2017). It is important to note, however, that I draw the conclusions from my study in the recognition that it was undertaken over a relatively small elevational gradient on a low number of species and did not include low elevation forest species that could potentially display thermal intolerance at their upper elevation 'cold' limits. Accepting this caveat, given the consistency of these results across regions and within a broad spectrum of avian taxa (now 240 + species), it seems unlikely that thermal barriers acutely set elevational ranges of tropical birds.

Because of the general capacity to cope with cold in endotherms (Porter & Gates 1969) an application of these results in birds to other tropical montane endotherms is appealing. However, the few physiological studies that have been undertaken have suggested direct physiological links to temperature in high elevation mice (Pasch *et al.* 2013) and cold adapted traits in bats (Soriano *et al.* 2002). Thus it remains unclear to what extent recent findings in birds apply across other endotherms, a factor which cannot be remedied without extensive further field studies.

6.1.2 Biotic drivers of elevational range restriction

My results offered support for a dual role of habitat selection and interspecific competition in setting elevational range limits. The role of species interactions setting elevational range limits in tropical species has been the subject of some debate, however, with some authors suggesting it reflects ecological specialisation rather than a sufficiently strong biological force to drive elevational patterns (Cadena & Loiselle 2007, Cadena 2007). Despite an increase in empirical research examining the role of biotic interactions maintaining elevational segregation (e.g. Jankowski *et al.* 2010, Freeman 2015b, Boyce & Martin 2019), my project is the first I am aware of to assess multiple factors in combination with interspecific competition (although see Pasch *et al.* 2013).

Early studies concluded that species interactions were the principal force limiting elevational ranges along tropical mountains (Diamond 1973, Terborgh 1985), supported by a growing body of experimental work (Jankowski *et al.* 2010, Freeman *et al.* 2016). My results, however, contribute to a contrasting (although not mutually exclusive) viewpoint that species interactions are unlikely to act alone in driving elevational range segregation (Jankowski *et al.* 2013, Freeman *et al.* 2019). The result that a mountain top species in this study (RCNT) appears to be restricted to high elevations by a combination of competitive interactions and ecological specialism reflects the suggestions of other authors (Robinson & Terborgh 1995, Freeman *et al.* 2019). Moreover, the results from my study also offer further support for the hypothesis that accumulation of tropical montane taxa is a result of range expansions (e.g. Cadena & Céspedes 2019) and that species interactions drive elevational divergence following secondary contact (Caro *et al.* 2013, Freeman 2015b). Finally, despite scepticism for the role of species interactions driving broad scale patterning in species distributions (e.g. Sexton *et al.* 2009), these results contribute to the alternative hypothesis that species interactions play an important role in the spatial constraints of species across tropical bird communities (Pigot & Tobias 2013).

I was not able, however, to find consistent evidence for habitat selection affecting the distributional limits of the species in my study (e.g. GBWW), suggesting that either the resolution of my methods were not sufficient to capture habitat variation, or that other biotic factors limit elevational ranges for some species. The latter point, in particular, represents a distinct shortfall in my study and in our understanding of the elevational ranges of tropical species more generally because little is known of other potential biotic drivers of elevational range determinants (Jankowski *et al.* 2013). One possible suggestion is that gaps between replacement species (non-parapatric distributions) are generated by interactions with pathogens (e.g. *Plasmodium* sp.), where thermal constraints limit mobile vectors at their upper 'cold' edge, creating truncated elevational distributions for higher elevation species. Famous examples of a similar scenario have been observed in Hawaii following the introduction of avian malaria (van Riper *et al.* 1986). Because my results were inconclusive, it is difficult to suggest a key biotic mechanism influencing elevational ranges. In many respects this result is intuitive, however, given that mountains and their conditions inherently vary between sites and regions (Rahbek *et al.* 2019). One hypothetical explanation is that GBWW is resigned to mountaintops as a result of 'ghost of competition past' (Connell 1980) having formerly been parapatric with White-breasted Wood Wrens *Henicorhina leucosticta* (WBWW) that occur at lower elevations at the study site.

6.1.3 Tropical mountains in a changing world

One of the most important aspects of this project is that it provides a starting point for interpreting the mechanisms underlying elevational shifts in species ranges with climate change. The mechanisms that underlie upslope shifts in tropical montane species remain unclear (Jankowski *et al.* 2013), although generally tropical species shift more in accordance with temperature than temperate species (Freeman & Class Freeman 2014). Because there is little evidence supporting a direct thermal limitation in tropical montane birds (Freeman 2015a, Londoño *et al.* 2017) it seems more likely that warming climates affect elevational range shifts indirectly (Freeman *et al.* 2018). This observation suggests that understanding the importance of various biotic limitations of species elevational ranges is critical in order to better project the range shifts of tropical montane species (Jankowski *et al.* 2013, Freeman *et al.* 2018). The exact biotic determinants are unclear, but may comprise a variety of factors such as species interactions, concurrent habitat shifts (itself comprising the combined influence of habitat loss, fragmentation and degradation) or interactions with ectotherms that are themselves more physiologically specialised to climate (e.g. Feeley *et al.* 2010, Jankowski *et al.* 2013, Fadrique *et al.* 2018, Polato *et al.* 2018).

In the context of this project, two of the study species (BHNT and GBWW) have shifted their elevational ranges upslope over the past decade (Neate-Clegg *et al.* 2018). The biotic drivers of their elevational ranges were habitat related (BHNT) or were not clear (GBWW) so while my study provided a starting point for interpreting these shifts, it ultimately was not able to provide a clear explanation for the potential drivers. Together this emphasises the importance of mechanistic studies exploring a variety of biotic range determinants beyond those explored in this project. Nonetheless, the methodological framework employed (broadly following Jankowski *et al.* 2013) allows for further range determinants to be explored and a starting point for further research.

6.2 PHYSIOLOGICAL DIVERSITY AND THE PACE OF LIFE OF TROPICAL BIRDS

The principal finding from the two chapters (**Chapter 3** and **4**) that focussed on aspects of the physiological ecology of tropical songbirds was that seasonal shifts in physiological traits may be species-specific, and that they appear to be unrelated to current environmental temperature. The results from **Chapter 3** suggest that seasonal shifts in physiological traits were not uniform across species. One species (BHNT) increased BMR in summer (breeding) compared to winter (non-breeding), whereas two other species (GBWW and CCBF) were consistent between seasons. Other physiological traits (body mass and haemoglobin concentrations) were also consistent between seasons for all species. In **Chapter 4** I sought to advance the understanding of seasonal shifts in metabolic rates in BHNT by exploring the relationship between territorial behaviour and changes in metabolic rates in male BHNT. Territorial aggression increased substantially in the breeding season, in concert with increases in BMR. In addition, metabolic responses to conspecific song were also greater in the breeding season, suggesting that there is a functional link between territorial behaviour and song recognition. This result is consistent with ‘performance’ energy management, where BMR positively covaries with energetically costly activity. The link displayed between metabolic rates and territorial behaviour is the first study I am aware of to directly relate metabolic rates and seasonality in life-history traits in a tropical species.

The historical perspective explaining the differences in the metabolic rates of tropical birds suggested they were a result of phenotypic adaption to the warmer, more seasonally benign climates at lower latitudes (White *et al.* 2007, Jetz *et al.* 2008). However, an emergent perspective is that the lower metabolic rates of tropical species are instead directly related to innate characteristics (e.g. Wiersma *et al.* 2007) and that low metabolic rates are consistent across elevation in tropical species (Londoño *et al.* 2015). Broadly, my results support this contemporary hypothesis; I found evidence for seasonal variation that was not linked to temperature variation and species did not appear directly related to the ambient temperatures in their elevational ranges. However, my results also contrast to some extent with this suggestion in the case of RCNT, which displayed several cold tolerance traits. In the case of RCNT, I interpret this result as a conserved trait as a result of historic isolation rather than contemporary adaption. Regardless of the mechanism, this finding suggests that low metabolic rates are not necessarily consistent across tropical birds and that climate processes may influence metabolic rates in some species. Previous studies on highland natives have suggested different relationships, where no apparent cold adapted traits were evident in species that evolved in highlands (Caro *et al.* 2013), or formerly highland natives that have secondarily adapted to warmer lower elevations (Dubay & Witt 2014). Such phenotypic adaption is poorly known in tropical species across elevation but suggests that at least historically climate may play a role in some physiological traits.

My results, particularly from **Chapter 4,** present an intriguing perspective on the physiological link with life-history characteristics. Several recent studies have indicated that phenotypic flexibility in metabolic rates in tropical species is substantial (Wells & Schaeffer 2012, Pollock *et al.* 2019), and it has been hypothesised that this is related to the intrinsic characteristics of different species (Anderson & Jetz 2005). My results offer some of the first direct support for this suggestion, by functionally linking territorial activity and increases in metabolic rates across season. Further work is required to establish why some species have flexible physiological traits while others do not. However, in its broadest sense my results support the suggestions of Anderson

& Jetz (2005), that the diversity of ‘metabolic niches’ is considerable in tropical latitudes. Moreover, this contributes to a growing recognition of the phenotypic flexibility in metabolic rates across birds (McKechnie 2007).

Ricklefs & Wikelski (2002) suggested that physiology and life-history traits are inextricably linked in a ‘nexus’, where physiological control mechanisms place limits on organismal performance. While my results on BHNT did not necessarily isolate the mechanism, they do, however, suggest that energy is managed in order to facilitate more energetically demanding territorial behaviours. A distinct shortfall in the interpretation of a mechanism in this is that I was not able to measure testosterone, which is likely (although not in all cases Moore *et al*. 2018) to increase territorial aggression (Hau *et al*. 2010). The differences in seasonal territorial aggression in BHNT contrasts to some extent with many lowland species, which often have low levels of testosterone across the year, allowing year-round territorial defence (e.g. Hau *et al*. 2000, 2004). One possible interpretation is that testosterone profiles in BHNT vary strikingly across the year, explaining the dichotomy of territorial aggression observed. Indeed, tropical montane species often have high testosterone profiles in comparison to lowland species (Goymann *et al*. 2004). High testosterone may reduce immunocompetence, but the increases in metabolic rate observed in BHNT may also represent an energy allocation strategy where reduced immunocompetence is countered by increase in maintenance (by upregulated BMR). Alternatively (although not mutually exclusive) the cooler conditions at higher elevations in the tropics may also reduce infection likelihood (e.g. Attenborough *et al*. 1997), allowing for longer periods of territorial defence with elevated testosterone levels. This is of course speculation, but merits further investigation considering the potential links with hormones and neural recognition (Louder *et al*. 2019).

6.3 FUTURE RESEARCH

6.3.1 Determinants of species elevational ranges

Despite longstanding interest in the high biotic turnover along tropical mountains, the factors maintaining these patterns remain poorly understood. The results from my project present a number of opportunities for future work, both in additions to my project, and the application of its methods elsewhere. A valuable extension to both **Chapter 2** and **Chapter 5** would be to investigate how interspecific competition manifests across the year. My results from **Chapter 4** displayed that BHNT significantly reduces territorial aggression in the non-breeding season (winter) and it thus remains unknown if competitive interactions with RCNT illuminated in **Chapter 2** remain constant across the year. By extension, more fine scale studies both on BHNT and RCNT examining ecological differences such as habitat usage or breeding ecology would provide an insight into the mechanisms that drive their habitat preferences (e.g. Rangel-Salazar *et al.* 2008). Such studies would be readily achievable at the field site. In unison, one of the clearest avenues for future work from this project would be to better identify the other biotic mechanisms that underlie species turnovers. This is not a simple project, although my study provides a starting point at least for future work in this system. For example, assays on parasitic vectors such as mosquitos could provide an insight into potential range limiting factors for non-parapatric species.

A further logical extension to my project would be the inclusion of more species, particularly from lower elevations. One of the principal additions could be WBWW, the lower elevation congener of GBWW (indeed this was one of the initial plans). This would allow for a test of the biotic and abiotic limitations for a lower elevation species at its 'cold' limits, which does occur in parapatry with a congener that my study was not able to include. Because my surveys in **Chapter 5** covered the entire avian community, data exist from which habitat variables and abundance could be readily applied (see Introduction **Figure 1.4**). In addition, a computational addition to my project would be to model how species elevational ranges may change in line with future climate projections. Much of the data are available to do this – for example we know the upslope shifts in some of the study species (Neate-Clegg *et al.* 2018) and temperature mismatches lapse rates could be applied to global datasets (e.g. https://www.worldclim.org/). There remains a critical lack of such mechanistic studies, and particularly in studies combining experimentally informed data.

An extremely valuable and plausible extension to the project longer term would be the inclusion of other taxa. The elevational ranges of groups of species across trophic levels such as dung beetles, herpetofauna and rodents are relatively well known at the field site (although in largely unpublished databases). The physiological methods that I employed could be adapted with relative ease to measure thermal tolerances (e.g. using different sizes of respirometry chambers). Because the sampling design at the field site is consistent across taxonomic groups, habitat data and thermal lapse rates that I have used could be immediately applied to new data on other species. This would provide a novel and unique insight into the elevational determinants across taxa, allowing tests of competing hypotheses across trophic levels and endo/ectotherms. Indeed, I am aware of no other studies globally that have assessed different biotic and abiotic factors driving elevation ranges across taxa at one site.

Extending aspects of this project to its broadest scale, each of the species in this study occurs at a number of sites throughout Central America, where their elevational ranges differ. For example, in the Tilarán mountains of Costa Rica, BHNT are restricted to a tighter elevational range than in my field site, where it is bounded at its upper and lower elevational limits by two other species of Nightingale-Thrushes (Jankowski *et al*. 2010). Similarly, WBWW also comes into direct contact with GBWW in the Tilarán mountains and aggressive interactions between the two species are present (Jankowski *et al*. 2010). Such examples offer natural replicated tests of different biotic and abiotic factors across tropical mountains, allowing different range determinants to be examined at different elevations (Mahli *et al*. 2010). There are numerous other mountains throughout central and western Honduras, and into Guatemala that would offer ideal systems on which to examine these different turnovers for which my project could be directly replicated.

6.3.2 Physiology and the pace of life of tropical birds

Our understanding of the physiology of tropical birds (seasonal or otherwise) remains in its infancy, presenting numerous intriguing avenues both in extensions to this project and beyond. An obvious extension to my study (as per **Chapter 3**) would be the inclusion of more species to investigate the variation of seasonal physiological shifts across species and also in obtaining winter measurements for RCNT. It was not possible to access the higher elevation sites during the non-breeding season (winter) but winter measures on RCNT, as well as additional tests on higher elevation species, would better clarify my conclusions that the flexibility in seasonal metabolic traits observed in lowland tropical birds is also present in highland species.

Additionally, more tests of congener pairs (ideally with the inclusion of genetic data to elucidate evolutionary history) would establish the ubiquity of the physiological divergence observed between BHNT/RCNT as a result of secondary contact. An ambitious study could also employ common garden experiments on BHNT and RCNT to test whether the physiological divergence observed is a result of evolutionary history rather than phenotypic adaption as I hypothesize (e.g. Wikelski *et al.* 2003). Finally, another extension to this aspect of the project would be an inclusion of other measures of energy expenditure such as field metabolic rates (through heart-rate telemetry) as well as measures of body temperature. Considering the results of **Chapter 4**, different measures of energy usage may offer an explanation for the differences observed in seasonal changes (or lack thereof) between and within species.

One of the clearest opportunities for future research from my project are replications of the experiments employed in **Chapter 4,** but with different species and across different territorial systems. A plausible hypothesis is that species that maintain consistent levels of territorial aggression year-round (e.g. Hau *et al.* 2000, Tobias *et al.* 2011) may have metabolic responses and seasonal metabolic rates that remain 'low and slow' across the year as an energy maintenance strategy. By extension, more invasive experiments could examine aspects of electrophysiology (see Louder *et al.* 2019) on the brains of tropical birds during exposure to conspecific song. Previous studies indicated that the brains of tropical birds are one of the few organs that are comparable to temperate species in size (Wiersma *et al.* 2012) offering an intriguing opportunity to investigate neural aspects of the slow pace of life and how this relates to territorial function. Indeed, **Chapter 4** only investigated a simple aspect of signal recognition between congeners and a control, however the diversity of territorial systems allow a number of unique and logistically achievable tests of hypotheses such as how species respond metabolically to neighbouring territories/'familiar' song or more aggressive songs at higher song rate/'the 'dear enemy effect' (e.g. Moser-Purdy *et al.* 2017). Finally, an additional extension would be to replicate the methods of **Chapter 2**, with the experimental methods in **Chapter 4** and investigate the energetics involved with interspecific competition and song recognition at the interspecific contact zone.

More broadly, a largely unexamined area of research is the relationship between metabolic rates and the variety of life-history characteristics *within* tropical birds. Previous comparative studies have investigated other aspects of physiological systems across tropical birds such as the endocrine system (e.g. Goymann *et al.* 2004, Goymann & Landys 2011), but the metabolic diversity associated with different life-history characteristics across tropical birds is little known. There is a reason to suggest that this diversity is important, however; higher BMR has been shown to covary with survival rates in tropical species (Scholer *et al.* 2019) but what drives this diversity in BMR is unknown.

6.4 CONCLUDING STATEMENTS

The findings from this thesis highlight both the complex nature of tropical montane systems and the interplay between physiological diversity and life-history characteristics of tropical birds. Firstly, aspects of this project focussing on the drivers of species turnovers display, intuitively, that species with different patterns of turnover may be a result of different mechanisms. Importantly though, my results indicate that these mechanisms appear primarily biotic and that physiological explanations for range restriction are unlikely, in contrast to classic hypotheses. Secondly, my results add further evidence to a growing appreciation for the physiological diversity of tropical species and provide evidence for the suggestion that physiological mechanisms may govern life-history characteristics. It is important to stress, however, that substantial knowledge gaps remain in site and/or species-specific studies to advance both these topics across the diversity of tropical fauna.

On a final, philosophical note, this study represents one small advance towards the substantial knowledge gaps still to be filled in our understanding of the ecology of tropical birds. Acutely surmised by Moore *et al*. (2018) *"..as the vast majority of the world's birds are tropical and the Neotropics are among the most biodiverse areas on earth, the future opportunities for major discoveries are limitless."*

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